ETHIOPIAN INSTITUTE OF WATER RESOURCES

ADDIS ABABA UNIVERSITY

DETERMINANTS AND WATERBORNE PATHOGENS TO THE CAUSE OF INFANT MORTALITY IN EASTERN ETHIOPIA

A PHD DISSERTATION SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD) IN WATER AND PUBLIC HEALTH

BY

Samuel Mebrahtom (Bsc,BA,MPH)

Supervisors

Professor Alemayehu Worku (Senior Advisor), Addis Ababa University, College of Health**,** Sciences, School of Public Health

Professor Daniel Gage (Co-Advisor), University of Connecticut, School of Engineering, Department of Molecular and Cell Biology

December, 2023

Addis Ababa, Ethiopia

DISSERTATION APPROVAL
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Determinants and Waterborne Pathogens to the Cause of Infant Mortality in Eastern Ethiopia

By: Samuel Mebrahtom

Ethiopian Institute of Water Resources, Addis Ababa University

Approved by the Examining Board

_____	_____
Chairman, examination Board	Signature and date
_____	_____
Advisor	Signature and date
_____	_____
External Examiner	Signature and date
_____	_____
Internal Examiner	Signature and date
_____	_____
Internal Examiner	Signature and date

ABSTRACT

This dissertation report is for the study entitled "Determinants and waterborne pathogens to the cause of infant mortality in eastern Ethiopia". The main objective of the study was to determine the cause of infant deaths at the population level and analyze patterns of associated factors among the most common deaths; identify facors contribute to diarrhea-related infant deaths; and detect pathogens that cause severe and fatal diarrhea from infant drinking water and analyze their relationship with water quality determinates. Understanding the cause of infant death at population level in developing countries including Ethiopia is still challenging, for the reason that most infants die at home and lost their life without having had contact with health facilities and no civil registration system. Infant cause of death in a range of setting and the pattern of associated factors among the common cause of death against the overall cause is not well known. Infant deaths are intrinsically linked to several causes and influencing factors that need extensive studies. Diarrhea has been shown to be one of the leading causes of infant mortality in Ethiopia, and its burden is still a serious concern. The risk of unsafe water supplies, inadequate sanitation, and insufficient hygiene practices has a substantial association with diarrheal deaths, which attributed to 88% of diarrhea-related deaths. Diarrhea is typically a waterborne disease that is caused by an extensive range of pathogenic microbes. *Cryptosporidium*, *Shigella*, a toxin-producing strain of *E. coli*, and rotavirus have all been reported as the most responsible for causing severe and fatal diarrhea in infants. Many infants died from illnesses caused by these pathogenic agents, which thrive in contaminated water. The first study objective employed a community-based prospective longitudinal survey, which was conducted with routinely enumeration of reported infant deaths for a period of two years (from September 2016 to August 2018) in Eastern part of Ethiopia. Using the two-stage cluster sampling technique, the study was undertaken in four randomly selected districts of West Hararghe zone in Oromia and two districts of zone 3 in Afar regional state. The study included a total of 362 infants who were deceased during the study period. Data was collected by trained enumerators by interviewing the mothers or guardians of the deceased infant using a 2014 standardize World Health Organization (WHO) Verbal Autopsy questionnaire. InterVA-4 model were used for processing and interpreting verbal autopsy data in order to arrive at the most likely causes of infant death. SPSS version 23 was also used for statistical analysis of frequency distribution and logistic regression for the association between covariates and outcomes. The second study objective employed community based unmatched nested case-control study design in Eastern Ethiopia. The cases were infants who died from diarrheal disease while controls were those who survived their first year of life from September 2016 to August 2018. A total of 305 study subjects (61 cases and 244 controls) were included in the study. Infants dying from diarrhea were compared to four neighborhood controls in terms of several risk components of Water, Sanitation and Hygiene. Data were collected from mothers/care takers of infants using pre-tested structured questionnaires, and entered onto CSpro version 5.1 and transform to SPSS version 23 to analyzed potential risk factors. A molecular (LAMP)-based cross-sectional study design was employed. A total of 410 water samples were collected from infant point-of-use at household

level and 37 samples from the corresponding water sources from June 2020 to May 2021. Data were collected from the household's mothers/care takers of infants using pre-coded structured questionnaires. The LAMP assay was applied for the detection of the targeted pathogens. The data were entered using CSpro version 6.1 and transform to SPSS version 23 for analyses. For the study objective one, the result shows that Of the overall (362) deceased infants' during the study period, 53.0% of deaths occurred during neonatal time while 47.0% died in the post-neonatal period. Acute respiratory infection including neonatal and post-neonatal pneumonia (38.4%), birth asphyxia (16.4%), diarrheal diseases (16.3%), prematurity (7.4%) and malaria (4.3%) were found to be the leading causes of infant mortality in the study area. The independent factors strongly associated with probable ARI, including pneumonia related mortality as compared to all-causes of death were infants with maternal age lower than 20 years old (p=0.001, AOR: 4.82, 95% CI: 1.88, 12.3) and infant being died outside of heath facilities (P=0.007, AOR: 2.85, 95% CI: 1.33, 6.12). The post-neonatal period (P=0.000, AOR: 15.5, 95% CI: 6.35, 37.8) and infant died in the wet season (P=0.006, AOR: 2.38, 95% CI: 1.28, 4.44) had strong relationship with dying from diarrhea-related death than those infants died from all non-diarrhea. The death due to malaria robustly associated with infants whose mothers age between 20-35 years old (P=0.024, AOR: 4.44, 95% CI: 1.22, 16.2) and infant who was dwelled in the districts of Afar region (P=0.013, AOR: 4.08, 95% CI: 1.35, 12.4). The factors that found to be significantly associated with infant death from diarrhoea after adjustment for confounding variables included the age of mother with <20 years old (*P=0.010*, AOR: 21.7, 95% CI: 2.10, 224.7), unsafe drinking water storage (*P=0.014*, AOR: 2.59, 95% CI: 1.22, 5.56), infants in households without point-of-use water treatment practices (*P=0.004*, AOR: 4.73, 95% CI: 1.66, 13.5), households with unimproved sanitation (P=0.050, AOR: 2.74, 95% CI: 0.99, 7.58), unsafe disposing of child feces (P=0.015, AOR: 2.88, 95% CI: 1.23, 6.75), improper management of solid waste (P=0.003, AOR: 3.33, 95% CI: 1.50, 7.07), households with improper management of liquid waste management (P=0.011, AOR: 3.38, 95% CI: 1.32, 8.66), households did not practiced hand washing at any critical times (P=0.015, AOR: 4.71, 95% CI: 1.34, 16.5) and households practice hand washing in lesser than three critical times (P=0.029, AOR: 2.99, 95% CI: 1.12, 8.04) as compared with their reference group. *Cryptosporidium* oocysts, *Shigella* species, a toxin-producing strain of *E. coli*, and rotavirus were detected in 28.5%, 30.0%, 26.3%, and 32.2%, respectively, of the water samples tested from infant point-of-use. All four pathogens together were detected in about 13.2% of the water samples. *Cryptosporidium* oocysts, *Shigella* species, toxin-producing *E. coli*, and rotavirus were detected in 27.0%, 32.4%, 29.7%, and 37.8%, respectively, of the water samples tested from water sources. For each targeted pathogen, there was a significant positive correlation between the infant's point of ingestion and the water sources it was drawn from. The presence of *Shigella* species, toxin-producing *E. coli*, and *Cryptosporidium* oocysts in the water samples was significantly and strongly associated with the unimproved water source. In conclusion, the highest cause of infant mortality was associated with diseases of the respiratory system, followed by diarrheal diseases. Most of the infant deaths that existed were as a result of diseases and conditions that are readily

preventable or treatable, similar to those reported worldwide. The patterns of significantly associated factors across the most leading cause-specific mortality against all-causes of death were dissimilar. Infants mother/caretakers whose the age with <20 years old, households without point-of-use water treatment practices and households did not practiced hand washing at any critical times shows the most higher odds of diarrhea-related infant deaths. Infants demonstrated high levels of exposure to contaminated drinking water by those recognized pathogens that cause the most severe and fatal diarrhea. Unimproved water sources remained the only strong predictors for the presence of these pathogens in infant drinking water. Therefore, strengthening maternal and child health programs with effective preventive interventions emphasizing the most common cause of infant deaths and those factors contributing to raising mortality risk is required. Due attention should be given to the reduction of diarrhea-related infant deaths through WASH intervention, taking into account the strong associated risk factors typically during the infantile period. Efforts should be made to improve water supplies, protect the sources, and educate caregivers of infants about safe drinking water practices and health.

Key words: Cause of infant mortality, Diarrhea-related infant death, Risk factors, Water, Sanitation and Hygiene, *Cryptosporidium*, *Shigella*, *Toxin-producing strain of E. coli*, Rotavirus, Drinking water, *LAMP*

ACKNOWLEDGEMENTS

I am grateful to my principal advisor, Professor Alemayehu Worku from Addis Ababa University's School of Public Health, and my co-advisor, Professor Daniel J. Gage from the University of Connecticut's Department of Molecular and Cell Biology, for their invaluable guidance and insightful feedback throughout my dissertation work.

I am also really appreciative to Addis Ababa University's Ethiopian Institute of Water Resources for giving me the opportunity to conduct this study. The Ethiopian Biodiversity Institution (EBI), Ethiopian Public Health Institution (EPHI), and Care Ethiopia-West Hararghe Field Office have my sincere gratitude and acknowledgement for their in-kind and technical resource assistance.

I would like to thank the West Harargehe zonal health department and the overall districts (Chiro, Mieso, Gemechis, Tullo, Amibara and Awash Fentale) health offices for their dedication and commitment in providing information and facilitate the study. I also extend my sincere thank for the study participants such as data collectors, supervisors and the respondents.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

AIDS	Aquired Immuno-deficiency Syndrome
ALRTI	Acute Lower Respiratory Infection
AOR	Adjusted Odds Ratio
ARI	Acute Respiratory Infection
ARTI	Acute Respiratory Tract Infection
cDNA	Complement Deoxyribonucleic acid
cHDA	Circular Helicase-dependent Amplification
CI	Confidence Interval
COR	Crude Odds Ratio
CSPro	Census Statistics Program
CSV	Comma Separated Variable
DNA	Deoxyribonucleic Acid
E. coli	Escherichia coli
EIWR	Ethiopian Institute of Water Resources
ETB	Ethiopian Birr
HAD	Helicase-dependent Amplification
ICD	International Classification of Disease
ICR	Information Collection Rule
IMDA	Isothermal Multiple Displacement Amplification
InterVA	Interpreting Verbal Autopsy
L/c/d	Liters per capital per day
LAMP	Loop-mediated Isothermal Amplification
NASBA	Nucleic Acid Sequence-based Amplification
NCBI	National Center for Biotechnology Information
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PSR	Polymerase Spiral Reaction
RCA	Rolling Circle Amplification
RNA	Ribonucleic Acid
RPA	Recombinant Polymerase Amplification
RTI	Respiratory Tract Infection
SD	Standard Deviation
SDA	Strand Displacement Amplification
SDG	Sustainable Development Goal
SMART	Single-mediated Amplification of RNA Technology
SPIA	Single Primer Isothermal Amplification
SPSS	Statistical Package for Social Sciences
VA	Verbal Autopsy
WASH	Water, Sanitation and Hygiene
WHO	World Health Organization

LIST OF APPENDICES

GLOSSARY OF TERMS

Infant mortality: defined as the death of a live-born baby before his or her first birthday.

Oligonucleotide: The term "oligonucleotide" or "oligo" usually refers to a synthetic laboratory-made DNA or RNA strand (211).

Molecular methods: can be defined as those methods that target macromolecules containing information about the identity of the microorganisms that produce them (131).

Loop-mediated isothermal amplification (LAMP): is a rapid molecular method in which reaction performed in a single-tube under isothermal condition for the amplification of nucleic acid (DNA or RNA) by using Bst DNA polymerase with strand displacement for the detection of certain pathogen (140)

Optimization: determination of optimum concentrations, temperature and time at which the LAMP functions to its best capacity both in terms of yield and specificity.

Primer: is a short single-stranded DNA fragment used in molecular laboratory techniques in which pair of primers hybridizes with the sample DNA and defines the region that will be amplified, resulting in millions and millions of copies in a very short timeframe (212).

Primer Sequence: defined as strand of short nucleic acid sequences that leads as a starting point for DNA synthesis.

Toxin Producing *E.coli*: *E.coli* are a diverse group of bacteria that normally live in the intestines of humans and animals. Although most strains of these bacteria are harmless, some produce toxins that can make human to be sick and cause diarrhea (loose stool/poop) such as Shiga toxin-producing *E. coli* (STEC).

Longitudinal survey: is a research design that involves repeated observations of the same variables (e.g people or groups) to detect any changes that might occur over a period of time.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Infant mortality refers to "the death of a live-born infant before their first birthday"(1,2).The infant mortality rate reflect as not only a measure of the risk of infant death, but it is also used more broadly as a crude indicator of community health status, poverty and socioeconomic status levels in a community, and the availability and quality of health services and medical technology(3). It is, therefore, recognized as uniquely vulnerable to several factors that impact health, represents a long-standing concern for public health, and is an important marker of the overall health of a society(2).

Globally, around 44% of all under-five deaths occurred during the neonatal period(4), and 75% occurred within the first year of life, with an estimated 4.1 million infants dying in 2017(5). More than 98% of these deaths occur in developing countries(6), and roughly half of those deaths occur in sub-Saharan Africa(6,7). Worldwide, the infant mortality rate has shown a decline from the rate of 63 deaths per 1000 live births in 1990 to 29 deaths per 1000 live births in 2017(5), yet the rate is at its highest level in the developing world(5,6). Evidence showed that Ethiopia has shown a remarkable improvement in the reduction of infant mortality(8–10). Despite this improvement, however, the infant death rate is still at its highest level, which stands at 59 deaths per 1000 live births, with a disproportionately higher rate among the population of some regions(9).The Afar and Oromia regions are among the highest in infant mortality.

Infant deaths are intrinsically linked to several causes and factors. A cause is a base that leads directly to death, while a contributor is a risk factor that makes the death more likely to occur(11). Many different causes of infant mortality exist, from infection to birth defects or accidents. Multiple risk factors contributed to the occurrence of infant deaths, such as maternal and infant characteristics, socioeconomic conditions, maternal and child health care, quality and access to medical care, nutrition, the availability of safe water, sanitation and hygiene, and other public health practices(12,13).

Determination of causes of death is a global concern, as around half of the world's children die without any formal registration of the cause of death(14). In such situation, verbal autopsy is the only available tool and best solution to determine the cause of death(15,16). Verbal autopsy interviews have been used by researchers and policymakers to determine the cause of death for many years(17). However, there is still a challenge in interpreting the cause of death from VA interviews in a reliable and consistent manner. Recently, a computer based algorism models have been developed. Among the available models, InterVA is a suite of computer models for interpreting verbal autopsy data that recognizes a range of indicators relating to a particular death, processes them in a mathematical model based on Bayes' theorem, and produces as its output the likely cause(s) of death(18). Taking its limitations into account from validation studies, the 2014 WHO VA instrument was developed to ascertain all individuals' causes of death and describe cause-specific mortality fractions at the population level(19).

According to global health estimates, there are many different causes of infant mortality(20). Among these, diarrhea is a major cause of morbidity and mortality in infants and children

worldwide(21). It is still appeared to be as one of the leading global killers (22,23) and disability-adjusted life-years lost(24,25). According to the World Health Organization, more than half a million of diarrheal-related deaths reported among under five children each year in worldwide(26,27). In 2019, diarrhea was responsible for about 7.4 percent of all global causes of deaths to children <5 years of age (20). Around 90% of all diarrhea-associated deaths occur in children under five years of age, particularly in low-and-middle income countries(28,29).

Despite a substantial reduction in the total annual number of diarrheal-related deaths observed in the world among children <5 years over the period 1990–2017, the number of deaths remained highest in some of the world's developing countries(30). According to the report, south Asia and sub-Saharan Africa were among the areas with the highest death rates, where 78% of childhood diarrheal deaths occurred (31). From all deaths, the top five countries where the most frequent diarrhea deaths occurred include Nigeria, India, Pakistan, the Democratic Republic of the Congo, and Ethiopia (31,32). While the majority of diarrhea cases occur in developing countries, developed nations also experience a considerable burden from diarrhea(33).

Diarrhea has been shown to be one of the leading causes of infant mortality in Ethiopia (20,34), and its burden is still a serious concern (35–38). Although the number of children under five who died from diarrhea decreased from 16% to 8% between 2000 and 2016, the decline in diarrhea-related deaths in this nation was found to be uneven(34). Diarrheal disease is the fourth leading cause of infant death in Ethiopia, responsible for a death rate of 136.6 deaths per 100,000 populations by the year 2019 (20). The risk of contaminated water supplies, poor sanitation, and poor hygiene practices has a very substantial association with diarrheal death, which is attributed to 88% of diarrhea-related deaths(39–41).

According to the WHO, diarrhea is commonly defined as the passage of loose or watery stools occurring three or more times in a 24-hour period and causing death by depleting body fluids, resulting in profound dehydration(42). It can be easily treatable and preventable(41–43). Most diarrhea-related deaths occur in small children(44,45). Infants (<1 year of age) are being placed at the highest risk of death from diarrhea among children under 5 years of age(33,46). Diarrhea can have a harmful impact on childhood growth and cognitive development(47). Hence, paying particular attention to this age group will have a marked effect on reducing infant mortality. There are multiple risk factors that are likely to be responsible for the cause of childhood diarrhea-related mortality. Along with biological and social factors, environmental factors such as unsafe water supply, poor sanitation, and insufficient hygiene practices are one of the main risk factors that contribute to the diarrheal death of infants(48–50), and are also the leading risk factors identified in the world(42,44,51).

Diarrhea is typically a waterborne disease that is caused by an extensive range of pathogenic microbes, which include viruses, bacteria, and parasites, and are responsible for high morbidity and mortality rates in infants and children(52). The etiologic agents causing diarrhea are typically transmitted by the fecal-oral route to another person upon ingestion of contaminated water and food(53). Infants (<1 year of age) exposed to etiologic agents are much more likely to develop severe disease than the general population(54). Some of these agents can cause immediate morbidity and mortality, while others may not be noticed for many years.

According to the existing studies, there are pathogenic microbes responsible for causing the most severe and fatal diarrhea among infants worldwide. These studies reported that the most common causes were rotavirus, the protozoan *Cryptosporidium*, and bacteria, including *Shigella* and a toxin-producing strain of *E. coli* (55–57). These four etiologic agents account for more than 60% of diarrheal deaths in children under 5 years of age worldwide, including Ethiopia(32).

Poor drinking water quality is continuing to pose a major threat to human health and remains one of the most significant challenges that the societies face (58). In addition, the role of drinking water as a carrier of disease-causing microbes is a major public health concern in many developing countries. Evidence suggests that a number of infants and children died from pathogenic organisms that occur in contaminated drinking water(59,60). Reportedly, unsafe water is continuing to be responsible for 72.1% of diarrhea deaths in children under 5 years old (31). The global initiative needs to conduct extensive studies on pathogenic organisms in drinking water and catalyze action to reduce morbidity and related deaths.

The molecular-based techniques that involve direct DNA or RNA detection have been essential for the correct identification of species-specific pathogenic agents in drinking water samples. Several molecular methods, such as PCR, real-time PCR, and multiplex PCR assays, have been developed to detect the pathogens accurately(61). In recent years, advanced molecular techniques have been developed that guide the use of methods that amplify the nucleic acid material at isothermal conditions. Among these techniques, loop-mediated isothermal amplification (LAMP) is a powerful nucleic acid amplification method that is simple, highly sensitive, and specific, less time-consuming than PCR-based methods, and less prone to inhibition from DNA preparations(62,63). It is ranked as the most frequently used method (64), and is also an important constituent for the efficient screening and testing of drinking water samples in resource limited settings(65).

The occurrence of pathogenic microbes in infant drinking water is affected by a combination of a wide range of natural and human influences on water quality (66), many of which are inadequately understood. Based on the district sector offices report document, the eastern parts of Ethiopia are vulnerable to a recurring lack of access to and use of clean water, and infant diarrheal morbidity and mortality are commonly reported.

1.2 Statement of the problem

Determining the cause of infant death has been the worst and most serious concern in developing countries, and yet little is known about the cause of death in sub-Saharan Africa(67). Understanding the cause of infant death in any setting is still difficult, and Ethiopia is one of the countries with a lack of consistent and reliable cause of death information(68,69). This is due to the fact that most people die at home(68,70–72) and lose their lives without ever having contact with the healthcare system(70,73). Additionally, the civil registration systems are typically nonexistent, and there is limited medical capacity to issue death certificates for the general population(68). Given this circumstance, it becomes extremely difficult to comprehend infant health issues in general and eastern Ethiopians' health issues in particular.

In a situation where the majority of deaths take place at home and civil registration systems are insufficient, verbal autopsy is the only tool available and the best alternative approach to determining the cause of death(15,16). It is also frequently applied to estimate deaths by specific causes. However, there is still a challenge in interpreting the cause of death from VA interviews in a reliable and consistent manner. Recently, verbal autopsy data interpretation techniques using computerized methods have been developed. Among the existing models, InterVA is one of a suite of tools for interpreting verbal autopsy data that recognizes a range of indicators relating to a particular death, processes them in a mathematical model based on Bayes' theorem, and produces as its output the likely cause(s) of death(18).

Infant deaths are intrinsically linked to several causes and influencing factors that need extensive studies. It is essential to look into the key causes of infant mortality at the population level and analyze the factors raising the risk of mortality due to each major cause. There is very little literature on the causes of infant deaths as compared to studies on neonates and children under the age of five, especially in Ethiopia. Few studies have examined the population-level causes of infant mortality. The existing studies were mainly conducted in hospitals and at demographic and health surveillance sites. Additionally, many studies have established the risk factors for dying among those who did not die, and no research has yet been done on the patterns of factors linked to the most frequent cause-specific infant mortality compared to all other causes of death. Without addressing this knowledge gap, it's difficult to plan the best solutions, and it's going to be hard to see the same level of progress in the reduction of infant mortality in the country that has been made in the last decade.

Infant deaths from diarrheal disease have remained as one of a top national priority over the last decades (9,38). Diarrhea caused by the water-borne diseases is the fourth leading cause of infant death in Ethiopia, responsible for death rate of 136.6 deaths per 100,000 populations by the year 2019 (20). Evidence suggests that unsafe water supplies, inadequate sanitation, and insufficient hygiene practices are contributing factors to 88% of deaths associated with diarrhea(39–41). In Ethiopia, an estimated 35.1% and 93.7% of households lack access to improved water supply and sanitation, respectively, and yet the rate of hand washing with soap remains low (17.6%) (38). The regions that reside in the eastern part of Ethiopia have been known as one of the areas where the highest rates of diarrheal morbidity and mortality are observed (38). According to the medical report of the health sector, diarrhea is one of the top ten causes of morbidity in the districts located in eastern Ethiopia. However, studies that look at risk factors for diarrheal death are rare (49). Indeed, a large number of studies have focused on children under five, mostly in the area of diarrheal morbidity (51), and studies on the factors that increase the risk of diarrhea-related death, particularly in infants, are scarce and have not yet been examined in the study area. Hence, there is a need for such a study because it will assist public health practitioners in better understanding how to select strategies and measures to reduce diarrhea-related mortality.

The pathogenic microbes known to cause the most severe and fatal diarrhea in infant have been identified (31,55,57), which include rotaviruses, *Cryptosporidium, Shigella*, and an *E. coli* strain that can produce toxins. These four etiologic agents together accounted for more than 60% of diarrheal deaths in children <5 years old in Ethiopia(32). Evidence suggests that a number of children died from an illness caused by these pathogenic agents that thrive in contaminated

drinking water(59,60). The presence of pathogens in infant drinking water is commonly associated with water quality determinants such as the improvement status of the water source, drinking water storage hygienic status, practices of water treatment at the point-of-use, water fetch time, water point location, and the presence of residual chlorine.

To date, there have been no studies that examined molecularly detected pathogens in infants' drinking water along with the corresponding water sources and their relationship with water quality determinants. Therefore, it is crucial to identify the most deadly pathogens in infant drinking water at the moment of use, correlation with water sources, and their association with the factors that affect their presence in drinking water using the molecular technique known as LAMP. Without solid evidence of such information, it would be difficult to develop effective interventions and target countermeasures against severe and fatal infant diarrhea.

1.3 Research Questions and Hypothesis

1.3.1 Research Questions

The following research questions were answered by this dissertation.

- What are the causes of infant death in the study area?
- What specific factors are differently associated with the most common cause of infant death as compared to mortality from all-causes?
- What factors significantly influencing diarrhea-related infant death?
- What is the prevalence of pathogens causing the most severe and fatal diarrhea (rotavirus, *Cryptosporidium* oocyst, *Shigella* species, and toxin-producing *E. coli)* in infants' drinking water samples at point-of-use and the corresponding water sources?
- What water quality determinants influence the presence of rotavirus, *Cryptosporidium* oocyst, *Shigella* species, and toxin-producing *E. coli* in infants' drinking water samples at household level?

1.3.2 Research Hypothesis

To better understand the implications of the research questions findings, the associated factors among the targeted infants will be examined through the following hypotheses:

Hypothesis 1: *There is no variation on causes of infant mortality from what is known nationally by type and magnitude.*

Hypothesis 2: *The pattern of factors associations has no variation in each common cause of infant mortality against all-causes of infant death together.*

Hypothesis 3: *There is no significant relationship among socio-demographic, water, sanitation, hygiene components, and diarrhea-related infant death*

Hypothesis 4: *There is no variation of prevalence of rotavirus, Cryptosporidium oocyst, Shigella species, and toxin-producing E. coli in infants' drinking water samples at point-of-use and the corresponding water sources.*

Hypothesis 5: *There is no significant association difference between water qualities determinates and the presence of each waterborne pathogen (rotavirus, Cryptosporidium oocyst, Shigella species, and toxin-producing E. coli) in infants' drinking water samples at point-of-use.*

1.4 Objectives of the study

1.4.1 General Objective

The general objective of this study was to determine the cause of infant deaths at the population level and analyze patterns of associated factors among the most common deaths; identify factors contribute to diarrhea-related infant deaths; and detect pathogens that cause severe and fatal diarrhea from infant drinking water and analyze their relationship with water quality determinates in eastern Ethiopia.

1.4.2 Specific Objectives

- To determine causes of infant death using InterVA-4 model and investigate the patterns of factors affecting the major cause-specific mortality as compared to mortality from all-causes (Paper I).
- To identify factors influencing diarrhea-related infant mortality (Paper II).
- To detect pathogens causing the most severe and fatal diarrhea (rotavirus, *Cryptosporidium* oocyst, *Shigella* species, and toxin-producing *E. coli)* in infants' drinking water samples at point-of-use and the corresponding water sources and analyze their relationship with water quality determinants (Paper III)

1.5 Scope of the study

This study dealt with the determination of the cause of infant deaths at the population level, examine patterns of associated factors among the most common deaths, pinpoint the factors that contribute to infant deaths related to diarrhea, find the pathogens that cause severe and fatal diarrhea from infant drinking water, and assess how these pathogens relate to factors affecting water quality. This study was conducted within its boundary of randomly selected districts in Eastern part of Ethiopia. These include Chiro, Mieso, Gemechis, and Tullo districts of the West Hararghe administrative zone of Oromia, as well as the Amibara and Awash Fentale districts located under zone 3 administration of the Afar region.

1.6 Rationale of the study

Evidence showed that the cause of infant deaths and the factors that contribute to common causes of death at the population level were not properly identified and recorded. Majority of the existed studies were conducted in hospitals and on demographic and health surveillance sites. Furthermore, the contributing variables to infant death from diarrhea were not thoroughly studied. Most of the studies focused on associated factors with diarrhea cases, which may have resulted in a lack of clear evidence for diarrhea-related death. This may lack to obtain clear evidence for diarrhea-related death. The most prevalent waterborne pathogens that responsible for infant deaths, especially in infant drinking water, were not identified or examined using the water quality determinants. Therefore, addressing this knowledge gap through a variety of methodological approaches helps to obtain sufficient information to implement interventions that aimed at reducing infant mortality.

1.7 Significance of the study

Information on the causes of infant deaths is essential for developing sound public health policies and strategies(15). Emphatically, detecting and generating empirical information on the cause of infant death and patterns of association factors on cause-specific mortality at the population level is the first priority and basically essential to taking evidence-based measures. This study, therefore, contributes to filling the knowledge gaps that exist on cause-specific fractions of infant mortality at the population level in a local setting as well as the pattern of the associated factors among the most common cause-specific infant deaths compared to deaths from all other causes. This unique approach would help to better understand the factors that largely have an effect on multiplying infants' risk of death from specific causes of illness or conditions, predominantly focusing on diarrhea-related infant deaths. This could be important for developing effective and efficient public health strategies and interventions, which will ultimately reduce the burden of infant mortality. Attention to this condition is important if the Sustainable Development Goal (SDG)-goal 3 target 3.2 (to reduce childhood mortality) is ever to be achieved by 2030(74).

Despite numerous studies showing a connection between risk factors and infant diarrhea cases, there is a gap in knowledge of the factors that may contribute to diarrhea-related infant mortality. Therefore, understanding the risk factors such as drinking water, sanitation, and hygiene associated with diarrheal-related infant mortality is an important step in reducing the infant mortality rate.

This study is assumed to fill the existing knowledge gap and enable policymakers and decision-makers to develop preventive strategies that can help infant survival against diarrhea.

Many waterborne pathogens may cause diarrhea morbidity that leads to death and focusing on the most serious and fatal ones are important to bring death rates down. Testing drinking water for the most severe and fatal etiologic agents is crucial for determining the best public health interventions to be implemented. There was absolutely no examination of the quality of the water that included the precise detection of pathogens that frequently cause morbidity and mortality, particularly in infants. Thus, this study contributes to closing the knowledge gap in this area. Additionally, this study offers helpful details on the key elements that significantly influence the presence of each targeted pathogen in infant drinking water. Therefore, this study helps policymakers to design the right strategies and may serve as a platform for future research.

1.8 Oprational Definition

Improved water source: defined as those that are likely to be protected from outside contamination, and from faecal matter in particular. This includes Piped water into dwelling/yard/plot, Public tap/Standpipe, Tubewell/borehole, Protected dug well, Protected spring, Rainwater and Bottled water; whereas unimproved water source includes Unprotected dug well, Unprotected spring, Surface water (river, dam, lake, pond, stream, canal, irrigation channels), Cart with small tank/drum, Tanker-truck (200)

Water Accessible: People access to 25 liters per capita per day within 1km radius from improved water supply sources for rural community (199).

Households adequate water treatment at point-of-use: Boiling, add bleach/chlorine, water filter (ceramic, sand, composite) and solar disinfection (200).

Safe Water Storage: Water stored in plastic, clay or metal pot narrow mouth (usually diameter of 3cm or less), have a lid or secured cover and a tap (spigot), cleaned and kept cover (38), while its reverse could be taken as unsafe water storage.

Improved Sanitation: flush/pour toilet, Ventilated Improved Pit (VIP) latrine, simple pit latrine with slab (slab that can be cleaned), and composting toilet. Whereas unimproved sanitation facilities include: Flush/pour-flush latrine that empties elsewhere without connection to a piped sewage system, septic tank, or pit, Flush/pour-flush latrine with unknown drainage, Pit latrine without slab/open pit, Bucket latrine (where excreta are manually removed), Hanging toilet/latrine, Open defecation in field or bush, into plastic bags (‘flying toilets’), and any other type of defecation (200).

Access to hand washing facilities near to latrine: Presence of hand washing station within 3 meters of the latrine with water and soap/substitute.

Critical time of Hand washing: Hand washing with soap or substitute at critical times (the most recommended occasions): after using latrine, after cleaning child bottom, before preparing food, before feeding child, before breastfeeding.

Safe disposal of children's faeces: Child used toilet/latrine, put/rinsed faeces into the toilet/ latrine and buried the faeces whereas "*unsafe" (put/rinsed into a drain or ditch, thrown into garbage, and left in the open)* (200).

Proper Solid Waste Management: households dispose their wastes to waste collection tank, provide to private waste collection groups, buried and/or burn, and composting.

Proper liquid Waste Management: Households dispose their liquid wastes through infiltrate to the ground, cesspool (a pit dug in the ground to receive liquid waste) and dumping to municipal disposal sites.

1.9 Organization of the dissertation

This dissertation report was organized in compliance with AAU guideline 2011 and was typically structured in the form of a monograph as per the requirements for the degree of Doctor of Phylosophy. Accordingly, this report is organized into three main sections. 1) Preliminary, which includes the title (cover page), declaration of the original literary work, abstract, acknowledgement, table of contents, list of figures, list of tables, list of abbreviations, list of appendices, and glossary of terms and words; 2) Main body, which was divided into six chapters, namely: Chapter 1: Introduction, Chapter 2: Literature Review, Chapter 3: Methodology, Chapter 4: Results, Chapter 5: Discussion, Chapter 6: Conclusion and References; finally the 3) Supplementary, which includes the List of publications and the Appendix

CHAPTER TWO: LITERATURE REVIEW

2.1 Cause of Infant Mortality: World Wide and Ethiopia

According to World Health Organization, the underlying cause of death is defined as the disease or injury that initiated the chain of morbid events leading directly to death(75). Infant mortality can be caused by a variety of conditions, such as infection, birth defects and accidents(11). The leading causes of infant mortality worldwide include preterm birth complications, interapartum-related events (birth asphyxia or inability to breathe at birth), infections such as sepsis or meningitis, and congenital abnormalities(5,11,20). The major causes of infant death in developed countries, which tend to occur in the neonatal period, are low birth weight, prematurity, birth complications, and congenital defects; in developing countries, they are vaccine-preventable infectious diseases, diarrhea and dehydration, and respiratory illness, all complicated by malnutrition(76). Among the overall causes of infant deaths in developing countries, about 86% are due to infections, premature births, complications during delivery, perinatal asphyxia, and birth injuries(77).

In Africa, less is known about the causes of infant deaths than about mortality rates and trends, and none of the sub-Saharan African nations, not even Ethiopia, has a reliable national system for registering deaths(68,69,78). According to findings of studies on the causes of infant and children deaths in nine regions of Africa; low birth weight, birth trauma, and congenital malformations were the major causes of neonatal mortality. In contrast, during the post-neonatal period, diarrheal illnesses are typically rated first, followed in variable order by ALRI-pneumonia, malaria, and measles(78).

A community-based birth cohort study conducted for a period of one year (from july 2005 to June 2006) in rural Aligarh, India, revealed that birth asphyxia, diarrhea, pneumonia, and preterm (including low birth weight and malnutrition) were the leading causes of infant mortality(12).These cause of death was ascertained using the standard verbal autopsy procedure. Among 37 reported deaths in children under one year of age, birth asphyxia (40.9%) and preterm (including low birth weight) (27.3%) were the two main causes of neonatal deaths in this study. The remaining causes of death (4.6%) included pneumonia, diarrhoea, tetanus, newborn sepsis, neonatal jaundice, and congenital abnormalities. In this study, pneumonia and diarrhea accounted for 80% of deaths during the post-neonatal era.

According to data analysis from global health estimates, there are some similarities between the major causes of infant mortality in Ethiopia and those in other developing nations and other parts of the world(20). In Ethiopia, the most frequent causes of infant mortality are repetitive and have evolved somewhat over the past decades. However, as they are now, the leading and most common causes of death are still neonatal conditions, lower respiratory infections, congenital malformations, diarrheal disease, meningitis, and measles. Based on global health estimates, the top ten causes of infant death in Ethiopia as of 2019 were protein-energy malnutrition, measles, meningitis, whooping cough, tetanus, lower respiratory infections, congenital abnormalities, diarrhea disease, and neonatal conditions(20)

A population-based cohort of 3684 births study that was conducted under the Kilite Awlaelo Health and Demographic Surveillance System were followed up to their first birthday or death, between September 11, 2009 and September 10, 2013 (79). In this study, a review of verbal autopsy by medical professionals was performed for 147 infant deaths and was reported that bacterial sepsis (32.5%), prematurity (23.7%), and birth asphyxia (13.8%) were the three leading causes of death during the neonatal period, while acute lower respiratory tract infections (ALRTI) (17.9%), bacterial sepsis (14.9%), and intestinal infections including diarrheal diseases (11.9%) were the leading causes in post-neonates.

A multilevel analysis of a prospective follow-up study that was conducted to identify the determinants and causes of neonatal mortality among 3463 neonates from September 2012 to December 2013 in Jimma Zone, Southwest Ethiopia (80). In this study, out of the total 110 neonatal death occurred, birth asphyxia (47.5%), neonatal infections (34.3%), and prematurity (11.1%) reported to be the three leading causes of neonatal mortality, accounting for 93%. Another prospective cohort study that was conducted among neonates born between April 2014 and July 2014 in seven hospitals, in Tigray region, Ethiopia indicated that prematurity (34%), asphyxia (31%), and infections (12%) were reported to be the main causes of neonatal death (81).

A retrospective cohort study among 2090 live born neonates admitted to Nekemte referral hospitals neonate care unit conducted from 2010 to 2014 revealed that, among the 183 deaths recorded, infections (60%), asphyxia (23%), preterm (16%), and congenital deformity (1%), were the causes of neonatal mortality (82). In a facility-based prospective follow-up study, preterm birth complications (28.6%), birth asphyxia (22.5%), neonatal infection (18.4%), meconium aspiration syndrome (9.2%), respiratory distress syndrome (7.1%), and congenital malformation (4.1%) were found to be the causes of neonate death among 98 deaths of neonates from 489 admitted to neonatal intensive care units of public hospitals from November 1 to December 30, 2018, in eastern Ethiopia (83).

2.2 Determination of Cause of Death

The majority of deaths in Ethiopia occur at home, and medical professionals do not routinely certify the reason of death, as a result, accurate causes of death information is frequently lacking (84). In principle, the medical professional who signs the death certificate is responsible for determining the cause of death(75). They specify which morbid conditions directly caused death and any antecedent conditions that contributed to the underlying cause of death. However, in the majority of poor nations, this is not practically feasible. As an alternative, verbal autopsy has emerged as a significant source of information regarding the causes of death in populations lacking medical certificate(75,85).

A verbal autopsy is an interview conducted with the deceased's family or caregivers using a standardized questionnaire to collect signs and symptoms and other important information that can later be utilized to determine the most likely underlying cause of death(75). It is primarily used as a research tool in the context of longitudinal population studies, intervention research, or epidemiological studies; secondly, it has developed into a source of cause-specific mortality data for use in policy, planning, priority setting, and benchmarking; and thirdly, it is gaining acceptance

as a source of cause-specific mortality data for tracking advancement and determining what works and what doesn't(19).

Verbal autopsy interviews have been conducted for many years by researchers and policymakers to determine the cause of death(17). However, there is still a challenge in determining the cause of death from VA interviews in a reliable and consistent manner. Verbal autopsy data interpreted by the physician review approach is time-consuming, costly, and inconvenient(16,86,87). In recent years, computerized methods for determining the cause of death have been introduced and provide an analysis solution that is more convenient, consistent, and rapid ways to interpret verbal autopsy data(86). The analytical software tools that are available for cause of death assignment without the involvement of physicians include InterVA, SmartVA/Tariff 2.0, InsilicoVA, King-Lu, Simplified Symptom Pattern (SSP), and Random Forest(87).

InterVA is one of a suite of computer models for interpreting verbal autopsy data and recognizes a range of indicators relating to a particular death, processes them in a mathematical model based on Bayes' theorem, and produces as its output the likely cause(s) of death.(18).

2.3 Patterns of associated factors (*Specific-cause of Infant death Versus all-cause*)

According to the reviewed literatures, numerous researches have examined the variables linked to a particular cause of infant mortality in comparison to those who did not die. There is, however, limited information on how the variables linked to the primary specific cause of infant mortality compare to the variables linked to deaths from other causes.

Acute respiratory infections (ARIs) have been proven to be a major cause of infant mortality. A case-control study was conducted between April 1, 2014 and December 31, 2016 to identify risk factors among children <5 years old who died of ARI at home in Buenos Aires, Argentina(88). In this study, 104 died childrens was participated and found that infants under the age of 12 months accounted for 87.5% of all deaths linked with ARI. Additionally, it was discovered that there were significant positive associations with living in a crowded home (AOR 3.73, 1.41-9.88), having an adolescent mother (OR, 4.89; 95% CI, 1.37-17.38), lacking running water in the home (OR, 4.39, 1.11-17.38), incomplete vaccination for age (OR, 3.39, 1.20-9.62), being admitted to a neonatal intensive care unit (OR, 7.17, 2.21-23.27), and not visiting the emergency care unit during the ARI episode (OR 72.32, 4.82-1085.6).

A cohort study conducted using linked administration data for 2003-2013 in Ontario reported that children born to teenage mothers were an independent risk factor for RTI-related death(89). A prospective post-mortem verbal autopsy study conducted in 307 deaths of young children in Bangladesh, from 2008 to 2012, found that delayed treatment seeking (by ≥ 2 days) behavior and access to care from multiple sources for treatment were 4.9 and 5.7 times more likely to be died from pneumonia than those who died from other causes, respectively(90).

According to various studies, post-neonatal infants were more likely to die from diarrhea than neonatal infants (91,92). Male sex and a low 5-minute Apgar score were found to be significant risk factors for diarrheal mortality in the study on infant and maternal risk factors for diarrhea-associated infant mortality in the United States during 2005–2006(91). According to this study,

infants with older maternal age had a higher chance of dying from diarrhea among infants with low birth weight, while infants with younger maternal age had a higher chance of dying from diarrhea among infants with normal birth weight. A combined time series analysis of seasonality, climate region, and clinical syndromes from 243,000 verbal autopsies in the nationally representative million death study between 2005 and 2013 was carried out in Mexico, and found that the distribution of diarrheal mortality was more pronounced in the summer(92).

According to a cross-sectional study done during 2009 in Benin City, Nigeria, males were more likely than females to experience severe birth asphyxia and mortality(93). In a similar vein, a one-year prospective observation study conducted from 2018-2019 in India found that boys died from birth asphyxia at a greater rate than females(94).With regard to prematurity, a study conducted from November 1 to December 30, 2018 in intensive care units in Ethiopia reported that from 98 deaths, low gestational age, low birth weight, being female, feeding issues, no antenatal care visit, and vaginal delivery among mothers with higher educational levels were identified as significant risk factors for prematurity-related deaths (83).

A special analytical study on the relationship between early childhood mortality and malaria conducted on Malawi DHS of the year 2000 reported that children were significantly at greater risk if their mother had a lower age relative to an older age(95). A pooled cohort study conducted between june 1999 and December 2004 in rural north-western Burkina Faso showed that 443 deaths were registered with malaria accounting for 49% of all deaths and malaria were significantly different between village clusters(96). According to a study on seasonal patterns of malaria and all-cause mortality from a longitudinal study with 60,000 individuals covers the period 1999-2003 of DHS in rural northwestern Burkina Faso indicated that infant mortality caused specifically by malaria showed distinct seasonal trends and was higher during the rainy seasons(97).

2.4 Diarrhea-associated Infant Death

As per the WHO, diarrhea is commonly defined as the passage of loose or watery stools occurring three or more times in a 24-hour period and causing death by depleting body fluids, resulting in profound dehydration(42).

According to the World Health Organization, there are nearly 1.7 billion cases of childhood diarrheal disease every year and more than half a million diarrheal deaths occur among children under 5 each year worldwide (42).Infants account for fifty percent of these fatalities (40). Diarrhea was responsible for about 9 percent of all worldwide deaths among children <5 years in 2019 (42). Diarrheal illnesses remain the second most common cause of death among children under five globally, following closely behind pneumonia (40,42). The death toll exceeds that of AIDS, malaria, and measles all together (40).

At the global level, the total annual number of deaths from diarrhea decreased by 67.9 percent among children <5 years between 1990 and 2017 (98); however, mortality rates are still highest in some developing countries, with 78% of these deaths occurring in south Asia and sub-Saharan Africa(31). The top five countries where the most diarrhea deaths among children occurred in 2016 include Nigeria, India, Pakistan, the Democratic Republic of the Congo, and Ethiopia (31,32).

Although the burden is highest in low-income populations who have limited access to clean water, sanitation facilities with poor hygiene practices, and weak emergency medical care, diarrhea is also a frequent reason for outpatient visits and hospital admissions in high-income countries(33).

Diarrhea has been identified as one of the major causes of infant mortality in Ethiopia (20,99). It was discovered that the fall in diarrheal mortality in this country was inconsistent, even though the percentage of children under five who died from the condition decreased from 16% to 8% between 2000 and 2016 (99). By the year 2019, diarrhea was the fourth most common cause of infant mortality in this nation, accounting for 136.6 newborn deaths per 100,000 populations (99).

2.5 Risk of Water, Sanitation and Hygiene on Diarrheal-related Infant-death

Lack of access to safe water and sanitation, along with poor hygiene practices, are the main contributors to the incidence of infectious diseases, resulting in infant and child mortality. Evidence suggests that unsafe water, inadequate sanitation, and insufficient hygiene are responsible for about 88 percent of deaths related to diarrhea (39–41).

A retrospective analysis of data from 145 countries reported that there were 1.50 million diarrhea-related deaths in the year 2012, of which 502,000 deaths were due to inadequate water, 280,000 to inadequate sanitation, and 297,000 to insufficient hand hygiene(100). A systematic analysis for the Global Burden of Disease study from 235 causes of death for 20 age groups in 1990 and 2010 revealed that unsafe water (72.1%) and unsafe sanitation (56.4%) were found to be the second and third leading risk factors of diarrheal death among children younger than 5 years, respectively, and a lack of access to hand washing facilities was responsible for 34% of the risk(101).

Few published studies exist that explore infant mortality from diarrhea and the risk associated with water, sanitation, and hygiene. A population-based case-control study conducted in 170 infant deaths from diarrhea (cases) and 340 controls from the period December 24, 1984 to December 23, 1985 in the metropolitan areas of Porto Alegre and Pelotas in southern Brazil reported that the unavailability of piped water was the only association that remained statistically significant with an increased risk of infant death from diarrhea after adjustment for confounding factors(49). In this study, those without easy access to piped water were found to be 4.8 times more likely to suffer infant death from diarrhea (95% confidence interval 1.7 to 13.8), and those with water piped to their plot but not to their house had a 1.5 times greater risk (95% confidence interval 0.8 to 3.0).

An ecological study conducted in 2001 to examined the variations in diarrhoea-specific infant mortality rates among municipalities in the State of Ceará, north-east Brazil, found that household’s access to inadequate water supply was an important determinant of infant mortality due to diarrhea (102). A study on the impact of water and sanitation on childhood mortality conducted using Demographic and Health surveys, 2003-2013 in Nigeria reported that both unimproved water and sanitation were linked to a significant 38% higher risk of mortality (103).

A study that used demographic and health data from 70 low and middle-income countries over the period 1986–2007 found that children living in dwellings with high-quality toilet facilities were associated with a 15–23% lower risk of mortality than those living in homes without toilet

facilities(104). According to the HALA ABOU-ALI assessment on the impact of water and sanitation on child mortality using DHS between November 1995 and January 1996 in Egypt, access to municipal water decreases the risk of death, and sanitation has been found to have a more pronounced impact on mortality than water(105).

Esrey et al analysed 144 studies and investigated the strong relationship between improved water supply and sanitation and children's health, resulted in a 55% reduction in child mortality, which suggests that water and sanitation have a substantial impact on child survival(106). An interventional (experimental) study conducted from a period of 2012-2014 in Kenya showed that household water treatment, particularly the use of chlorinating water solutions, significantly lowers infant mortality by 65%(107).This study suggested that this may have been due to reduced diarrhea, although other adopted health behaviors could also have contributed to the reduction in mortality. The study of infant and child mortality in Ethiopia using the 2000 and 2005 EDHS data revealed that the availability of safe drinking water (pipe) and toilet facilities (flush and latrine pit) decreased the risk of infant mortality by 34% in comparison to other categories (without safe drinking water and without the availability of toilet facilities)(108).

2.6 Waterborne pathogens causing the most severe and fatal diarrhea

Various waterborne pathogens cause diarrhea and fluid loss, which all too frequently lead to severe dehydration and mortality. Rotavirus, adenovirus, norovirus, *Amoebiasis*, *Cryptosporidium*, *Shigella*, *Cholera*, *Campylobacter*, *Clostridium difficile*, *Aeromonas*, *non-typhoidal Salmonella*, *enterotoxigenic*, and *enteropathogenic E. coli* are the main organisms responsible for child deaths from diarrheal disease (32). Four microbial pathogens are reported to be responsible for the majority of the severe and deadly diarrhea cases among infants worldwide, which include rotaviruses, the protozoan *Cryptosporidium*, and two bacteria: *Shigella* and an *E. coli* strain that produces toxins (57,109).

According to the WHO, rotaviruses are the most common cause of severe diarrhea disease in infants and young children worldwide(110).The Global Burden of Disease study estimated that rotavirus would cause more than 258 million infections and nearly 128,515 deaths among children under 5 years old globally by the year 2016 (31). Rotavirus are responsible for up to 500,000 diarrhea deaths in developing countries each year (111).

In most developing countries, rotaviruses affect the vast majority of children, particularly before the first birthday. A global review indicated that in countries with very high child mortality, 69% of rotavirus-positive admissions in children <5 years of age occurred in the first year of life (112). An observational trend study of rotavirus-associated deaths from 1990–2019 indicated that rotavirus was shown to be the leading global pathogen of diarrhea-associated mortality, representing 19.1% of all diarrhea-related deaths in 2019 (113). This study also showed that rotavirus caused a higher death burden in African, Oceanian, and South Asian countries in the past three decades. Ethiopia is among the five countries with the highest rotavirus burden, accounting for six percent of the global rotavirus deaths (114,115). Rotavirus is responsible for 28% of hospitalizations and 6% of deaths of under-five children in Ethiopia(114), where it takes the lives of more than 28,000 children each year (115).

Cryptosporidium accounts for 30–50% of deaths in infants and children worldwide, which is the second leading cause of diarrhea and deaths in children, following rotavirus (116,117). A meta-analysis study estimated that *Cryptosporidium* infection in children under 5 years was associated with 44.8 million diarrheal episodes and 48,300 deaths globally in 2016 (118). In this analysis, it was reported that 75% of episodes and 88% of deaths occurred in Africa, and the effect is higher in infants than in older children. MØlbak K et al indicated excess mortality in children who had a *Cryptosporidium* infection in infancy, and this mortality persisted into the second year of life (119). *Cryptosporidium* is endemic in Ethiopia and exhibits one of the highest rates, with an estimated 1.35 million episodes and 1,143 deaths by the year 2016 (118).

Bacterial pathogens such as *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) are frequently associated with diarrheal illness and are a major source of morbidity and mortality globally (120). By the year 2016, *Shigella* was responsible for an estimated 74.7 million episodes and 63,713 deaths among children younger than 5 years, which accounted for 13% of deaths from diarrhea cases (31,120).

ETEC causes about 75 million episodes in children under 5 years of age globally, resulting in between 18,700 and 42,000 deaths as per the MCEE and IHME estimates, respectively (121). ETEC was responsible for about 4.2% (2.2–6.8) of diarrhea deaths in children younger than 5 years (120). A study to quantify ETEC and *Shigella* morbidity and total mortality showed that Ethiopia exhibited an estimated *Shigella* episode of 419,014 and total *Shigella* deaths of 1,576 of under 5 children, whereas 383,244 episode and 1,393 total deaths for ETEC's in 2016 (122).

2.7 Detection of Specific-Waterborne Pathogens in Infant Drinking Water

Waterborne pathogens pose a severe and expanding threat that is spreading infectious diseases and continuing to have an impact on people health all over the world (123). Most of these pathogens are enteric in origin, which implies that they are excreted in faecal matter and then enter new hosts by ingestion (also known as the faecal-oral pathway) (53). Water is a primary means of spreading these pathogenic agents, either directly through ingesting contaminated water or indirectly through its usage in the manufacturing and/or processing of food (124). According to varies studies, there is a significant frequency of diarrheal disease among children and infant due to the usage of contaminated water (36,42,125).

Since it can be challenging to identify and isolate many waterborne pathogens, researchers and public health officials frequently check nonpathogenic fecal markers of bacteria such as total coliform, fecal coliform, *E. coli*, and fecal *Streptococci* and *Enterococci* (126,127). The isolation of the aforementioned indicator organisms uses conventional techniques for testing water quality, such as MPN, filter membrane, and present and absent assays. These techniques, however, cannot identify particular pathogenic species in drinking water (128,129). Additionally, the presence of these microbial indicators does not necessarily indicate the presence of pathogens (130), and their absence does not necessarily indicate that there are no pathogens in the water (131). As studies suggested, the absence of indicator organisms in a water quality examination does not necessarily mean that the water is safe, especially from protozoa and viruses(132).

The introduction of molecular assays has significantly improved and simplified the detection and identification of microorganisms in the environment (133). Nucleic acid-based amplification techniques, such as PCR, have been developed to detect microbial pathogenic species directly in drinking water samples among a wide spectrum of molecular techniques (134). This method procedurally consists of the concentration of the target organism from the sample, nucleic acid extraction from the target organism, genomic segment amplification, and quantification of the amplified genomic segments (135).

Recently, new advances have led to the use of methods that amplify the nucleic acid material at isothermal conditions. This techniques include nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), isothermal multiple displacement amplification (IMDA), rolling circle amplification (RCA), helicase-dependent amplification (HDA), single primer isothermal amplification (SPIA), single-mediated amplification of RNA technology (SMART), circular helicase-dependent amplification (cHDA), recombinant polymerase amplification (RPA), polymerase spiral reaction (PSR), and loop-mediated isothermal amplification (LAMP) (131).

The use of molecular methods has grown, particularly when looking for the detection of waterborne pathogens(136,137). Direct DNA detection-based molecular approaches, which are now used in water analysis, do have the advantage of providing a definitive signal of whether a particular organism is present or not. They can be used to assess the water's microbiological quality, the effectiveness of pathogen elimination in drinking water, and the safety of wastewater treatment plants. In comparison to culture and other conventional approaches, PCR offers a number of benefits for the detection of microbial pathogens in water (138). The results of a study comparing the PCR method with the MPN (Most Probable Numbers) method for identifying coliform bacteria in water showed that, of the total of 18 samples of well drinking water tested, 3 samples were positive for the MPN method and 8 samples for the PCR method (139). This result clearly demonstrated that PCR is more sensitive than MPN.

Eiken Chemical Co., Ltd. developed the "LAMP" method, which stands for Loop-mediated Isothermal Amplification, which is a simple, rapid, specific, and cost-effective nucleic acid amplification method (140). This method is characterized by the use of four different primers specifically designed to recognize six distinct regions on the target gene, with the process being carried out at a constant temperature (ranging between 60 - 65 °C) using a strand displacement DNA polymerase. The techniques are often based on the identification and measurement of particular DNA or RNA fragments from the pathogen's genome. The particular segments are amplified in vitro in order to attain the detection level. This technique enable the quick and precise detection of pathogens that pose a threat to public health (137). It follows these steps: (i) concentrating the target organism from the environmental water sample into an appropriate volume (if necessary); (ii) extracting the target organism's RNA or DNA; (iii) amplifying the selected genomic segment(s); and (iv) detecting (or quantifying) the amplified genomic segment(s) (141).

2.8 Prevalence of *Cryptosporidium*, *Shigella*, Toxin Producing *E.coli* and Rotavirus in drinking water at household's level and water sources

2.8.1 Prevalence of *Cryptosporidium* species in drinking water

A study conducted in 2010 by Xiao-Ping Zhang et al investigated *Cryptosporidium* species in drinking water and source water by a procedure of micromembrane filtration, immune magnetic separation (IMS), and immunofluorescent assay (IFA) in Shanghai (142). In this study, no *Cryptosporidium* oocysts have been detected from 156 drinking water sample of the communities, but they were found in 6.7% from 70 source water samples. In Mongolia, a cross-sectional study implemented a household risk factor survey at 250 home sites between April and October, 2017 indicated that five of water samples of households (2%) were positive for *Cryptosporidium* species using multiplex real-time PCR (143).

A cross-sectional laboratory based study conducted by Kifleyohannes T. et al. in Tigray, Ethiopia, during the period from October 2018 until January 2019 found that 2 (5%) of 37 water samples tested positive for *Cryptosporidium* using the standard ISO 15553 method (144). The water sample testing carried out around Addis Ababa, Ethiopia, in 2011 using immunofluorescent antibody testing (IFAT) reported that 15 (21%) of the 72 tap water samples and 6 (35%) of the 17 storage tank samples examined for *Cryptosporidium* oocysts were found to be positive (145). Another study that was conducted with the same technique at the "Legedadi" water source of the municipal drinking water system around Addis Ababa from Feburary to April, 2005 showed that *Cryptosporidium* was found in all (100%) water samples from a total of 22 samples (146). Amenu D. et al conducted cross- sectional study between February and May, 2011 in the rural communities of Dire Dawa Administrative Council, Eastern Ethiopia and investigated 16.7% *Cryptosporidium* from 18 tap (household) water samples and 69.4% from 72 source water samples that was detected using biochemical test (147).

In Fayoum Governorate, Egypt, a descriptive analystical study conducted to determine the presence of protozoal agents in tap water and storage tanks during the period from May, 2015 to October, 2015 found that 53.6% of the water samples from 65 tap water and 53.3% of water samples form 30 water tanks (water storage) were contaminated by *Cryptosporidium* species (148). A study aimed to estimate the risk of *Cryptosporidium* infection through the consumption of drinking water was conducted drinking water survey, which lasted from June 2003 to December 2004, in rural site of Swizerland. In this study, it was reported that oocysts of Cryptosporidium specious were detected in all water samples from the three sites using nested PCR (149).

A water sample survey conducted in four countries in Southeast Asia between April and October 2013, and found 24.4% *Cryptosporidium* from 221 water samples using real-time PCR (150). The authors that reviewed various studies in 2008 showed the prevalence of *Cryptosporidium* species in drinking water ranged from 1.4%-100% (151). The principal source of *Cryptosporidium* contamination is believed to be animal and is commonly found in rivers and lakes, especially when the water is contaminated with animal waste (152).

2.8.2 Prevalence of *Shigella* species in drinking water

Negera E. et al conducted a descriptive analytical study on the microbiological assessment of drinking water with reference to diarrheagenic bacterial pathogens in a rural district of Shashemane, Ethiopia, from October 2012 to February 2013. In this study, *Shigella* species were not found in 42 household water samples while 6.5% of the water samples from 93 water sources were observed to be positive using a biochemical test (153). A cross-sectional study conducted to assess the bacteriological quality of drinking water in Jigjiga City from May-August, 2013 revealed that 3.33% of the water samples from 60 households and 12.3% of samples from 65 water sources (pipeline, reserviour, beyolle, main source) were contaminated by *Shigella* species (154). Another cross-sectional study in Ziway town, Ethiopia, which was collected water samples from April to November, 2013 reported that about 4.8% samples from 21 tap water tested positive for *Shigella* species tested using biochemical method (155).

In Quetta, Pakistan, a cross-sectional study that attempted to isolate and identify *Shigella* species from food and water samples using conventional biochemical tests during January 2017 to June 2017 discovered that 22% of water samples from a total of 50 households were contaminated with *Shigella* species (156). *Shigella* flexineri was identified at a rate of 6.4% from 340 household water samples in Peshawar, Pakistan that was conducted between January 2016 and May 2017 through biochemical, serological and 16SrRNA gen sequencing (157). H.B. Niguendo-Yongsi investigated *Shigella* species in 0.24% of 508 drinking water samples using biochemical tests that was conducted from June 2005-July 2008 in Yaounde, Cameroon (158). *Shigella* species were isolated from 125 domestic drinking-water samples stored in impoverished rural households in Venda of Limpopo Province, South Africa, at a frequency of 5%, in a study that was conducted over a six-month period (from June to November 2002), according to Potgieter et al.(159). In a study on the quality of drinking water from three locations from December 2016 till November 2017, in the Giza Governorate, namely Kerdasa, Kafr El-Gebel, and Dahshur, authors Nouran H. Assar and Mohammed found that 34.7% of the water samples from 864 households were contaminated by *Shigella* species(160).

A number of studies have been conducted on the detection of *Shigella* species in drinking water sources. A study conducted in Kampala, Uganda, from November 2014 to May 2015 reported that about 10% of the water sources tested positive for *Shigella* spp. ipaH, all from the accepted positive detection data points (n = 441) tested by using microfluidic quantitative polymerase chain reaction (MFQPCR) (161). A cross-sectional study that was carried out from January to August 2017 in Enugu state of Nigeria showed that about 9.4% of the water samples were positive for the presence of *Shigella* species (162). Wahome et al isolated 6.9% of *Shigella* species from 144 groundwater used by residents of Ongata Rongai, Kajiado North County, Kenya (163).

Mahagamage et al assessed the contamination status of *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. in 45 surface and 72 groundwater of the Kelani River Basin, Sri Lanka, in both dry (February-March, 2015) and wet (May-June 2015), and the finding indicated that none of the water samples were contaminated by *Shigella* species (164). A survey of water quality assessments conducted in Nakla Paurosova, Sherpur, Bangladesh, from July to November 2018 revealed that *Shigella* species were found in 30% of the samples from 30 water sources (165).

2.8.3 Prevalence of Toxin Producing Strain of *E.coli* species in drinking water

In the water samples that were collected from 248 households in South Wollo, Ethiopia, about 17% tested positive for *E. coli* enterotoxins using polymerase chain reaction (PCR) (166). Only 1% of diarrheagenic *Escherichia coli* were found in 42 Escherichia coli isolates from 242 drinking water samples in a study conducted in the period between October 2018 and April 2019 in the city of Ouagadougou, Burkina Faso (167). A cross-sectional study conducted on the microbial quality of water in rural communities in Trinidad, from March to April 1998 confirmed the presence of toxic-producing *E. coli* (O157 strains) in only 2% of samples from the total of 253 *E. coli* isolates tested (168). Vadivelu J. et al investigated enterotoxigenic *Escherichia coli* in the domestic environment of a Malaysian village and found that 19% of samples from the household's water were positive (169). In Bangladash, Lothigius A. et al reported that enterotoxigenic *Escherichia coli* were detected in 87.5% of the water samples from 26 households in a study conducted between November 2005 and February 2006 in an endemic area using real-time PCR (170).

The presence of toxin-producing *E.coli* in water sources has been indicated in several studies. A cross-sectional study that aimed to assess the occurrence of waterborne pathogens in Lake Ziway and the drinking water system of Batu (Ziway) Town, Ethiopia, which was conducted from April to November 2013 revealed that about 5.5% of samples from 57 water sources were positive for E. coli O157:H7 (155). Similarly, a study conducted in Modjo, Ethiopia, reported that 4.2% of this pathogen was isolated from water samples using PCR (171). Studies in elsewhere showed that toxin-producing E.coli were detected from water source samples in South Africa at 25.6% (172); Uganda at 33% (173); northern Ghana at 75% (174); India at 33.3% (114); Brazil at 6.2%, 1.0%, and 0.65% (175–177); and southeast of the island of Puerto Rico at 52% (178).

2.8.4 Prevalence of Rotavirus in drinking water

There is evidence of rotavirus in household drinking water from numerous researches. Rotavirus was discovered using LAMP method in 16.7% of the samples drawn from 12 human drinking water in the Shandong, China (179). A prospective study of the circulation of rotavirus in water was carried out during the usual epidemic period, from January 3 to March 7, 1994 in southeast France, and using reverse transcription-PCR testing of the drinking water in the households of 56 children who suffered from rotavirus gastroenteritis resulted that rotavirus genome was present in 7.1% of the water samples(180). Out of 20 drinking water samples collected from different areas of Karachi, Pakistan, rotavirus was detected in 5% of the drinking water samples tested(181). According to studies conducted in Colombia, rotaviruses were found in 27.3% of the water samples taken from household taps(182), and 20.5% of the 288 samples analyzed from 102 Colombian municipalities were positive for both enterovirus and rotavirus(183).

Studies indicate rotavirus has been found in the water sources that supply drinking water to homes. Verheyen J et al detected rotavirus in 2.1% of 287 drinking water sources used in rural areas of Benin, West Africa (184). In the study of the molecular epidemiology of Group A rotavirus in water sources in southern Africa, it was reported that 1.7% of the 296 water samples were positive (185). The prevalence of rotavirus in water sources in different investigations conducted in various regions of Egypt was found to be 15.6% in five Egyptian governorates (186), 8.3% in Giza Tap water (187), and pooled estimate for systematic review at 23.3% (188).

In the western region of Accra, Ghana, Dongdem J et al used multiplex RT-PCR to find and characterize the human rotavirus in tap water (189). In this study, the authors collected treated water samples from five zones within the distribution network of Weija Water Works and found that 48.1% of the 27 water samples tested were positive for rotavirus. Rotavirus prevalence in source water was reported in several studies conducted in different regions of the world, including 8.1% in Costa Rica(190) and 20.3% in Beijing, China (191). Additionally, it has been reported that rotavirus was present in 23% in Karachi, Pakistan (181), 9.47% of the water samples taken from several water sources in Peshawar, Pakistan (192), and 26.6% in Faisalabad, Pakistan (193).

2.9 Association of Waterborne pathogens presence in drinking water and water quality determinates

Only a few studies have looked at the relationship between the specific pathogens in drinking water and the factors that influence their presence. A cross-ectional study of Barnes AN, et al found that households use of an improved water source remained significant in the multivariate model and had a protective effect on the presence of *Cryptosporidium* in drinking water (OR 0.16; CI 0.04–0.68; p = 0.01) (143). A matched case-control study conducted from March-May 2010 to examined the relationship between contamination of hands and water with *E.coli* virulence genes indicated that improved water sources significantly reduced the detection of *E. coli* virulence genes in stored drinking water (194).

2.10 Summary of the Reviewed Literatures

In summary, the literature that describes the causes of infant deaths in local settings is limited. This is due to the fact that most infant deaths occur at home and routine medical certification of the cause of infant death is lacking. The most effective solution to this issue is a verbal autopsy analyzed by computerized analytical software tools like InterVA. Uncertainties exist regarding factors influencing the most prevalent cause of infant death and deaths from all other causes. In particular, no details are provided regarding the factors affecting infant death from diarrhea in comparison to all other causes of infant death. Although the degree of significance varies among research studies, it has been demonstrated that water, sanitation, and hygiene components are all closely related factors that significantly affect infant morbidity and mortality. There are, however, very few published studies that link the factors like water, sanitation, and hygiene to the risk of diarrheal infant death. Though various waterborne pathogens can cause diarrhea that leads to death, four are known to cause severe and deadly diarrhea in infants: Cryptosporidium, Shigella, an E. coli that produces toxins, and rotavirus. Some of the main ways that these pathogenic agents spread are by direct intake of contaminated water and indirect use of contaminated water in food production and/or processing. There has been a rise in the use of molecular techniques to detect pathoges in drinking water. This facilitates the execution of research-based public health programs. The connection between specific pathogens found in drinking water and variables affecting water quality has not been extensively studied.

The primary motivation behind pursuing objective one was to obtain information about the cause of infant mortality in the study area. In this case, the most prevalent caused are identified and influencing factors is analysed. More specifically, information on the prevalence of infant deaths due to diarrhea and the factors influencing its occurrence relative to all other causes of death

combined is required. Meanwhile, we analysed the factors influencing diarrhea-related infant death against those who survived their first year of life (Objective II).Detecting the pathogens that cause the most severe and deadly infant diarrhea in the study area's drinking water (Objective III) and any potential connections to factors influencing water quality are of special importance when it comes to infant diarrhea deaths.

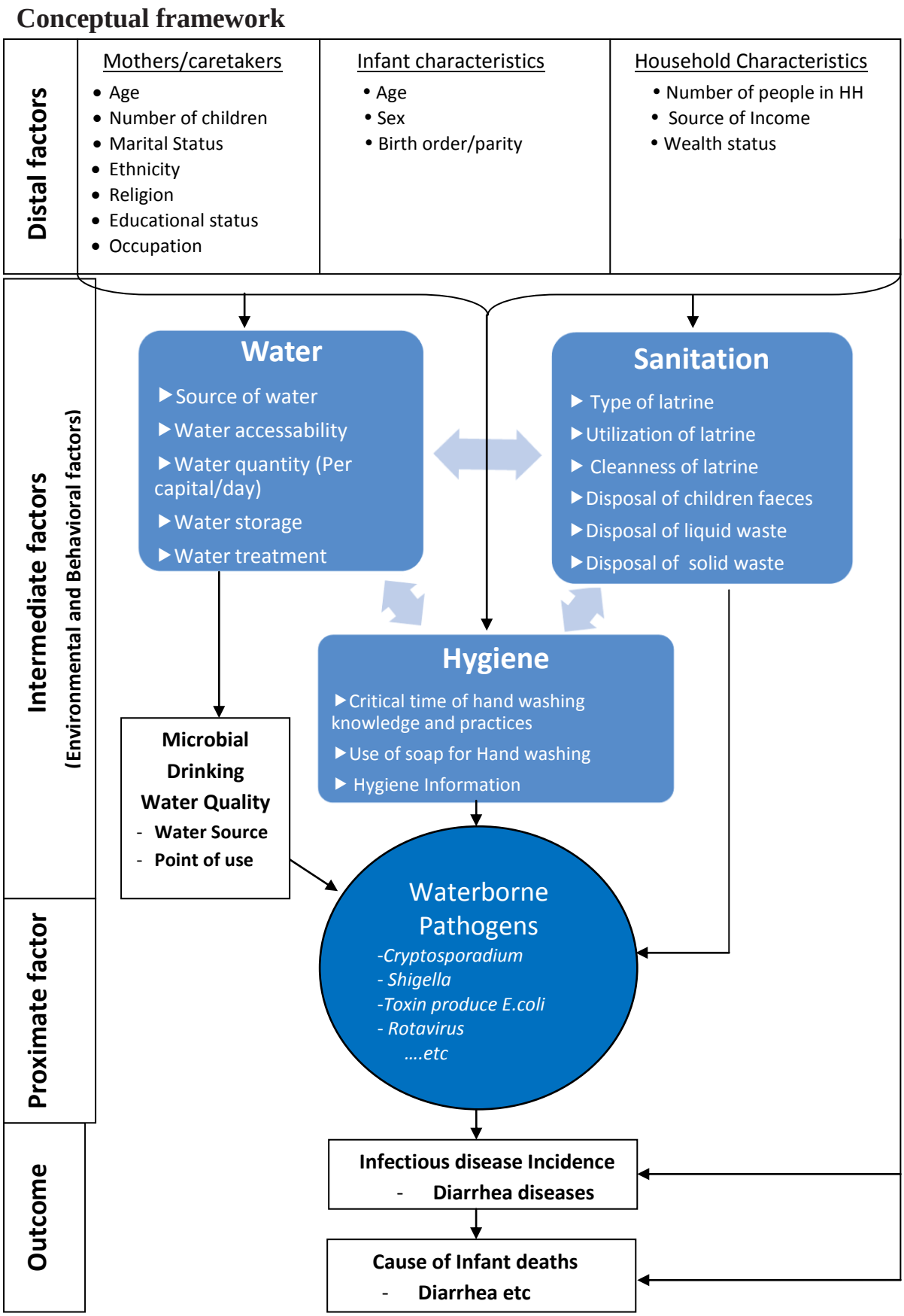
2.11 Conceptual Framework

The conceptual framework of the study was developed based on a literature review of existing studies that dealt with the overall objective of the study. The major subjects of the study, important concepts, or significant variables, as well as their potential relationships, are all represented in the conceptual framework. The framework proposed by Mostley and Chen is generally considered to be the most comprehensive and systematic and has formed a powerful organizing structure for mortality studies by demographers and epidemiologists (195).

For analysis purposes, the conceptual framework has typically been approached as distal factors (characteristics of mothers/caregivers, infants, and households), intermidiate factors (environmental and behavioural factors such as water and sanitation and hygiene practices), proximate factors (waterborne pathogens), and outcome variables (incidence and cause of infant death). According to the conceptual framework, one of the distal variables for any cause of infant mortality was the socio-demographic characteristics of mothers/caretakers, infants, and households. Furthermore, based on the literature reviewed, the relative importance of predictive variables for each cause of infant mortality may vary depending on the existing conditions in a community. Maternal/caretakers characteristics (age, marital status, ethnicity, religion, educational status, and occupation), infant characteristics (age, sex of the child, birth order), and household characteristics (source of income, wealth index, number of people in the household) all play significant roles in determining each cause of infant mortality.

The environmental factors, such as water and sanitation, and the behavioral factors related to hygiene practices were identified as the intermediate factors for infant death, particularly from infant diarrhea. The extent to which an infant is affected by diarrheal diseases that lead to death is determined by factors related to water (water source improved/unimproved, water accessibility, water quantity/water consumption per capital per day, water storage, and water treatment), sanitation (latrine availability, latrine utilization, sanitation status/improved/unimproved, disposal of children's feces, solid water management, and liquid water management), and hygiene behavioral factors such as critical time of hand washing knowledge and practices and use of soap/detergents.

The integration of water, sanitation, and hygiene status has an effect on the condition of microbial drinking water quality. Infant may be exposed to various waterborne pathogens due to poor water quality at water sources and points of use in the households. The water pathogens are proximate factors for infant death caused by diarrhea. The microbial water quality determinantes such as water sources (improved/unimproved), drinking water storage status, water treatment practice, retention time of fetched water, and place of the water point have been shown to greatly influence the presence of waterborne pathogens in drinking water (See Figure 1).



Source: adaptation from Mostley WH and Chen LC (1984) and Schell, C.M., et al (2007)

Figure 1: Conceptual framework for distal, intermediate and proximal factors to the cause of infant mortality

CHAPTER THREE: METHODS AND MATERIALS

3.1 Statistical Methodology

3.1.1 Study area

This study was carried out in six randomly selected districts in the West Hararghe administrative zone of Oromia region and Zone 3 of Afar region, which are situated in the eastern part of Ethiopia. These districts include Chiro, Mieso, Gemechis, and Tullo districts of the West Hararghe administrative zone of Oromia, while Amibara and Awash Fentale districts are comprised under zone 3 administration of the Afar region. Based on the 2007 National Population and Housing Census of Ethiopia (196), the projected population estimate for each district for the year 2019 is as follows: Chiro (243,151), Mieso (191,978), Gemechis (263,615), Tullo (212,234), Amibara (85,964), and Awash Fentale (40,448). As per the national context, 3.4% of the total population is under the age of one year (196).

The study area has two main climate seasons: a dry season and a rainy season. The dry season ranges from October to February, while the rainy seasons have two periods: the period from June to September is the main rainy season, and some rainy weather usually occurs from March to May (197). As of 2019, the health facilities available to each district are: Chiro (one hospital, 7 health centers, and 39 health posts); Meiso (one hospital, 3 health centers, and 33 health posts); Gemechis (6 health centers, and 36 health posts); Tullo (8 health centers, and 30 health posts); Amibara (one hospital, 3 health centers, and 13 health posts); and Awash Fentale (2 health centers, and 11 health posts). The most often observed cases in these districts are acute upper respiratory tract infections, lower respiratory infections such as pneumonia and bronchitis, diarrheal diseases, malaria, urinary tract infections, and skin infections. Exact information on the coverage of access to improved water supply, sanitation, and good hygiene practices in each district is difficult to ascertain due to the inconsistent nature of secondary data.

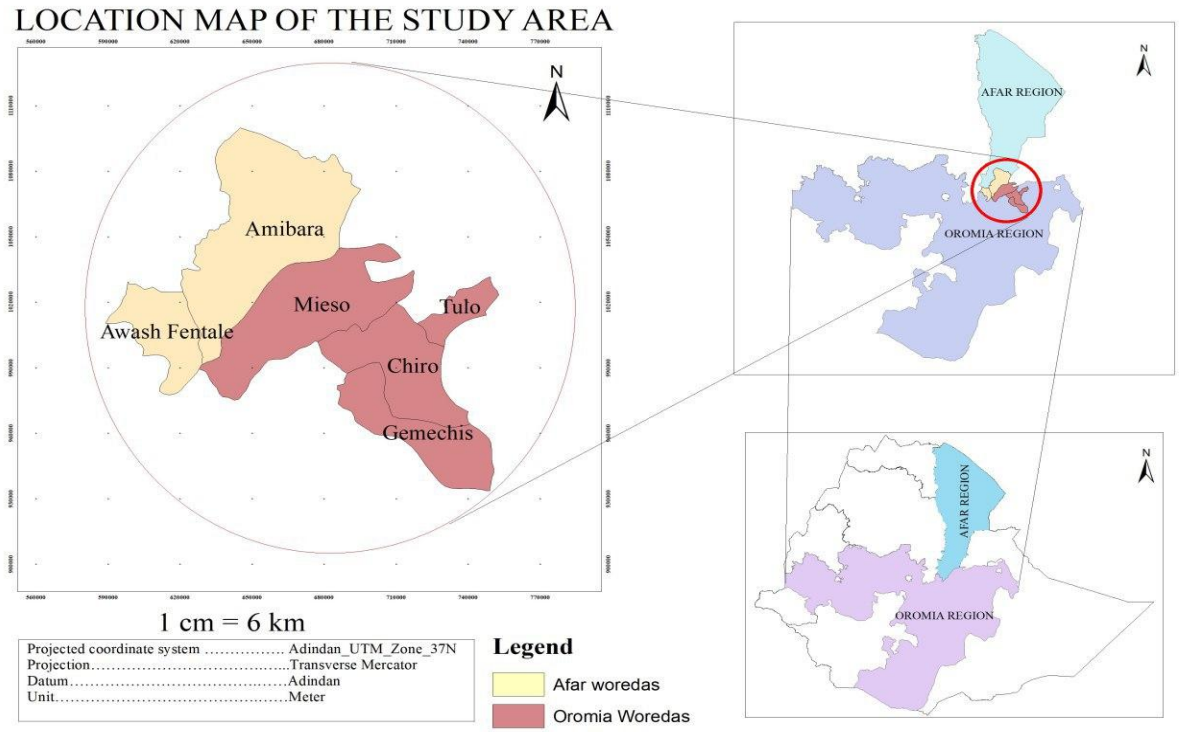


Figure 2: Location map of the study area

3.1.2 Study design and period

A community-based prospective longitudinal survey design was employed (Objective I), which was conducted with routine enumeration of reported and notified infant deaths for two years (from September 2016 to August 2018). A nested unmatched case-control study design was carried out (Objective II), which was nested from a longitudinal survey to identify the risk of water supply, sanitation, and hygiene on diarrhea-related infant death. Molecular testing-based cross-sectional study design (Objective III), which applied the LAMP technique for the detection of four microbial pathogens (*Cryptosporidium*, *Shigella*, toxin-producing strains of *E. coli*, and Rotavirus) from infants' drinking-water samples at point-of-use in the households and the corresponding water sources, was done during the period from June 2020 to May 2021 in eastern Ethiopia.

3.1.3 Study population

For objective I, the study populations were all deceased infants (<1 year of age), and the primary respondent to the VA questionnaire was their mother/primary caregiver who was with the deceased in the period leading to death or a witness to a sudden death or accident. For objective II, the study population (mother-infant pair) consisted of cases and controls drawn from the longitudinal survey conducted in the randomly selected study area. Cases were infants who died from diarrheal disease (all 61 cases that have been identified in the study area during the study period were included), while controls were considered for those who survived their first year of life. For objective III, the study population was comprised of randomly selected infants (<1 year of age). The sampling unit was households with infants, and the basic sampling population units (i.e., elements from which required information was ascertained) were their mothers/primary caregivers.

The inclusion and exclusion criteria are described as follows:

Inclusion criteria

- All households with mother's live-borne infant's pair in the selected area are included in a one month interval follow-up through house-to-house visit until the end of their infancy.
- Infant who deceased before their first birthday is included.
- All deaths due to diarrheal disease until the end of their Infancy (Objective II)
- All households with ongoing practicing of drinking-water to their children age of less than one year in the selected area are included in detecting waterborne pathogens.

Exclusion criteria

- Infants who have less than one year of follow up, either due to external-out-migration or loss-to follow up has been excluded from the study.
- All causes of deaths other than diarrhea-related death is included in the study of risk factors of diarrheal disease associated- infant death (for objective II).
- Households currently practicing exclusive breast feeding excluded in waterborne pathogens detection in drinking-water samples.(for objective III)

3.1.4 Sample size determination

The sample size estimation for objective I used Cochran's sample size formula for single population proportions using OpenEpi Version 3.3a. The sample size was determined based on the proportion of the most common cause of infant mortality previously identified in the cohort study conducted in Ethiopia (79), which would yield the largest sample size. Accordingly, the sample size is calculated with the assumption that the proportion of 17.7% for the infant death caused by bacterial sepsis from all infant deaths (79), a ± 5% precision, a 95% level of confidence, and a design effect of 1.5. Thus, the calculated sample size was 336, and 10% was added for the non-respond rate, which resulted in a final sample size of 370 infant deaths. This would be obtained from 6271 live births, as the national infant death rate was 59 per 1000 live births (9). Therefore, this survey covered 197,201 populations as calculated by cross-multiplication from the national crude birth rate of 31.8 live births per 1000 inhabitants (38).

For objective II, the sample size was determined using Openepi version 3.03a by applying the sample size computation to case-control study. The sample size was calculated for each variable by taking the result of the study conducted previously that would yield the largest sample size. Accordingly, the sample size was established based on the following assumptions: the household latrine proportion was found to be 5.93% in the household of cases and 15.4% in the household of controls group taken from the previous study (49), the two-sided significance level (1-alpha) = 95%, the power (1-beta, % chance of detecting) = 80, the ratio of unexposed to exposed in the sample = 1:4, to detect an odds ratio of 0.35. Thus, the minimum sample size required for the study was estimated to be 126 cases and 504 controls. The finite population correction factor was needed, thus, the number of cases was adjusted three times by the proportion of deaths due to diarrhea and the proportion of all-cause mortality using the following formula.

$$Nf = \frac{no}{1+\frac{no}{N}}$$

Where: *Nf = final sample size (cases)*
no= initial simple size (126 cases)
N= Total number of all-cause infant mortality (362)

Accordingly, the calculated result of 61 cases (deaths due to diarrhea) was needed. Because of many reasons of uncertainty and possible non-response, 10% were added, and thus a final sample size of 67 cases and 268 controls were required.

For objective III, the sample size (i.e., the number of households to be included in the study to represent the population of interest) was calculated using Openepi version 3.03a with the single population proportion. The sample size was calculated considering the assumption that the estimated prevalence of feacally contaminated drinking water in 80% of households with children in the overall study group at the baseline conducted in one of the districts in eastern Ethiopia (198), an error risk parameter of 1.96 (for an error risk of 5%, i.e., 95% confidence limits), a desired precision of 5%, and a design effect of 1.5 resulted in a sample size of 369 households with infants, and 15% of the non-response rate was added, and thus, 424 drinking-water samples were taken from infants' drinking water at point-of-use in the households.

3.1.5 Sampling techniques

For the study of objective I, a two-stage cluster sampling procedure with probability proportional to size (PPS) technique was used. In the first sampling stage, six districts were selected randomly using the lottery method from the two zones (four districts from the West Hararghe zone of the Oromia region and two districts from zone 3 of the Afar region). The second sampling stage involved selecting "Kebeles" (the smallest administrative unit) at random from districts that had been chosen at random, taking each "kebele" as one cluster. A total of sixty kebeles were selected from the chosen districts. Every infant death that occurred in each kebele (cluster) was routinely recorded by making a visit to every home once a month and conducting a verbal autopsy interview using a questionnaire after the culturally prescribed period of mourning had passed, up to a point within 15–30 days of the infant's death.

The sampling techniques for the objective II study exploited all diarrheal deaths of infants, which were selected as cases, while density sampling was used for the control groups. The entire cases (infants who died from diarrhea) that were ascertained by electronic verbal autopsy were directly taken from the longitudinal survey (Objective I). Infants who had survived their first year were eligible for selection as the control group, which was randomly selected from the adjacent area linked to that of the infants who died due to diarrhea. For each case, four controls were randomly selected from the longitudinal survey database, and their socio-demographics and several components of water, sanitation, and hygiene were compared.

A two-stage cluster sampling method with a probability proportional to population size was used for objective III. In the first sampling stage, thirty "kebeles" (the small administrative units) or clusters that infant diarrhea death occurred in objective I were allocated to each targeted district on the basis of proportional allocation to the available "kebele" population size. In the second sampling stage, infant-containing households within the chosen "kebele" were selected using simple random sampling.

3.1.6 Data Collection Methods

Longitudnial survey data: Live births in the study area are first registered until they reach the final count of 6271. Following that, each household with livebirth was visited once a month until the end of their first year of life. All infant deaths were routinely recorded and, conducting a verbal autopsy questionnaire after the culturally mandated period of grieving had passed. Meanwhile, the household survey questionnaire was administered for both deceased infant and those required number of survived in the first year of life. The household survey data were collected by trained data collectors using a structured and pre-tested questionnaire and conducted under closely overseen by supervisors. The questionnaire was prepared based on WHO, UNICEF and national standards, as well as adopted from relevant literatures (9,199,200).

Verbal autopsy data: Data was collected by interviewing the mothers or primary caretakers of the deceased infant using a standard 2014 WHO verbal autopsy questionnaire (19). This questionnaire elicits data on the age and sex of the deceased, diseases, signs and symptoms, as well as the circumstances observed preceding death. For the objective I study, either of the two questionnaires

was utilized: one for deaths of children under the age of four weeks and one for deaths of children between the ages of four weeks and 11 years. All the standard questionnaires were translated into the local languages "Amharic" and "Afan Oromoo", and retranslated back into English by relevant experts to verify that the meanings of the questions were retained and check their consistency.

Training in conducting questionnaire-based VA interviews and data collection procedures was provided to the data collectors and supervisors. The pre-testing of the questionnaire took place in a neighborhood with characteristics resembling those of the study region and population. The health extension workers, who are stationed at their respective settings, routinely visit each household every month for events and report and notify all infant deaths. After receiving this, the data collectors conducted VA interviews between 15-30 days after the date of every infant death. The data collection was closely monitored by supervisors, and each filled-out questionnaire was thoroughly checked for completeness as well as consistency and go back to the field for correction when errors were detected.

Water sample data: the household and infant characteristics data along with the water samples were collected by trained enumerators using pre-coded structured questionnaires and a water sample leveling format, respectively.

Measurement of outcome variable: The cause of infant death were measured using the InterVA-4 model which follows the VA cause of death categories defined in the WHO-2014 standard together with WHO cause codes and corresponding ICD-10 categories, which tend to establish the most likely cause for a particular case with its own likelihood (Objective I). For objective II, the outcome variable cases (infant death from diarrhea) were taken from objective I. For objective III, the prevalence of four targeted pathogens was measured using laboratory testing with molecular based- LAMP techniques.

3.1.7 Study Variables

3.1.7.1 Independent (Predictors) variables of the study

- Mother's and the deceased infant's characteristics (Objective I)
- Environmental effect variables (Water Supply and Sanitation and Behavioral factors (Hygiene practices) (Objective II)
- Primary source of drinking water (improved or unimproved), drinking water storage hygienic status, practices of water treatment at point-of-use, water fetched time, water point location and presence of residual chlorine (objective III).

3.1.7.2 Dependent (outcome) variables of the study

- Common specific-cause of infant mortality (Objective I)
- Diarrhea-related infant mortality (Objective II)
- Presence of microbial pathogens in drinking water (*Cryptosporidium* Oocyst, *Shigella* species, toxin-producing strain of *E.coli* and Rotavirus) (Objective III)

3.1.8 Data Quality Control

Data quality was assured by regulating both random and systemic errors before, during, and after the data collection. Data quality was maintained through a properly designed data collection tool. Thirty experienced enumerators and five supervisors with relevant educational backgrounds and language proficiency were recruited. The data collection procedures were developed, and intensive training was provided for the data collectors and supervisors. Questionnaires were translated into the local language and then back to English in order to maintain consistency. Pre-testing was also conducted to thoroughly familiarize the data collection instruments and procedures. The targeted respondent who provided data about the deceased was mothers/primary caretaker, who would provide more credible, reliable, and accurate data (19). The VA interviews were conducted after the mourning period had passed, with shorter recall periods. On-the-spot monitoring of data collectors and field editing were carried out in every study area by the assigned field supervisors. The supervisors provided immediate feedback and technical support as needed. Any identified errors were discussed, and immediate measures have been taken.

For objective III, the data collectors as well as supervisors were recruited with health-related educational backgrounds and language proficiency. Three days training was given to the data collectors on data collection and water sample collection procedures (ANNEX II), and a pre-test was carried out in a community with similar characteristics. The data collection procedures were developed, and the collected data were reviewed by the principal investigator. Any identified errors were discussed, and immediate measures were taken, such as revisiting the households before leaving the village to make corrections and complete the questionnaire, discarding the water sample if exposed to contamination, and collecting it in a new sterile bottle.

3.1.9 Data Management and Analysis

For the objective I study, the InterVA-4 model was used for the entire processing and determination of the cause of death from the collected verbal autopsy data. The collected data was entered into a comma-separated variable (.csv) file database in the InterVA-4 program, which prepared a data entry sheet with columns in identical sequence to the questions in the questionnaire. Following the installation procedure outlined in the InterVA-4 user guide (18), the probable causes of death for each deceased infant were determined. As InterVA requires specifying basic epidemiological parameters for malaria and HIV/AIDS prevalence in the population as "very low", "low," or "high" , this study leveled "high" for malaria as the infant death prevalence in Ethiopia for recent years is 1% (201), which is laid at 1:100. For HIV/AIDS, "low" is set as the death prevalence being less than 1% (202), which is pointed at around 1:1000 of all deaths in the population in the context of the guideline.

The InterVA-4 model follows the VA cause of death categories defined in the WHO-2014 standard together with WHO cause codes and corresponding ICD-10 categories, which tend to establish the most likely cause for a particular case with its own likelihood. To estimate cause-specific infant mortality fractions, we summed the likelihood of each cause for every infant death and divided this by the total number of deaths. The data from the Excel spreadsheet was also exported to SPSS version 23 for statistical analysis. Descriptive statistics were carried out to

express data as frequency distributions, means, standard deviations, or percentages with 95% confidence intervals. Binary logistic regression was used to analyze the association factors of maternal and infant characteristics among mortality from each of the ARIs, including pneumonia, diarrhea disease, birth asphyxia, prematurity, and malaria, against all other causes combined. For each outcome, separate models were adjusted to identify factors independently. Factors that had a significant level of $p<0.2$ in bivariate analysis in each model were candidates for the multivariable analysis of logistic regression. The models include Model 1: Association of factors with deaths due to ARTI versus all other causes of death, Model 2: Association of factors with deaths due to diarrhea versus other cause of death, Model 3: Association of factors with deaths due to birth asphyxia versus other cause, Model 4: Association of factors with deaths due to prematurity versus other cause of death and Model 5: Association of factors with deaths due to malaria versus other cause of death. In such a case, those factors with $p<0.05$ were considered statistically significant and had an independent association with the outcome variables.

For objective II, the collected data were entered into CSPro version 6.1 then transformed to SPSS version 23 for analysis. Descriptive statistics such as frequency distribution and cross-tabulation were used to summarize the study variables. Both bivariate and multivariable logistic regression was used to estimate crude and adjusted odds ratios with 95% confidence intervals for the association between risk factors and diarrhea-related infant death. Bivariate logistic regression for each variable was analyzed, and any factors that showed a marked association (p-value <0.25) were considered candidates for multivariable logistic regression. The Hosmer-Lemeshow goodness-of-fit test was checked and indicated that this model is valid, seeing that the p-value is greater than 0.05. Variables that resulted in a P value <0.05 in the multivariable logistic regression were affirmed as significantly associated with the outcome variable (diarrhea death).

For objective III, the raw data were entered into CSPro version 6.1 and then transformed to SPSS version 23 for analysis. Descriptive statistics such as frequency distribution and cross-tabulation were used to summarize the study variables. A phi coefficient correlation was done to analyze the association between point-of-consumption and water sources in the presence of pathogens. Binary logistic regression was applied to establish crude and adjusted odds ratios with 95% confidence intervals for the association between water quality determinants and each targeted pathogen. In bivariate conditional logistic regression, each variable in each model with a p-value <0.25 was considered eligible for multivariable logistic regression. These models include Model 1: Association of factors with the presence of *Cryptosporidium* oocyst in the water sample, Model 2: Association of factors with the presence of *Shigella* species in water sample, Model 3: Association of factors with the presence of toxin producing strain of *E. coli* in water sample, Model 4: Association of factors with the presence of rotavirus in water sample and Final Model: Association of factors with the presence of all targeted pathogens (*Cryptosporidium*, *Shigella*, toxin producing strain of *E. coli* and rotavirus) in the sample water sample. Variables that demonstrated a p-value <0.05 in the multivariable logistic regression were declared to be significantly associated with the outcome variable (presence of targeted pathogens).

3.2 Molecular – LAMP Laboratory Methodology

3.2.1 Selection of Target Genes

For the detection of each pathogenic microbe, a specific gene target region was selected. The selection was based on the gene that was most commonly used to identify the target genes that expressed proteins unique to the organism of choice and helped to differentiate each pathogenic microbe's presence in drinking water (150,178,180,203). The chosen specific targeted genes included the gene encoding the 18S rRNA presented in *Cryptosporidium* spp., the ipaH gene in *Shigella*, the stx2A and stx2B genes in toxin-producing strains of *E. coli*, and the *VP7 glycoprotein* gene for rotavirus. These genes served as sites for specific primer designs that were used in LAMP assays.

3.2.2 Primer Design

The primer sequences for LAMP amplification were designed based on the *Cryptosporidium 18S rRNA gene* accession number *GenBank: L16996.1*, *Shigella ipaH gene* accession number GenBank: *M76443.1*, *Toxin-producing strains of E. coli stx2A and stx2B gene* accession number GenBank: *FN252458.1*, and *Rotavirus VP7 glycoprotein gene* accession number GenBank: *AB018697.1* from NCBI. The primers were designed by means of the Primer Explorer Version 5 software, available at https://primerexplorer.jp/e/. The software was used to design one set of primers (five to six primers per target organism) (see Table 1).

Table 1: Primers and Sequence used for LAMP assays to detect *Cryptosporidium*, *Shigella*, Toxin-producing of *E.coli* and Rotavirus

Primer Name	Primer Sequence	Target Gene
Cryptosporidium		18S rRNA
FIP	CCTCGTTCAAGATCAATAATTGCAA-ATGGGTAATCTTTTGAATATGCA	
BIP	TCCTAGTAAGCGCAAGTCATCAG-ATTCAATCGGTAGGAGCG	
F3	GTATATATTCCTGTTTCGAAGGA	
B3	TCCGAATAATTCACCGGATC	
LB	GCTGATTACGTCCCTGCCCTTTG	
Corresponding nucleotide position of Cryptosporidium parvum 18s rRNA gene, 1746bp (Accession No. 16996.1)		
Shigella		ipaH
FIP	AAGCTCCGCAGAGGCACTGA-CACGCAATACCTCCGGATTC	
BIP	AGCAGTCTTTCGCTGTTGCTGC-CCGGAGATTGTTCCATGTGA	
F3	GCCTTTCCGATACCGTCTCT	
B3	TGATGGACCAGGAGGGTT	
LF	TGCAGCGACCTGTTCACG	
LB	CACTGAGAGCTGTGAGGACCG	
Corresponding nucleotide position of Shigella flexneri ipaH gene, 1312bp (Accession No. M76443.1)		
Toxin producing strain of E.coli		Stx2A and stx2B
FIP	AGACGAAGATGGTCAAAACGC-GCAGTTATTTTGCTGTGGA	
BIP	CGGGTTCGTTAATACGGCAA-CGGGCACTGATATATGTGT	
F3	TCGGTGTCTGTTATTAACCA	
B3	TGGAAACCGTTGTCACAC	
LF	TGATAGACATCAAGCCCTCGTA	
Corresponding nucleotide position of Escherichia coli stx2A gene for Shiga toxin 2 A-subunit and stx2B gene for Shiga toxin 2 B-subunit, strain CB10686, 1450bp (Accession No. FN252458.1)		
Rotavirus		VP7 glycoprotein
FIP	TTGGTTGCTAGCTTCAATTGGATAA-CGCATATGCTAACTCTACTCAA	
BIP	TGGTGAATGGAAAGATACATTGTCA-TGTACTCTTTAAAGTAGACCGAT	
F3	CCAATAACAGGATCAATGGATAC	
B3	GGATCGACAGAAAATTCAACAA	
LB	TGTTTCTTACAAAAGGCTGGCCAAC	
Corresponding nucleotide position of Human rotavirus A gene for VP7, 981bp (Accession No. AB018697.1)		

3.2.3 Oligonucleotide primers Synthesis

Laboratory-made DNA or RNA strands or oligonucleotide primers for each targeted gene were prepared based on the designed primer sequence. All oligonucleotide primers were synthesized by Africa's Genomics Company, Inqaba Biotec East Africa Ltd. (ANNEX III).

3.2.4 Drinking Water Sample Collection

The drinking water samples were taken from two locations: the primary infant drinking vessels at the site of intake in the home and the corresponding water source. One liter of water was collected in a sterile bottle with no chemical additives. Water samples were collected and stored in accordance with the ICR (Information Collection Rule) guidance (204). Briefly, before moving on to the next phase, the obtained water samples were kept at a temperature of 1-4^{o}C in a dark cold box and transported within eight hours after the time of collection.

a) Water Sample collection from source

b) water sample from households

Figure 3: Water sample collection using 1000ml sterile bottel

3.2.5 Filtration

Once the water sample was received, filtration was carried out for each sample using a Zeta Plus 1MDS electropositive microfilter media disc with a 47-mm size (3M Purification Inc., Meriden, CT, U.S.A.). This filter can simultaneously capture and recover multiple microbes from a water sample (205). This filter disc was placed on the sterile funnel unit of the membrane filter support disc assembly. The filtration was carried out by applying a vacuum pump until the water sample was finished, and finally, the filter disc was removed using sterile forceps and placed in a sterile petri dish. This is followed by storing at 4°C in the dark for not more than 12 days before undergoing the elution process (http://www.lenntech.com).

a) Filtration process setup

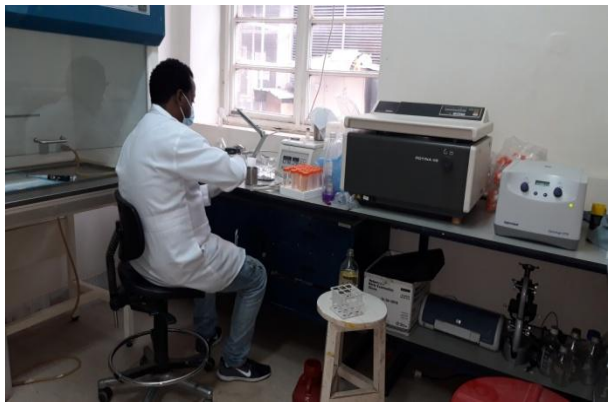
b) 1MDS filter membrane after filtration

Figure 4: Filtration using 1MDS electropositive microfilter media disc

3.2.6 Elution

An eluting solution was used to remove the adsorbed pathogenic microbes from the filter disc surface. For a single elution, a 200 mL eluting/backflushing solution was made by mixing [50 mL 1.5% beef extract (Accumix, CA-69574, India) with 0.05 M glycerin (Fluka Chemika, CA-49780, Switzerland) autoclaved at 121^0c for 15 min + 50 mL 0.01% Tween 80 (Mana Scientific Product, CA-9005-65-6, India) + 50 mL 0.1% sodium polyphosphate/NaPP (Sigma Aldrich, CA-305553, USA) + 50 mL 0.001% antifoam agent (Bio-Rad Laboratories, CA-94547, USA) to reduce foaming effect]. The pH of the eluting solution was adjusted to 8 using an Accumet pH benchtop meter (Thermo Fischer Scientific, Canada), which could reduce the potential destruction of bacteria (205). The cleaned and sanitized bronze filtration support disc housing was used to hold the adsorbed filter disc once it had been mated with the filter funnel. The eluent solution was allowed to come into contact with the filter disc for 10 minutes prior to starting the elution pumping procedure. The eluent solution was then added to the filter funnel and pumped into the sterilized filter housing in the opposite direction of the sample flow until it became clear. The 200 mL eluent was finally put into four sterile 50 mL Falcon tubes (Merck KGaA, Corning 430290, Germany) to be centrifuged (Eppendorf AG- 22331, Hamburg, Germany) for the concentration of targeted pathogens in the filtered eluate.

a) eluting solution preparation



b) eluent solution added to the filter funnel

Figure 5: Elution of 1MDS electropositive microfilter media disc

3.2.7 Concentration of Pathogens from the Filter Eluate (Centrifuge)

The eluted effluent underwent a secondary concentration stage over several centrifugation cycles. A preliminary centrifugation (Eppendorf AG- 22331, Hamburg, Germany) was performed on four tubes (Merck KGaA, Corning 430290, Germany) containing 50 mL of eluate at 4,100×g for 30 min. Following that, the Falcon tubes (Merck KGaA,Corning 430290, Germany) were carefully withdrawn from the centrifuge (Eppendorf AG- 22331, Hamburg, Germany), and the supernatant was properly eliminated ~25 mL from each one using a serological pipette. The aliquots of the re-suspended materials were pipetted up and down each tube to transfer them into two 50 mL tubes (Merck KGaA, Corning 430290, Germany), after which a second centrifugation (Eppendorf AG-22331, Hamburg, Germany) was performed under the identical circumstances. The residual material was gently shacked and transferred into two sterile 15 mL tubes before being centrifuged at 4,100 g for 30 minutes (AG- 22331, Hamburg, Germany) with the supernatant, which had been carefully aspirated to a volume of 35 mL.

In order to avoid disrupting the pellets, the supernatant was once again aspirated to a total of 12 mL from each of the 15 mL tubes. After being centrifuged at 4,100 g for 30 minutes, the leftover supernatant (3 mL) was placed into a single 15 mL tube (Merck KGaA, Corning 430055, Germany) and removed. The supernatant was then gently aspirated into each of the 15 mL tubes (Merck KGaA, Corning 430055, Germany) until around 300 µL remained, and the tubes were then kept at -20°C pending DNA/RNA extraction.

Figure 6: Eluted effluent underwent several centrifugation

3.2.8 DNA/RNA Extraction

Genomic DNA for *Cryptosporidium*, *Shigella,* and a toxin-producing strain of *E. coli* and RNA for rotavirus were extracted from 300 µL eluate (concentrated water samples) using the DaAN Gene RNA/DNA Purification Kit (MDSS GmbH, Germany), according to the manufacturer's instructions. The RNA genome for rotavirus was converted to cDNA using first-strand cDNA synthesis protocols [E6300] (206). This conversion is needed for the reason that cDNA is a more convenient way to work with the coding sequence than RNA, which could be very easily degraded by omnipresent RNases. The extracted DNA/RNA and cDNA samples were stored at –20°C until amplification was done.

Figure 7: DNA/RNA extraction using the DaAN gene RNA/DNA purification kit

3.2.9 LAMP Assay

3.2.9.1 Reaction mixture for LAMP (Operated on ice)

The reaction mixture consisted of all the components necessary to make new strands of DNA/cDNA in the LAMP process. The reaction mixture is composed of the following reagents: 1) Master Mix, containing 1x Thermopol reaction buffer (10 mM KCl, 10 mM $(NH4)_2SO4$, 20 mM Tris-HCl, 2.0 mM MgSO4, 0.1% Triton X-100; New England Biolab), 6mM MgSO4 (100mM;New England Biolabs Inc.), 0.8 M betaine (Glentham Life Sciences Ltd, Corsham, UK), 1.4 mM dNTP's (10 mM; New England Biolabs Inc.), Bst 2.0 DNA polymerase large fragment (8U; New England Biolabs Inc), 50x LAMP fluorescent dye (New England Biolabs Inc.), Primer mixture [1.6 µM FIP, 1.6 µM BIP, 0.4 µM LPF, 0.4 µM LFB, 0.2 µM F3 and 0.2 µM B3; Africa's Genomics Company, Inqaba Biotec East Africa Ltd], Nuclease free H_2O (Fisher Scientific, CAS 7732-18-5, USA) and 2) Template genomic DNA/cDNA. The reaction mixture for each target pathogen was prepared in an ice-filled box to a final volume of 25 µL (see Table 2).

Table 2: Individual reaction mixture composition of LAMP assay for the detection of selected pathogens

Prepare Mixture	**Quantity (µl)**			
1. Primer Mixture Ingredient (Per reaction)	Cryptosporidium	Shigella	Toxin- producing *E.coli*	Rotavirus
1.6µM FIP	1.0µL	0.8µL	1.0µL	1.0µL
1.6µM BIP	1.0µL	0.8µL	1.0µL	1.0µL
0.4µM LPF	-	0.4µL	1.0µL	1.0µL
0.4µM LFB	1.0µL	0.4µL	-	-
0.2µM F3	1.0µL	0.1µL	1.0µL	1.0µL
0.2µM B3	1.0µL	0.1µL	1.0µL	1.0µL
Total	5.0µL	2.6µL	5.0µL	5.0µL
1. Create the Reaction Mixture Ingredients (Per reaction)				
1X Isothermal Buffer	2.5µL	2.5µL	2.5µL	2.5µL
6mM MgSO4	1.5µL	1.5µL	1.5µL	1.5µL
1.4mM DNTPs	3.5µL	3.5µL	3.5µL	3.5µL
	4.0µL	4.0µL	4.0µL	4.0µL
0.8M Betaine	1.0µL	1.0µL	1.0µL	1.0µL
Bst 2.0 DNA polymerase	4.0µL	6.4µL	2.0µL	2.0µL
Nuclease free H2O	16.5 µL	18.9 µL	14.5 µL	14.5µL
Total				
2. Template DNA	3µL	3µL	5.0µL	-
3. Template cDNA	-	-	-	5.0 µL
4. LAMP fluorescent dye	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Overall reaction mixture	25µL	25µL	25µL	25µL

Figure 8: Reaction mixture operated on ice

3.2.9.2 Optimization of LAMP assay for each pathogen

To establish the best amplification reaction conditions, the concentrations, temperature, and time of the reaction mixture were optimized for the LAMP assay. The reaction mixture for each targeted pathogenic microbe was tested under different conditions of concentration, amplification temperature, and time. The temperature tested ranged from 60 to 65°C for 60 minutes, followed by a heat inactivation step at 80°C for 5 to 10 min. The genomic DNAs/RNA-cDNA extracted from the positive control of each targeted pathogen and DNA/RNA extracted from nuclease free water (Fisher Scientific, CA-7732-18-5, USA) as a negative control were used for the optimization of LAMP. After several trials, the optimized LAMP reaction mixture condition for each targeted pathogen was presented in Table 2. The optimum temperature and time for the best amplification result for *Shigella* species, *Cryptosporidium* oocysts, and rotaviruses were 60 °C for 60 minutes followed by 80 °C heat inactivation for 5 min while a toxin-producing strain of *E. coli* was best worked at 64°C for 60 minutes followed by inactivation at 80°C for 5 min. In all optimization procedures, there was no amplification result observed in the negative control.

3.2.9.3 Positive and Negative Control

To determine the positive control, the live pathogen strains (*Cryptosporidium*, ATCC PRA-67DQ; *Shigella*, ATCC 12022; a toxin-producing strain of *E. coli*, ATCC 43895; and Rotavirus, a positive stool sample stored in a laboratory) were obtained from the Ethiopian Institute of Public Health (EPHI) and Ethiopian Biodiversity Institute (EBI) biobanking repository laboratories. This strain was diluted in one liter of distilled water and processed by filtration, elution, and centrifuging (Eppendorf AG- 22331, Hamburg, Germany), followed by DNA/RNA extraction (MDSS GmbH, Germany), and amplification. A negative control was done using nuclease-free water (Fisher Scientific, CA-7732-18-5, USA) instead of the DNA template. A positive control and a negative control were included in every LAMP reaction to ensure that false-negative and false-positive results were eliminated.

3.2.9.4 LAMP operational procedure and reaction

The final concentration of the LAMP reaction mixture of 25 µL was dispensed into each labeled Loopamp reaction tube (EIKEN Chemical Co.,Ltd. Tochigi, Japan). The reaction was carried out by inserting the reaction tubes in a Bio-RAD iCycler Thermal Cycler (Bio-Rad Laboratories, Inc. USA). Based on the optimization result for *Shigella*, *Cryptosporidium*, and rotavirus, the reaction was run by heating at 95°C for 3 minutes, subsequently incubating at 60°C for 60 minutes, inactivating at 80°C for 5 min and then cooling at 4°C for 5 min to terminate the reaction. The same procedure was undertaken for the detection of a toxin-producing strain of *E. coli* by activating at 64°C for 60 minutes and inactivating at 80 °C for 5 min.

Figure 9: Amplification operated using Bio-RAD iCycler Thermal Cycler

3.2.9.5 Detection Using U.V Trans-illuminator

After completion of LAMP, amplified DNA/cDNA were detected using a UV transilluminator (SYNGENE, Synoptics Ltd., UK) and photographed, and finally the numbers were noted down for all positive samples.

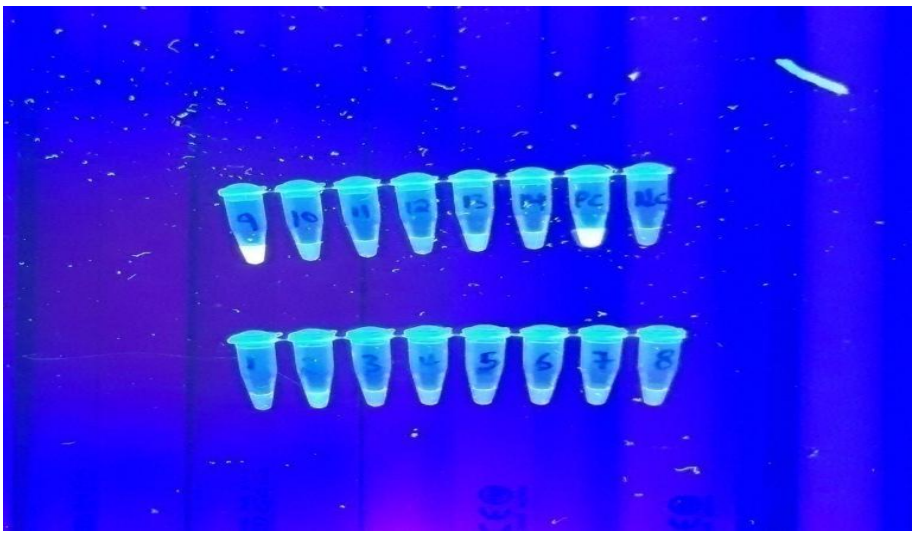


Figure 10: LAMP detection of Shigella species in water sample with flurescent dye added seen with ultraviolet light; NC: negative control, PC: positive control; sample number 9 is positive

3.2.10 Sensitivity and Specificity of LAMP

The LAMP reaction sensitivity greatly relies on the previous studies that explored the high sensitivity of LAMP for *Cryptosporidium* oocyst (207), *Shigella* species (203), toxin-producing strains of *E. coli*(203,208,209), and RNA-cDNA of Rotavirus (210). The specificity of the LAMP assays for *Shigella* was examined with three closely related bacterial species: *Salmonella* species, *Vibrio cholerae***,** and toxin producing *E. coli*. The result demonstrated a positive for only *Shigella* species and a negative for the aforementioned bacterial species. The specificity of the toxin producing strain of *E. coli* was determined by testing it with three other bacterial species: *Salmonella* species, *Vibrio cholerae***,** and *Shigella* species, which solely resulted in a positive for the toxin-producing strain of *E. coli* and a negative for all the other bacterial species. The LAMP assay for *Cryptosporidium* oocyst and rotavirus was examined with *Giardia* cysts and adenovirus, respectively, which demonstrated only positive results for *Cryptosporidium* oocyst and rotavirus. Therefore, the assay established in this study was found to detect only the sequence of the targeted pathogens and no cross-reaction with other pathogenic microbes, representing its high specificity.

3.2.11 Quality control for LAMP

For the laboratory quality assurance of the LAMP assay, the ICR (Information Collection Rule) microbial laboratory manual was followed (204).The operating procedures were kept exactly as stated in the protocol. All LAMP laboratory equipment was maintained in a safe and working condition. Each DNA/RNA extraction and LAMP test was carried out by knowledgeable and skilled personnel in a secured separate room set-up to avoid contamination. Samples with positive results were confirmed by analyzing them with the same procedures as the positive control. All test results were photographed and recorded directly into an electronic registry format. The methods of recovery for each organism comprised of filter material, eluent, and centrifuge steps were applied considering the other study (205), which can yield the highest recovery efficiency.

3.3 Ethical Consideration

Ethical approval to conduct the study was received from Ethics Review Board of Addis Ababa University - Ethiopian Water Resource Institute (EIWR N^{o}. 134/08/16), and also ethical clearance was provided by the research ethics committee of Oromia Regional Health Bureau. Before the study begins, official permission was secured from each study district administrative as well as health offices. Informed consent from each study subjects were obtained after clear and adequate explanation of the objectives and purpose of the study were provided using the participant's information sheet. Personal data, in particular name, geographical information and contact information about the respondent, is kept and be encrypted to protect privacy and ensure confidentiality. The water samples were tested and analyzed anonymously, and the original paper records were stored in a locked file cabinet. The personal identifiers are removed from study documents and also computer-based files were stored in a password encrypted a laptop to protect the participants' confidentiality.

3.4 Dissemination of findings

The overall finding of this study will be disseminated to the targeted districts as well as for the country level policy makers and concerned bosies to utilize the findings in one way or another as deemed necessary. Maximum effort is done to publish the finding in scientific trustworthy journal.

CHAPTER FOUR: RESULTS

4.1 Cause of Infant Deaths and patterns of associated factors

4.1.1 General Characteristics of Respondents and Study Population

During the study period, a total of 362 deceased infants' mothers/caretakers were interviewed, yielded 97.8% response rate. The highest proportion (90.4%) of the respondents was parents to the deceased infants.' The mean age (± SD) of the respondent was 27.3 (±4.3) years old. The age distribution of the deceased infants' mothers/care taker was highest in the age category of 20-34 years old (86.7%). The majority of the respondents were married at 91.2%. More than four-fifths (87.9%) of the respondents were reported not to have been attended formal education at some point in their lives. Almost 88.6% of respondent's economic activity was home-maker (housewife) in a year prior to infant's death.

Of the overall recorded infant deaths, about 192 (53.0%) deaths were occurred during neonatal life while 170 (47.0%) in the post-neonatal period. The deceased infant comprised of 205 (56.6%) males and 157 (43.4%) females with an overall sex ratio of 1.31:1. Over half (55.4%) of the infant death were occurred in the wet season. Almost seven out of nine deaths (77.1%) took place outside of health facilities, of which the majority (71.8%) was died at home (see Table 3).

Table 3: Frequency distribution of the respondents and the deceased infant characteristics in Eastern Ethiopia, 2016-18

Socio-demographic Characteristics	Neonate (<1month) (n=192)		Post-neonate (1-12 months) (n=170)		Total (<1 year) (N=362)	
	n	%	n	%	n	%
Respondent characteristics						
Age of the mother/care taker						
<20 Years old	12	6.3	12	7.1	24	6.6
20-34 Years old	169	88.0	145	85.3	314	86.7
≥35years old	11	5.7	13	7.6	24	6.6
Maternal Marital Status						
Married	181	94.3	149	87.6	330	91.2
Single	6	3.1	10	5.9	16	4.4
Divorce	2	1.0	9	5.3	11	3.0
Widowed	3	1.6	2	1.2	5	1.4
Mother's level of education						
No education	174	90.6	144	84.7	318	87.8
Primary school	15	7.8	23	13.5	38	10.5
Secondary school	3	1.6	2	1.2	5	1.4
College	0	0.0	1	0.6	1	0.3
Maternal Occupational Status						
Home maker (Housewife)	176	91.7	145	85.3	321	88.7
Employed	4	2.0	12	7.1	16	4.4
Mainly unemployed	12	6.3	13	7.6	25	6.9
Deceased Infant Characteristics						
Infant sex						
Male	117	60.9	88	51.8	205	56.6
Female	75	39.1	82	48.2	157	43.4
Seasons of death						
Wet season	107	55.7	94	55.3	201	55.5
Dry season	85	44.3	76	44.7	161	44.5
Place of death						
At home	131	68.2	129	75.9	260	71.8
At health facilities	55	28.7	28	16.5	83	22.9
On the route to health facility	6	3.1	8	4.7	14	3.9
Other places	0	0.0	5	2.9	5	1.4

4.1.2 Specific Cause of Infant Deaths: Result from Verbal Autopsy (InterVA-4)

Based on the result of the InterVA-4 model, the mean (± SD) of the likelihood value for the assigned cause of death across cases was 96.5% (±4.8%). The likelihood rate across cases varied with the range between 85-100%. In this analysis**,** the probable causes of infant deaths were classified into an age group of neonatal (<1month) and post-neonatal (1-11months). The analysis revealed that there were considerable major differences in the causes of infant death structure by age group.

4.1.2.1 Neonatal Cause of Death (n=192)

Across all the study area, nearly half of neonatal deaths (47.0%) occurred within 24 hours of birth, and about 10.4% die more than 24 hours after birth but within 48 hours from birth. For a further 14.6% deaths occurred more than 48 hours from birth but within the first week of life and for the rest 28.0% deaths were after the first week, but within first 28 days.

Among neonatal deaths, the three major causes of deaths were neonatal pneumonia (33.9%), followed by birth asphyxia (32.3%) and prematurity (14.6%). The fourth leading cause of death was meningitis and encephalitis which was responsible for 5.2 percent of death under this age group. Congenital malformation and neonatal sepsis were found to be another cause of neonatal mortality with 4.7 percent and 4.2 percent respectively. Diarrhea diseases caused about 3.1 percent of neonate death and for the remaining 2.1 percent no cause could be determined which concluded under other and unspecified neonatal cause of death.

4.1.2.2 Post-Neonatal Cause of Death (n=170)

In the post-neonatal age group, the leading cause of death was acute respiratory infection including pneumonia, which is responsible for 45.3% of all deaths in this age category. Close to one-third (32.4%) of death were due to diarrheal diseases which accredited to the second largest cause of deaths and malaria positioned at the third major cause of death, which accounted for one of every ten deaths (10.0%).

The result revealed that about 2.9 percent of deaths were due to severe malnutrition. The other illness that attributed to death under this age group was measles, which took a toll of 2.4 percent followed by pulmonary tuberculosis (1.8%) and non-obstetric sepsis (1.2%). Few deaths were ascertained due to HIV/AIDs related and meningitis and encephalitis each accounted for 0.6% of death. Deaths due to accidents found to be 2.4%, typically from contact with venomous animals and plants (1.8%) and accidental poisoning and exposure to noxious substance (0.6%). The remaining (0.6%) appeared to be other and unspecified infectious disease cause of death.

4.1.2.3 Overall Cause of Infant Death (n=362)

Of the total infant died, the findings indicate the highest mortality load among infants was diseases of respiratory system, particularly acute respiratory infection including pneumonia in post-neonate and neonatal pneumonia, which accounted for 20.7% and 17.7% respectively and causing in a combination of 38.4 percent infant deaths in the study area.

The mortality risk due to birth asphyxia was the next leading cause of deaths, accounted for 16.4% of infant death. Almost one-sixth (16.3%) of the infant were died as a result of diarrheal diseases. About 7.4% of deaths were associated with prematurity. Deaths due to malaria, meningitis and encephalitis, and sepsis appeared to be another identified cause of death conditions that contributed to 4.3%, 3.0%, and 2.5% of infant death, respectively. Moreover, congenital malformation (2.4%), severe malnutrition (1.3%) and measles (1.0%) were also observed as causes that directly lead to

infant deaths. Pulmonary tuberculosis (0.8%) and HIV/AIDS related death (0.3%) were the least ranked conditions among the deceased infant, and further 1.1% was causes due to accidents.

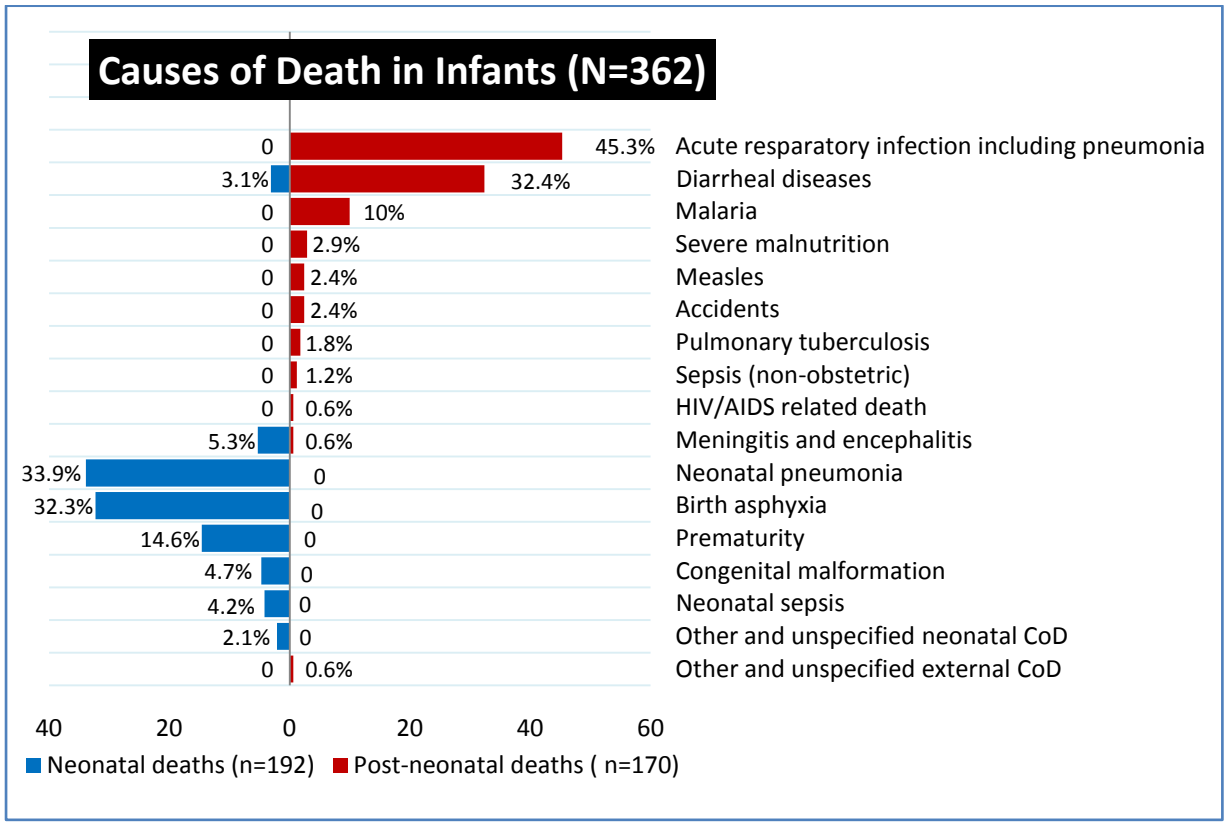


Figure 11: Percentage distribution of cause of infant death

As per consolidated into a broad WHO cause category, Infectious and parasitic diseases causes exhibited 48.7 percent —with a high burden on post neonate age group, followed by neonatal cause of death (47.4%), nutritional and endocrine disorder (1.4%), external causes of death (1.1%) and the remaining (1.4%) were reportedly unknown cause (see Table 4).

Table 4: Cause-specific infant mortality fraction by WHO VA category and age group in Eastern Ethiopia, 2016-18

Code	Causes of Infant Death *(WHO VA cause category)*	Frequency %(CI,95%) Distribution of Infant Death		Total %(CI, 95%)
		Neonate	Post Neonate	
01	Infectious and parasitic diseases	**4.5(1.9-5.9)**	**44.2(38.1-48.2)**	**48.7(41.8-52.1)**
	01.01 Sepsis	-	0.5(0.0-1.3)	0.5(0.0-1.3)
	01.02 Acute respiratory infection, including pneumonia	-	21.3(16.5-24.9)	21.3(16.5-24.9)
	01.03 HIV/AIDS related death	-	0.3(0.0-0.8)	0.3(0.0-0.8)
	01.04 Diarrheal diseases	1.7(0.03-2.2)	15.2(11.5-18.9)	16.9(12.5-20.1)
	01.05 Malaria	-	4.7(2.3-6.5)	4.7(2.3-6.5)
	01.06 Measles	-	1.1(0.03-2.2)	1.1(0.03-2.2)
	01.07 Meningitis and encephalitis	2.8(1.1-4.5)	0.3(0.0-0.7)	3.1(1.3-4.8)
	01.08 Pulmonary tuberculosis	-	0.8(0.0-1.7)	0.8(0.0-1.7)
03	Nutritional and endocrine disorders	**-**	**1.4(0.13-2.5)**	**1.4(0.13-2.5)**
	03.01 Severe malnutrition	-	1.4(0.13-2.5)	1.4(0.13-2.5)
10	Neonatal causes of death	**47.4(40.7-51.0)**	**-**	**47.4(40.7-51.0)**
	10.01 Prematurity	7.7(5.1-10.7)	-	7.7(5.1-10.7)
	10.02 Birth asphyxia	17.1(12.6-20.2)	-	17.1(12.6-20.2)
	10.03 Neonatal pneumonia	17.9(13.8-21.6)	-	17.9(13.8-21.6)
	10.04 Neonatal sepsis	2.2(0.6-3.4)	-	2.2(0.6-3.4)
	10.05 Congenital malformation	2.5(0.8-3.9)	-	2.5(0.8-3.9
12	External causes of death	**-**	**1.1(0.03-2.2)**	**1.1(0.03-2.2)**
	12.01 Contact with venomous animals and plants	-	0.8(0.0-1.7)	0.8(0.0-1.7)
	12.02 Accidental poisoning and exposure to noxious substance	-	0.3(0.0-0.9)	0.3(0.0-0.9)
99	Cause of death unknown	**1.1(0.03-2.2)**	**0.3(0.0-0.9)**	**1.4(0.13-2.5)**
	Total deaths	**53.0(45.6-55.9)**	**47.0(40.7-50.9)**	**100**

4.1.3 Pattern of associated factors with specific-causes of deaths against all-causes of death

Independent factors in multivariable analysis have been identified through pattern of selected factors associated with each leading specific-causes of infant death (ARTI including pneumonia, diarrheal death, birth asphyxia, prematurity and malaria) as compared to those infant who died from all other cause of death in combined.

Result from ARI-specific mortality model showed that, infant mortality from Acute respiratory infection, including pneumonia shows a strong significant association with younger maternal age (<20 years old), deaths out of heath facilities and unmarried women than all other causes of infant death. Lower maternal age (<20 Years old) was almost 5 times more likely to die of Acute respiratory infection, including pneumonia than all other causes of infant death combined (*P*=0.001, AOR: 4.82, 95% CI: 1.88, 12.3). Infants being died out of health facilities were also associated with nearly 3 times higher risk of ARTI death as compared to mortality from all other causes (*P*=0.007, AOR: 2.85, 95% CI: 1.33, 6.12). Significant interaction were observed for those died infants with unmarried mothers, but resulted in lower chance (*P*=0.041, AOR: 0.46, 95% CI: 0.22, 0.97) of ARTI-related death than those who died from other all-causes (see Table 5).

Analysis from diarrhea-specific mortality model indicated that post-neonates period and wet seasons have more pronounced significant association with diarrhea death than all-cause of death. The post-neonatal period had almost 16 times higher risk of diarrhea death than non-diarrheal deaths (*P*=0.000, AOR: 15.5, 95% CI: 6.35, 37.8). Wet seasons had significantly 2 times higher chance of infant death due to diarrhea than other cause of death (*P*=0.006, AOR: 2.38, 95% CI: 1.28, 4.44) (see Table 5).

Infant mortality from birth asphyxia shows a statistical significant relationship, but less likely to occur, with male sex (*P*=0.039, AOR: 0.52, 95% CI: 0.28, 0.97) and being died out of heath facilities (*P*=0.002, AOR: 0.32, 95% CI: 0.16, 0.66), as compared to mortality from all other causes. Infant died out of health facilities was the only associated factor with prematurity death, but lower odds (*P*=0.001, AOR: 0.24, 95% CI: 0.10, 0.57) (see Table 5).

Infant who died from malaria is more closely associated with age of mothers between 20-35 years old and infant who resided in districts of Afar region. Infant with age of mothers between 20-35 years old were 4 times (*P*=0.024, AOR: 4.44, 95% CI: 1.22, 16.2) higher to die of malaria than malaria-unrelated death. Similarly, infant who resided in districts of Afar region were 4 times higher to die of Malaria than non-malaria death (*P*=0.013, AOR: 4.08, 95% CI: 1.35, 12.4). Significant association is observed on the risk of death from malaria in wet season, but the likelihood of dying resulted less (*P*=0.024, AOR: 0.22, 95% CI: 0.06, 0.82) (see Table 5).

Table 5: Multivariable logistic regression analysis of factors associated with cause-specific mortality against all other cause of infant death in Eastern Ethiopia, 2016-18

Factors	Adjusted odds Ratio (95% CI)				
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Maternal Age					
<20 Years old	4.82 (1.88, 12.3)*	0.14 (0.02, 1.11)	-	-	1.43 (0.17, 11.8)
20-34 Years old	1.03 (0.43, 2.50)	1.14 (0.36, 3.64)	-	-	4.44 (1.22, 16.2)*
≥35years old	1	1	-	-	1
Marital status					
Unmarried	0.46 (0.22,0.97)*	0.85 (0.33, 2.18)	6.92(0.91, 52.4)	-	-
Married	1	1	1	-	-
Mother's education					
No education	-	-	0.59(0.19, 1.84)	0.24(0.03, 1.85)	-
Educated	-	-	1	1	-
Occupational status					
Housewife	-	-	0.39(0.11, 1.37)	-	-
Others	-	-	1	-	-
Infant Age					
Post-neonate	1.40(0.90, 2.18)	15.5(6.35, 37.8)*	-	-	-
Neonate	1	1	-	-	-
Sex of Infant					
Male	-	1.21(0.65, 2.27)	0.50(0.27, 0.92)*	-	-
Female	-	1	1	-	-
Seasons of death					
Wet seasons	-	2.38(1.28, 4.44)*	-	-	0.22 (0.06, 0.82)*
Dry seasons	-	1	-	-	1
Place of death					
Outside of health facilities	2.85 (1.33,6.12)*	1.49 (0.46, 4.77)	0.34(0.17, 0.67)	0.24(0.10, 0.57)*	-
Health facilities	1	1	1	1	-
Administration Division					
Districts in Afar region	-	-	-	-	4.08 (1.35, 12.4)*
Districts in Oromia region	-	-	-	-	1

- *Factors that hadn't p-value of <0.2 from bivariate analysis & not eligible in multivariable*

*Model 1: Association of factors with deaths due to **ARTI** versus all other causes of death*
*Model 2: Association of factors with deaths due to **diarrhea** versus other cause of death*
*Model 3: Association of factors with deaths due to **birth asphyxia** versus other cause*
*Model 4: Association of factors with deaths due to **prematurity** versus other cause of death*
*Model 5: Association of factors with deaths due to **malaria** versus other cause of death*
**statistically significant at p<0.05*

4.2 The risk of Water, Sanitation and Hygiene on Diarrhea-related infant mortality

A total of 305 study subjects (61 cases and 244 controls) were included in the study, which yields the non-response rate 8.9% for both cases and controls. Among these studied subjects, 61 were infants who died as the result of diarrheal disease (cases), and 244 were those who survived their first year of life (controls) ,which was nested in a longitudinal survey database (from September 2016 to August 2018) residing in Eastern Ethiopia.

4.2.1 Socio-demographic characteristics associated with infant diarrheal death

Of the interviewed infants' mothers/caretakers, the mean age (± SD) of the respondent among cases (infant who died due to diarrhea) was 26.8 (±3.9) years old and 27.8 (±4.5) for controls (infant who survived their first year of life). The majority of the infants' mothers of the cases fall within the youth age group of 20-34 (90.2%), while it was (82.0%) for the controls. About 44.3% of cases and 48.4% of controls had history of having borne two to four viable offspring (Parity). The mean (± SD) family size of households with infants in the cases was 4.70 (±1.98) and 4.99 (±1.96) for controls. Majority of the study participants were married at 96.7% of cases and 98.0% of controls. Oromo ethnic group comprises the largest proportion of the study subjects (90.2% of cases and 87.3% of controls). Muslim followers were larger in the study participants at 98.4% for cases and 88.5% for the control group. The majority of the respondents (88.5% of cases and 74.2% of controls) were not educated. Likewise, most of the spouse of the cases (78.7%) was uneducated as compared with the controls (65.2%). Almost equal proportion of the cases (90.2%) and the controls (90.6%) were housewives by occupation. Spouse's occupational status between the two-study groups indicated that about (90.2%) cases and (85.7%) controls were found to be farmers/own farm labor. High proportion of controls (50.8%) compared with that of cases (45.9%) had an average household monthly income of more than and equal to 570 ETB.

The bivariate analysis between socio-demographic characteristics and diarrhea-associated infant death indicated that religion as well as mothers and spouse's level of education were significantly associated with infant's diarrheal death. In this analysis, infant death from diarrhea was found to be higher among Muslim mothers/caretakers than Christian (*P=0.046,* COR: 7.78, 95% CI:1.03, 58.3). There were greater odds of infant death from diarrhea in infants whose mothers/caretakers were uneducated than in infants whose mothers/caretakers had completed some education. (*P*=0.021, COR: 2.69, 95% CI: 1.16, 6.21). The odds of uneducated spouse was also higher among death of infant from diarrhea as compaired to those of spouse who had attained a certain level of education (*P*=0.046, COR: 1.97, 95% CI: 1.01, 3.85) (see Table 6).

Table 6: Frequency distribution and bivariate analysis of socio-demographic characteristics with diarrheal cases and controls in Eastern Ethiopia, 2016-18

Socio-demographic Characteristics	Case		Control		Crude Odds Ratio (95% CI)	P-value
	n	%	n	%		
Age of the mother						
<20 Years old	5	8.2	18	7.3	7.22 (0.77, 67.1)	0.082
20-34 Years old	55	90.2	200	82.0	7.15 (0.95, 53.9)	0.056
≥35years old	1	1.6	26	10.7	1	-
Parity						
1st	22	36.0	72	29.5	1.38 (0.63, 3.02)	0.428
2nd-4th	27	44.3	118	48.4	1.03 (0.49, 2.19)	0.939
≥5	12	19.7	54	22.1	1	-
Household Family Size						
≥5	18	29.5	91	37.3	0.70 (0.38, 1.29)	0.258
<5	43	70.5	153	62.7	1	-
Maternal Marital Status						
Married	59	96.7	239	98.0	0.62 (0.12, 3.26)	0.570
Unmarried	2	3.3	5	2.0	1	-
Ethnicity						
Oromo	55	90.2	213	87.3	1.33 (0.53, 3.36)	0.540
Others	6	9.8	31	12.7	1	-
Religion						
Muslim	60	98.4	216	88.5	7.78 (1.03, 58.3)*	0.046
Christian	1	1.6	28	11.5	1	-
Mother's level of education						
No education	54	88.5	181	74.2	2.69 (1.16, 6.21)*	0.021
Educated	7	11.5	63	25.8	1	-
Spouse's level of education						
No education	48	78.7	159	65.2	1.97 (1.01, 3.85)*	0.046
Educated	13	21.3	85	34.8	1	-
Mother's Occupation						
Housewife	55	90.2	221	90.6	0.95 (0.37, 2.46)	0.922
Others	6	9.8	23	9.4	1	-
Spouse's Occupation						
Farmer/own farm labor	55	90.2	209	85.7	1.54 (0.61, 3.84)	0.359
Others	6	9.8	35	14.3	1	-
Households Average Monthly Income (ETB)						
<570ETB	33	54.1	120	49.2	1.22 (0.69, 2.14)	0.492
≥570ETB	28	45.9	124	50.8	1	-

**Risk factors significantly associated at p-value≤0.05*

4.2.2 Environmental Variables (Water Supply, Sanitation and Hygiene) Associated with Risk of Infant death due to Diarrhea

The distribution of cases and controls as well as bivariate analysis in the different categories of Water supply, Sanitation and Hygiene presented as follows:

4.2.2.1 Risk of access and use of water supply associated with infant's diarrheal death

Almost equal proportion of case (78.7%) and control (78.3%) group of infant's households were used improved water sources. The household's time to access water source resulted with 30 minutes or less (65.6% of case and 62.7% of control). About 85.2% of cases and 82.0% controls fetched water within 1km radius from their dwelling. Most of the infants in the households with less than 25 liters water consumption per capita per day among case and control group appears to be 88.5% and 75.4%, respectively. The vast majority of cases (91.8%) and controls (79.1%) among infants in the households found with water inaccessibility (i.e households not access to at least 25 liters per capita per day within a distance up to 1km radius). High proportion of cases 65.6% compared with that of control (40.2%) found to have unsafe drinking water storage. Majority of controls (65.2%) compared with case (52.5%) reported to know at least one and more households point-of-use drinking water treatment methods. However, about 80.3% of the cases and 61.5% of controls' group have ever practiced water treatment at household's point-of-use.

In the bivariate analysis, the exposure variables that showed significant association with diarrheal-related infant death were households Water Consumption Per Capita per day with less than 25 l/c/day (P=0.031, COR: 2.52, 95% CI: 1.09, 5.82), households water inaccessibility (P=0.028, COR: 2.96, 95% CI: 1.13, 7.77), households with unsafe drinking water storage (P<0.001, COR: 2.84, 95% CI: 1.58, 5.10), households reported as did not practices any water treatment at point-of-use (P=0.007, COR: 2.56, 95% CI: 1.29, 5.06) as compared to the reference group (see Table 7).

Table 7: Frequency distribution and bivariate analysis of drinking water access and use with diarrheal cases and controls in Eastern Ethiopia, 2016-18

Water Supply Characteristics	Case		Control		Crude Odds Ratio (95% CI)	P-value
	n	%	n	%		
Households Water Source						
Unimproved	13	21.3	53	21.7	0.98 (0.49, 1.94)	0.945
Improved	48	78.7	191	78.3	1	-
Time to access water						
Above 30 minutes	21	34.4	91	37.3	0.88 (0.49, 1.59)	0.678
30 minutes or less	40	65.6	153	62.7	1	-
Distance to access water						
Above 1km radius	9	14.8	44	18.0	0.79 (0.36, 1.72)	0.546
Within 1km radius	52	85.2	200	82.0	1	-
Water quantity (Water Consumption Per Capita per day)						
Less than 25 l/c/day	54	88.5	184	75.4	2.52 (1.09, 5.82)*	0.031
25 l/c/day and above	7	11.5	60	24.6	1	-
Households Water Accessibility¥						
Not accessible	56	91.8	193	79.1	2.96 (1.13, 7.77)*	0.028
Accessible	5	8.2	51	20.9	1	-
Drinking Water Storage						
Unsafe	40	65.6	98	40.2	2.84(1.58, 5.10)**	0.000
Safe	21	34.4	146	59.8	1	-
Household Point-of-use water treatment knowledge						
Do not know at all	29	47.5	85	34.8	1.69 (0.96, 2.99)	0.068
Knows at least 1 and more methods	32	52.5	159	65.2	1	-
Household Point-of-use water treatment Practices						
Do not treat	49	80.3	150	61.5	2.56 (1.29, 5.06)*	0.007
Treat Water	12	19.7	94	38.5	1	-

**Risk factors significantly associated at p-value≤0.05*
***Risk factors significantly associated at p-value<0.001*
¥ Water Accessible: HHs access to at least 25 liters per capita per day within 1km from improved water sources

4.2.2.2 Risk of sanitation associated with infant's diarrheal death

About 65.6% of the cases and 76.2% of controls infants in the households had their own latrine. Nearly a similar proportion of households in cases and controls have practiced open defecation (21.3% for cases and 20.1% for controls). The household's latrine utilization appears to be 60.7% in cases and 71.3% in the control group. Less than half of the study subject (39.3%) in cases and about 49.6% in controls have found with cleaned latrines. Hand washing facilituies near to latrine comprises the less proportion in case (18%) than in the control (50%). The majority of cases (83.6%) compared with controls (66.4%) in the households found with unimproved sanitation status. Unsafe disposes of child feces in the households appear in large in cases (70.5%) than in control (39.8%). About 54.1% of cases and 79.9% of controls in the households dispose solid wastes in improper way. Majority of cases (82.0%) as compared with controls (57.4%) found with unsafe disposal of liquid wastes by the households.

Comparison of variables that were statistically significant with infant death due to diarrhea in crude analysis includes households with unimproved sanitation (*P=0.011*, COR: 2.58, 95% CI: 1.25, 5.35), households with unsafe disposing of child feces (*P<0.001*, COR: 3.62, 95% CI: 1.97, 6.64), households with improper management of solid waste (*P<0.001*, COR: 4.69, 95% CI: 2.59, 8.49), infants in the households who disposed liquid waste unsafely (*P=0.001*, COR: 3.38, 95% CI: 1.68, 6.80), unavailability of hand washing facility near to latrine (*P<0.001*, COR: 5.46, 95% CI: 2.57, 11.6) as compared to their reference group (see Table 8).

Table 8: Frequency distribution and bivariate analysis of sanitation with diarrheal cases and controls in Eastern Ethiopia, 2016-18

Sanitation Characteristics	Case		Control		Crude Odds Ratio (95% CI)	P-value
	n	%	n	%		
Latrine Ownership						
No latrine	22	36.1	67	27.5	1.49 (0.82, 2.69)	0.188
Have Latrine	39	63.9	177	72.5	1	-
Open Defecation Practices						
Yes	13	21.3	49	20.1	1.08 (0.54, 2.15)	0.831
No	48	78.7	195	79.9	1	-
Household Latrine Utilization						
No	24	39.3	70	28.7	1.61 (0.89, 2.89)	0.109
Yes	37	60.7	174	71.3	1	-
Household Latrine Cleanness						
No	18	29.5	64	26.2	1.42 (0.72, 2.81)	0.316
Yes	24	39.3	121	49.6	1	-
Not applicable	19	31.2	59	24.2		
Hand washing Facility near to latrine						
No	31	50.8	63	25.8	5.46 (2.57, 11.6)**	0.000
Yes	11	18.0	122	50.0	1	-
Not applicable	19	31.2	59	24.2		
Sanitation Status						
Unimproved sanitation	51	83.6	162	66.4	2.58 (1.25, 5.35)*	0.011
Improved sanitation	10	16.4	82	33.6	1	-
Households Disposing of Child feces						
Unsafe	43	70.5	97	39.8	3.62 (1.97, 6.64)**	0.000
Safe	18	29.5	147	60.2	1	-
Solid waste Management						
Improper management	33	54.1	49	20.1	4.69 (2.59, 8.49)**	0.000
Proper management	28	45.9	195	79.9	1	-
Liquid waste Management						
Improper management	50	82.0	140	57.4	3.38 (1.68, 6.80)*	0.001
Proper management	11	18.0	104	42.6	1	-

**Risk factors significantly associated at p-value≤0.05*
***Risk factors significantly associated at p-value<0.001*

4.2.2.3 Risk of hygiene associated with infant's diarrheal death

The result showed that about 29.5% in case group and 9.8% in controls of the respondents reported as did not washing their hands at any critical time. Handwashing practices were scored less than three critical time of hand washing, which shows almost similar proportion in both comparative groups (59.0% for cases and 59.5% for controls). The majority of cases group (41.0%) reported as not used any agnets during handwashing as compared with controls (26.6%).

In the bivariate analysis, infants whose mothers/caretakers in the households did not practice hand washing in any critical time at all shows higher odds than households practiced three and more critical time of hand washing (*P<0.001*, COR: 8.04, 95% CI: 2.99, 21.6). Infants in the households practiced less than three critical time of hand washing was two times more likely to dying from diarrhea than households practiced three and more critical time of hand washing (*P=0.002*, COR: 2.66, 95% CI: 1.13, 6.26). The occurrence of diarrhea death among infant's whose mothers/ caretakers did not use any agents (water with soap or ash/abrasives) during hand washing had higher odds than those who used agents (*P=0.030*, COR: 1.91, 95% CI: 1.07, 3.43) (see Table 9).

Table 9: Frequency distribution and bivariate analysis of hygiene characteristics with diarrheal cases and controls in Eastern Ethiopia, 2016-18

Hygiene Characteristics	Case		Control		Crude Odds Ratio (95% CI)	P-value
	n	%	n	%		
Critical Time of Hand washing Practices						
Do not practiced hand washing in any critical time	18	29.5	24	9.8	8.04 (2.99, 21.6)**	0.000
Practiced less than three critical time of Hand washing	36	59.0	145	59.5	2.66 (1.13, 6.26)*	0.002
Practiced 3 and more critical time of Hand Washing	7	11.5	75	30.7	1	-
Agents used during Hand washing						
Not used any agents	25	41.0	65	26.6	1.91 (1.07, 3.43)*	0.030
Used (water + soap or ash/abrasives)	36	59.0	179	73.4	1	-

**Risk factors significantly associated at p-value≤0.05*
***Risk factors significantly associated at p-value<0.001*

4.2.3 Multivariable's Logistic Regression Analysis

In the multivariable logistic regression, variables that were significantly associated with infant diarrheal death identified includes age of mother with <20 years old, unsafe drinking water storage, infants in households without point-of-use water treatment practices, households with unimproved sanitation status, households with unsafe disposing of child feces, households with improper management of solid and liquid waste, households with no and lesser as well as not hand washing practices at critical time.

In this analysis, infants whose age of mother being lower than 20 years old had significant relationship with higher odds to occur infant death due to diarrhea as compared to those reference group of age ≥35 years (*P=0.010*, AOR: 21.7, 95% CI: 2.10, 224.7). However, mother's religious status, mothers and spouse's level of education did not show statistically significant association with infant's diarrheal death, particularly after adjustment.

Infants in households with unsafe drinking water storage and households treating their drinking water at point-of-use showed significant association with infant diarrheal death. The households exposed to unsafe drinking water storage were 2.59 times more likely to be occurred infant death from diarrhoea as compared to that of safe drinking water storage (*P=0.014*, AOR: 2.59, 95% CI: 1.22, 5.56). Those infant with households who did not treat their drinking water at point-of-use were 4.73 times more likely to having infant death from diarrheal than those treated their drinking water (*P=0.004*, AOR: 4.73, 95% CI: 1.66, 13.5).

The occurrence of diarrheal death among infants in households with unimproved sanitation status had higher odds than households with those households with improved sanitation (P=0.050, AOR: 2.74, 95% CI: 0.99, 7.58). Compared to households with the safe disposing of child faeces, those disposing unsafely were found with an increased odds of infant death due to diarrhoea (P=0.015, AOR: 2.88, 95% CI: 1.23, 6.75). Infant diarrheal death were higher odds to happen in the households disposing solid wastes improper than those properly managed (P=0.003, AOR: 3.33, 95% CI: 1.50, 7.07). Households with improper management of liquid waste management found with three times more likely to occur diarrhea-associated infant death as compared to those with proper liquid waste management (P=0.011, AOR: 3.38, 95% CI: 1.32, 8.66).

Infants whose mother/caretaker did not practiced hand washing at any critical times was four times greater to be dying from diarrhea than those who had practice more than three critical times of hand washing (P=0.015, AOR: 4.71, 95% CI: 1.34, 16.5). The odds of occurring infant death from diarrhea was also higher among study participants who had practice hand washing in less critical times (lesser than three times) than those who practices three and more critical times (P=0.029, AOR: 2.99, 95% CI: 1.12, 8.04) (see Table 10).

Table 10: Bivariate and multivariable logistic regression for the risk factors associated with diarrhoea- related cases and controls in Eastern Ethiopia, 2016-18

Variables	Case		Control		Odds Ratio (95% CI)	
	n	%	n	%	Crude	Adjusted
Age of the mother						
<20 Years old	5	8.2	18	7.3	7.22 (0.77, 67.1)	21.7 (2.10, 224.7)*
20-34 Years old	55	90.2	200	82.0	7.15 (0.95, 53.9)	1.59 (0.37, 6.77)
≥35years old	1	1.6	26	10.7	1	1
Religion						
Muslim	60	98.4	216	88.5	7.78 (1.03, 58.3)*	1.57 (0.18, 13.9)
Christian	1	1.6	28	11.5	1	1
Mother's level of education						
No education	54	88.5	181	74.2	2.69 (1.16, 6.21)*	1.92 (0.45, 8.19)
Educated at some level	7	11.5	63	25.8	1	1
Spouse's level of education						
No education	48	78.7	159	65.2	1.97 (1.01, 3.85)*	1.39 (0.41, 4.65)
Educated at Some level of schooling	13	21.3	85	34.8	1	1
Water quantity (Water Consumption Per Capita per day)						
Less than 25 l/c/day	54	88.5	184	75.4	2.52 (1.09, 5.82)*	4.79 (0.59, 38.7)
25 l/c/day and above	7	11.5	60	24.6	1	1
Households Water Accessibility						
Not accessible	56	91.8	193	79.1	2.96 (1.13, 7.77)*	0.95 (0.09, 9.66)
Accessible	5	8.2	51	20.9	1	1
Drinking Water Storage						
Unsafe	40	65.6	98	40.2	2.84(1.58, 5.10)**	2.59 (1.22, 5.56)*
Safe	21	34.4	146	59.8	1	1
Household Point-of-use water treatment knowledge						
Do not know at all	29	47.5	85	34.8	1.69 (0.96, 2.99)	1.14 (0.44, 2.93)
Knows at least 1 and more methods	32	52.5	159	65.2	1	1
Household Point-of-use water treatment Practices						
Do not treat	49	80.3	150	61.5	2.56 (1.29, 5.06)*	4.73 (1.66, 13.5)*
Treat Water	12	19.7	94	38.5	1	1

Table 10 (Continued)

Variables	Case		Control		Odds Ratio (95% CI)	
	n	%	n	%	Crude	Adjusted
Latrine Ownership						
No latrine	21	34.4	58	23.8	1.49 (0.82, 2.69)	0.68 (0.09, 5.37)
Have Latrine	40	65.6	186	76.2	1	1
Household Latrine Utilization						
No	24	39.3	70	28.7	1.61 (0.89, 2.89)	3.87 (0.59, 25.5)
Yes	37	60.7	174	71.3	1	1
Hand washing Facility near to latrine						
No	31	50.8	63	25.8	5.46 (2.57, 11.6)**	4.59 (1.69, 12.5)
Yes	11	18.0	122	50.0	1	1
Sanitation Status						
Unimproved sanitation	51	83.6	162	66.4	2.58 (1.25, 5.35)*	2.74 (0.99, 7.58)*
Improved sanitation	10	16.4	82	33.6	1	1
Households Disposing of Child feces						
Unsafe	43	70.5	97	39.8	3.62 (1.97, 6.64)**	2.88 (1.23, 6.75)*
Safe	18	29.5	147	60.2	1	1
Solid Waste Management						
Improper management	33	54.1	195	79.9	4.69 (2.59, 8.49)**	3.33 (1.50, 7.07)*
Proper management	28	45.9	49	20.1	1	1
Liquid Waste Management						
Improper management	50	82.0	140	57.4	3.38 (1.68, 6.80)*	3.38 (1.32, 8.66)*
Proper management	11	18.0	104	42.6	1	1
Critical Time of Hand washing Practices						
Do not practiced in any critical time	18	29.5	24	9.8	8.04 (2.99, 21.6)**	4.71 (1.34, 16.5)*
Practiced less than three critical times	36	59.0	145	59.5	2.66 (1.13, 6.26)*	2.99 (1.12, 8.04)*
Practiced in 3 and more critical time	7	11.5	75	30.7	1	1
Agents used during Hand washing						
Not used any agents	51	83.6	78	32.0	1.91 (1.07, 3.43)*	1.92 (0.86, 4.27)
Used (water + soap or ash/abrasives)	10	16.4	166	68.0	1	1

**Risk factors significantly associated at p-value≤0.05*
***Risk factors significantly associated at p-value<0.001*

4.3 Molecular detection of Pathogens “Causing most of infants’ Severe and fatal diarrhea” in infant drinking water and their relationship with water quality determinants

4.3.1 General Characteristics of Study Population and Households

This study included 410 infant-containing households, resulting in a 97.0% response rate. The reasons for the non-response were a refusal to engage in the interview and the disposal of some water samples. The study subjects' mean age (± SD) was 8.35 (± 2.59) months. The majority of the study population fell within the age group of 6–12 months at 73.4%. The sex composition consisted of 217 (52.9%) males and 193 (47.1%) females, making the overall sex ratio of 1.1:1.

Among the 410 households with infants surveyed, the majority (37.3%) were getting their drinking water from a piped public tap/standpipe. This was followed by unprotected springs, used by 28.8% of the households; protected springs (12.0%); and piped water in their own yard or premises (10.2%). Almost one-third of the households (32.7%) have no access to an improved source of drinking water. Nearly all (99.8%) of the households did not treat drinking water before they provided it to the infant. The majority of the households (94.9%) were observed to be storing their drinking water in an unsafe manner. About 71.7% of the households had fetched water at the present time prior to the survey. Most of the households (83.7%) collected their water from water points in public spaces. Almost all (99.8%) of the household water samples tested had residual chlorine that was not within the standard required level (between 0.2 and 0.5 mg/l) (see Table 3).

Table 11: Demographic and drinking water characteristics in the study population of Eastern Ethiopia, June 2010- May 2021

Characteristics	Frequency(n)	Percentage (%)
Age of Infant		
<6months	109	26.6
6-12 months old	301	73.4
Sex of Infant		
Male	217	52.9
Female	193	47.1
Primary water Source type		
Piped water into dwelling	4	1.0
Piped water into yard or plot	42	10.2
Piped water, public tap/standpipe	153	37.3
Piped water kiosk or retailer	7	1.7
Protected dug well	8	2.0
Protected spring	49	12.0
Bottled water	13	3.2
Unprotected dug well	1	0.2
Unprotected spring	118	28.8
River water	2	0.5
Irrigation canal	13	3.2
Water Source Improvement status		
Improved water source	276	67.3
Unimproved water source	134	32.7
Point-of-use water treatment Practices		
Do not treat	409	99.8
Treat Water	1	0.2
Drinking Water Storage		
Unsafe	389	94.9
Safe	21	5.1
When water is fetched?		
Today	294	71.7
Yesterday	87	21.2
Days ago	29	7.1
Where the water point is found?		
In the dwelling	17	4.1
Private yard/plot	37	9.0
Neighbor's yard/shared compound	13	3.2
Public Space	343	83.7
Residual chlorine		
Not between 0.2 and 0.5mg/l	409	99.8
Between 0.2 and 0.5mg/l	1	0.2

4.3.2 LAMP Results in Infants Point-of-use Water Sample

Of the total 410 water samples tested using the LAMP technique, 28.5% [95%, CI, 24.2-32.9] were found to be positive for *Cryptosporidium* oocysts, about 30.0% [95%, CI, 25.6-34.4] were positive for *Shigella* species, 26.3% [95% CI, 22.1-30.6] were positive for toxin-producing strains of *E.coli*, and 32.2% [95% CI, 27.7- 36.7] were positive for rotavirus. In total, 13% [95% CI, 9.9-16.4] of the water samples were positive for all four pathogens mentioned above, which were detected in the same water sample (see Figure 12).

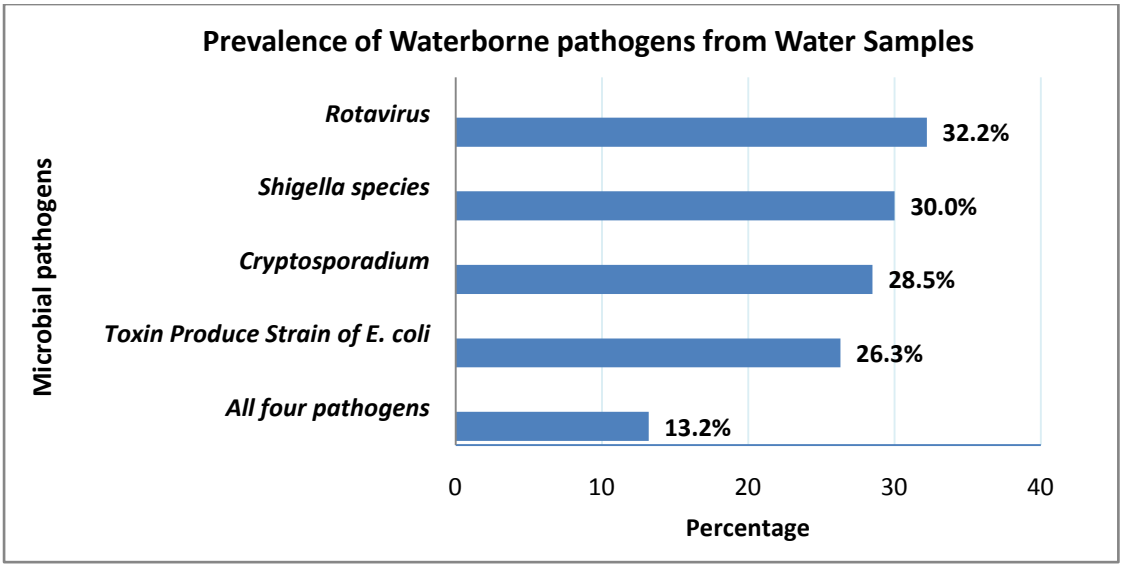


Figure 12: Prevalence of waterborne pathogens from infant drinking water sample at household

4.3.3 LAMP Results in Water Sources

The study included a total of 37 water samples that were collected from various types of water sources used by the households for infant ingestion in various geographic locations. These include a public tap/standpipe designated from borehole 16 (43.2%), an unprotected spring at 11 (29.7%), a protected spring at 5 (13.5%), a reservoir designated from boreholes 2 (5.4%) and 1 (2.7%) for each protected dug well, an irrigation canal, and a river. The positive and negative results of pathogens in each source with their location are presented in table 12. About 35.1% of the water samples were observed to be drawn from unimproved water sources. The majority of the reported water sources (83.8%) did not undergo routine cleaning. The majorities (97.3%) of the water sources were untreated on a regular basis, and 73% lacked catchment or fencing.

Out of the 37 water source samples tested, the result showed that 10 (27.0%), 12 (32.4%), 11 (29.7%), and 14 (37.8%) were positive for *Cryptosporidium* oocysts, *Shigella* species, toxin-producing strains of *E. coli*, and rotavirus, respectively. In total, 13.5% of the water samples tested from water sources was positive for all four pathogens (See Figure 13).

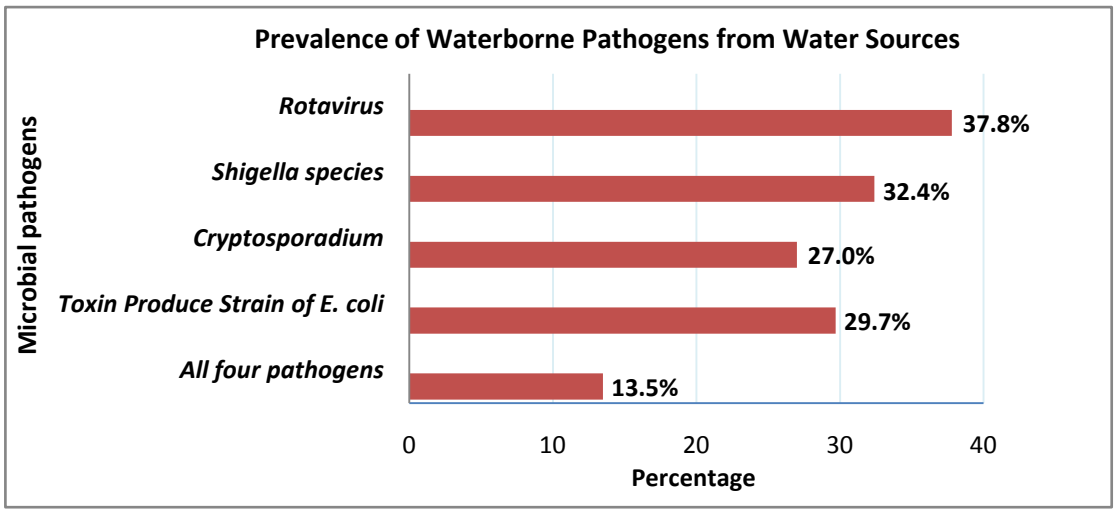


Figure 13: Prevalence of waterborne pathogens from water sources

Table 12: Pathogens positive and negative results in each source with locations

S.N	Source	Date	Location		Type of Pathogens			
			Latitude (N)	Longitude (E)	*Cry*	*Shigella*	Toxin Produce *E.Coli*	Rotavirus
1	Public tap designated from borehole	6/10/2020	05279	4056270	-	-	-	-
2	Public tap designated from borehole	6/10/2020	06233	04058529	-	-	-	-
3	Public tap designated from borehole	6/11/2020	02718	04054693	-	-	-	-
4	Public tap designated from borehole	6/11/2020	03513	04054801	-	-	-	-
5	Public tap designated from borehole	6/12/2020	08185	04049375	-	-	-	-
6	Protected spring	6/13/2020	08185	04049375	-	-	-	-
7	Protected spring	6/13/2020	05963	04053957	-	-	-	-
8	Unprotected spring	6/14/2020	05268	04047664	-	-	+	-
9	Unprotected spring	6/14/2020	06251	04048688	-	-	-	+
10	Reservoir designated from borehole	7/24/2020	0629208	1030652	-	-	-	+
11	River	7/26/2020	0629030	1042074	-	+	-	-
12	Public tap designated from borehole	7/27/2020	0635854	1045299	+	+	+	+
13	Public tap designated from borehole	3/2/2021	0690282	1020122	-	-	-	-
14	Unprotected spring	3/3/2021	0692900	1015471	-	-	-	-
15	Unprotected spring	3/4/2021	0707247	1021605	-	-	-	-
16	Public tap designated from borehole	3/5/2021	0702962	1022129	-	-	-	+
17	Unprotected spring	3/6/2021	0699855	1018567	-	-	-	-
18	Unprotected spring	3/7/2021	0691712	1012443	-	-	-	+
19	Unprotected spring	3/8/2021	0701043	1016519	+	+	+	+
20	Public tap designated from borehole	3/9/2021	0687259	1013652	+	+	+	+
21	Public tap designated from borehole	3/10/2021	0680964	1018487	+	+	-	-
22	Public tap designated from borehole	3/11/2021	0682593	1011179	+	+	+	+
23	Public tap designated from borehole	3/12/2021	0683120	1010054	-	-	-	+
24	Unprotected spring	4/2/2021	-	-	-	-	-	-
25	Unprotected spring	4/3/2021	-	-	+	-	-	+
26	Unprotected spring	4/16/2021	-	-	-	-	-	+
27	Unprotected spring	4/17/2021	-	-	-	+	-	-
28	Public tap designated from borehole	4/18/2021	-	-	+	+	+	-
29	Protected spring	4/19/2021	-	-	-	-	-	-
30	Public tap designated from borehole	4/20/2021	-	-	+	+	+	-
31	Protected spring	4/21/2021	-	-	+	+	+	-
32	Protected spring	4/22/2021	-	-	-	-	+	-
33	Irrigation canal	5/3/2021	-	-	-	-	-	-
34	Reservoir designated from borehole	5/4/2021	-	-	+	+	+	+
35	Protected hand pump	5/5/2021	-	-	-	-	-	+
36	Public tap designated from borehole	5/6/2021	-	-	-	+	+	+
37	Public tap designated from borehole	5/7/2021	-	-	-	-	-	-

4.3.4 Correlation of pathogens presence between water source and point-of-use sample

The phi coefficient correlation showed a significant positive relationship between the primary water source and infant drinking water at the point-of-use in the household for the presence of targeted pathogens. The presence of Cryptosporidium in water source samples and drinking water at the point-of-use, thus, showed a statistically significant and moderately positive correlation (Phi= 0.527; p=0.000). The presence of *Shigella* species showed a strong positive correlation between the water source and the point-of-use (Phi= 0.524; p=0.000). The presence of a toxin producing strain of *E. coli* also significantly correlated between water source samples and point of use at the household level (Phi= 0.424; p=0.000). Between the water source and the point-of-use drinking water at the household level, rotavirus exhibits a significant positive correlation, although one that is weak in magnitude (Phi= 0.113; p=0.023).

4.3.5 Multivariable's Logistic Regression Analysis

In binary logistic regression, bivariate analysis indicated that an unimproved water source and the length of time water was retained before use were significantly associated with the occurrence of *Cryptosporidium*, *Shigella***,** and toxin-producing strains of *E. coli*. Variables such as unsafe water storage, the retention time of water before use, and fetching water in public spaces showed a significant association with the presence of rotavirus. An unimproved water supply and the water retained before an infant drinks were significantly associated to the presence of overall pathogens in drinking water.

After adjustment, in the multivariable logistic regression, households with infants who used water from unimproved sources were significantly more likely to increase the presence of *Cryptosporidium* oocysts (p=0.000, AOR-Adjusted odds ratio: 4.02, 95% CI: 2.29, 7.04), *Shigella* species (p=0.000, AOR: 4.21, 95% CI: 2.43, 7.29) and toxin-producing strains of *E. coli* (p=0.004, AOR: 2.14, 95% CI: 1.27, 3.59). However, no statistically significant association was observed in households using an unimproved water source with the occurrence of rotavirus. The households water samples that tested positive for *Cryptosporidium* were significantly associated with those who fetched water days ago (p=0.001, AOR: 0.03, 95% CI: 0.00, 0.26) and one day ago (p=0.010, AOR: 0.51, 95% CI: 0.30, 0.85). Similarly, the presence of a toxin producing strain of *E. coli* showed a significant relationship with fetching water on the days ago (p=0.008, AOR: 0.18, 95% CI: 0.05, 0.64). The presence of rotavirus showed a significant association with those households that fetched water one day ago (p=0.002, AOR: 0.46, 95% CI: 0.28, 0.76) prior to the survey time. No significant relationship is observed between the presence of each targeted microbial pathogen in drinking water samples and the household's drinking water storage status as well as the household's fetching water places (see Table 12).

In the final model, the presence of the overall (four) pathogens among water samples showed a significant association with households using unimproved water sources, which were approximately four times (p=0.001, AOR: 3.96, 95% CI: 1.73, 9.07) higher than those households that used improved water sources. The water samples tested from the household's drinking water that were fetched one day ago were significantly associated with the presence of all targeted pathogens in infants drinking water (p=0.009, AOR: 0.44, 95% CI: 0.23, 0.81).

Table 13: Multivariable logistic regression analysis for the presence of targeted pathogens in water samples and water quality determinants in Eastern Ethiopia

Variables	Model 1 AOR (95% CI)	Model 2 AOR (95% CI)	Model 3 AOR (95% CI)	Model 4 AOR (95% CI)	Final Model AOR (95% CI)
Water Source					
Unimproved	4.02 (2.29, 7.04)*	4.21 (2.43, 7.29)*	2.14 (1.27, 3.59)*	-	3.96 (1.73, 9.07)*
Improved	1	1	1		1
Drinking Water Storage					
Unsafe	-	-	-	1.78 (0.68,4.68)	-
Safe				1	
When water is fetched					
Days ago	0.03 (0.00,0.26)*	-	0.18 (0.05,0.64)*	1.08 (0.46,2.53)	-
Yesterday					
Today	0.51 (0.30,0.85)*	0.66 (0.39,1.11)	0.65 (0.39,1.09)	0.46 (0.28,0.76)*	0.44 (0.23,0.81)*
	1	1	1	1	1
Where the water point is found?					
Public Space	-	-	-	1.07 (0.59,1.97)	-
In the dwelling/ yard				1	

- *Factors that hadn't p-value of <0.2 from bivariate analysis & not eligible in multivariable*

Model 1: Association of factors with the presence of Cryptosporidium oocyst in the water sample

Model 2: Association of factors with the presence of Shigella species in water sample

Model 3: Association of factors with the presence of toxin producing strain of E. coli in water sample

Model 4: Association of factors with the presence of rotavirus in water sample

Final Model: Association of factors with the presence of all targeted pathogens (Cryptosporidium, Shigella, toxin producing strain of E. coli and rotavirus) in the sample water sample

** Statistically significant at p<0.05*

CHAPTER FIVE: DISCUSSION

5.1 Cause of infant deaths and patterns of associated factors

The exact cause of death is ascertained by postmortem autopsy (213). However, this is not practically applicable in most developing countries and, as an alternative Verbal Autopsy has become one of the major sources of data for causes of death, which also used for identification of major health problems and comparison of local and national mortality ratio differences (85).Most of the published on cause of death using verbal autopsy data was interpreted by physician review, and this approach is time-consuming, costly and inconvenient (16,86). In recent years, the electronically determination of death cause has been introduced and provide an analysis solution that is more convenient, consistent, and rapid ways to interpret VA data (86). In our study context, InterVA-4 model is preferred to use for assigning cause of infant death as this model is relatively better than other available models (SmartVA/Tarrif, InSilicoVA), particularly for neonate and children death, when contrasting in terms of the degree of chance corrected concordance (CCC), CCVA (Computerized coding of verbal Autopsy) population Accuracy, PCVA (Physician-certified verbal autopsy) performance (214,215). In view of that, the present study was undertaken to determine the cause of death using InterVA-4 model and analyze the pattern of associated factors between the most leading cause-specific mortality against all-causes of death.

In this study, many different causes of infant death were observed, from infection to birth defects or accidents. The study result revealed that the proportion of death in the neonatal stage was higher in contrast to post-neonatal period. Similar view was seen in other studies (12,79,216,217). These findings clearly proven as neonatal period is the most vulnerable time for a child death (218).The most common causes of infant death in the neonatal period are different from those that occur in the post-neonatal. Analyses in the interpretation of specific causes of infant mortality indicated that neonatal pneumonia, birth asphyxia and prematurity were the three major causes of deaths during the neonatal period. Regardless of proportional differences, birth asphyxia and prematurity as a common appeared to be leading causes, which have similarity to those of studies conducted in some parts of Ethiopia (10,81–83), and other developing countries (12). Some studies described infections diseases (sepsis, pneumonia, meningitis, tetanus and diarrhea) are the leading cause (10,81,219), which had methodologically and/or application tools differences with this study. Evidences from several studies conducted in Ethiopia reported that prematurity, birth asphyxia, and neonatal sepsis were the leading causes for neonatal death (79,220). These results are consistent to the present study except neonatal sepsis as the proportion of deaths due to sepsis appeared to be less in the study area.

Neonatal Pneumonia is a devastating condition (221), and remained one of the most cause of death in Ethiopians children (222). It is a serious respiratory infectious disease in a neonate which can be prevented by simple measures such as treatment of maternal infections, careful obstetric care and general infection control measures in neonatal facilities. The higher risks of death from birth asphyxia might be due to lower institutional delivery coverage and mother's education is essential which might be expected in large reduction in some circumstances. The burden of prematurity seemed to be mostly due to caregivers failed to recognize the danger signs related to prematurity

and its consequences. It has been recognized that behaviors that encourage a healthy pregnancy plays an important role in preventing premature labour.

In the post-neonatal period, acute respiratory infection including pneumonia, diarrheal diseases and malaria were the most common causes of death. Despite means of verification and proportional differences, these results showed a similar pattern with the finding of other studies within nationwide (222). Acute respiratory infection including pneumonia and diarrheal diseases were mostly appeared as a common cause during post-neonate period, which showed in other inland studies (79,222), and somewhere else (12,78,216,217). The present study indicated as Malaria was the third major cause of death in this age category, which are quite different from deaths identified in other studies of the country as bacterial sepsis were observed in one of the top three causes of post-neonate death (79) as well as malnutrition (223). Similar view was observed in studies conducted in other countries (12,216,217). As per consolidation into a broad cause category, Infectious and parasitic disease causes were the leading causes of death during the post-neonatal period, which is in agreement with previous studies (79,222).

In overall infant death, acute respiratory infection including pneumonia, birth asphyxia and diarrhea disease were the most common caused in the present study. Apart from magnitude and rank order disparity, the findings of acute respiratory infection including pneumonia and diarrhea disease as a major probable cause of infant death has similarity with those of the results reported in other parts of the country (10,222–225), and elsewhere (12,226). It can be observed that Prematurity, Malaria, Meningitis and encephalitis, Sepsis and Congenital malformation were other condition resulting deaths of Eastern Ethiopia children, which are more or less the same with causes occurred in most parts of developing countries (78,227,228).

The higher risk of death due to acute respiratory infection in the current study might be due to less awareness of the disease transmission and prevention, weak case management in health services, less understanding on early recognition of pneumonia cases and inappropriate care-taking by the parents (88). Acute respiratory infection including pneumonia may exist due to lack of adequate through and cross ventilation system in the dwelling (229), overcrowding or suffocation in bedroom and weak maternal health, which have a potential favors to transmit the infection (230).

Diarrheal diseases were another most important cause of infant death as reflected by the facts that almost one-sixth of infants reported to have been dead. Infant and children are more likely to die due to lack of safe water and sanitation, along with poor hygiene practices, that result in deteriorative synergy that leads to diarrheal disease (231). Health seeking behavior such as poor early sough of health care, less measure to diarrhea disease management including oral rehydration therapy (ORT) for rehydration were responsible for diarrhea-related mortality (232). Other deaths due to severe malnutrition, Measles, Pulmonary tuberculosis and HIV/AIDS appeared to be tribulation of infant's survival on the basis of the present study result. However, deaths due to tetanus were not observed. This disease was remained as a noticeable death causes of Ethiopian children (8,222,225,233). The nonappearance of death due to tetanus is most likely associated with being controlled through tetanus toxoid (TT) routine immunization program. Likewise, the less

infant death from malnutrition attributed to high-impact nutrition interventions delivery through an integrated package by government and non-governmental organization.

The present study also compares the patterns of factors associated with the leading specific cause of infant death against all other cause of death. Our finding revealed that the patterns of association among each cause-specific mortality were quite different when evaluate with that of its comparison group (all-cause of death in combined). Infants with lower maternal age (<20 years old) were five-fold an increased risk of death from ARI, including pneumonia in contrast to infant with higher maternal age (≥35 years old). This finding suggested as lower maternal age group has largely contributed to multiplying ARI-specific infant mortality risk than all other causes of death. Studies revealed that children having adolescent mother was a strong risk factor for ARI-related death (88,89). This could be possibly due to the assumption that lower aged mothers tend to have lower experience in pneumonia-related health care of children and, therefore, greater susceptibility to severe forms of infections that could be drives to mortality. The risk of infant death due to ARI again rises with those infant who died out of heath facilities, which shows three-fold at an increase risk of ARTI, including pneumonia death than ARTI-unrelated infant death. This, in turn, indicated the contribution of death that occurred at home, which might probably due to lack or delay in seeking appropriate medical care utilization. This showed to be highly increases risk of dying from pneumonia than died from other causes (90).

Another important finding was that, the post-neonatal period is the most predominant factor in contributing to the greater elevated by nearly sixteen times of diarrheal death risk than the corresponding risk of all-causes of death. The strong influence of this age period on diarrhea-related death had been examined in other studies (91,92). Our study also revealed that wet seasons are strong significant risk factors by twice for diarrhea-related death than all other causes. The study conducted in India and Mexico where largely confirmed that deaths caused by diarrhea are strongly linked to seasons of death with more pronounced during cooler months (92,234). This might be due to the fact that diarrhea diseases had more potential to transmit in wet seasons, and increase its incidence that initiated the series of events leading to death.

Our study has also found that being male sex is significantly association with death from birth asphyxia; however, the risk of dying has been less likely as compared to other cause of death. This linkage could be attributed to behavioral, biological, socio-cultural, and genetic factors, which needs further study. We also observed that male is the most predominance in dying from birth asphyxia; similar finding was observed in various studies (93,94). Our analysis indeed showed significant association in place of death that occurred out of health facilities for birth asphyxia against other causes; though the likelihood of its effect is less. The study similarly demonstrated that place of death in out of health facilities are the only significant factor with lower chance for premature death as compared to all-cause mortality.

It has been also observed that infant mortality resulted from malaria significantly related to infant's mothers/care takers age group of 20-34 years with four times highest-risk chance in contrast to maternal age ≥35 years old, as evaluated from that of all-causes of death. A study on the link between early childhood mortality and malaria in Malawi reported that children were significantly

at greater risk if the mother was lower age relative to older age (95). The geographical administration division appears to be statistically significant to the risk of malaria-specific infant death. Infants resided in the study area of districts in Afar region were four times substantial raised risk of malaria deaths as compared to districts in Oromia region, as evaluated against all other causes. A study in Burkina Faso has demonstrated the significant variations in all-cause and malaria-specific mortality across village clusters (96). The differences by geographical administrative division might be due to variability in socio-demographic and economic factors, the prevalence of malaria incidence, weather condition, and variability of quality of health care service. However, further studies are needed to better understand for these disparities. Significant interaction was seen between wet seasons of infant death with the risk of malarial-specific death. However, this risk is less likely as observed in comparison to the all-cause of death. A study in West Africa indicated that malaria-specific infant death had significant seasonal trends and higher in the rainy seasons (97).

5.2 The risk of Water, Sanitation and Hygiene on Diarrhea-related infant mortality

The present study has attempted to look for possible contributing risk factors for diarrheal-related infant death, predominantly, on water supply, sanitation and hygiene. Although several risk factors were significantly associated with infant's diarrheal death in the bivariate analysis, some considerable factors that could predispose infants to death were identified after adjusting for confounders. These factors included age of mother with <20 years old, unsafe drinking water storage, infants in households without point-of-use water treatment practices, unimproved sanitation, unsafe disposing of child feces, households with improper management of solid and liquid waste and households practices hand washing not at all as well as lesser in critical time of hand washing.

In our study, infants whose mothers/caretakers age is lower than 20 years old had twenty one times higher for the occurance of infant's death from diarrhea as compared to the reference group (age ≥35 years). Regardless of the magnitude of odds, this finding is consistence with a case-control study conducted elsewhere, which reported that those infant's mother with lower maternal age were significantly associated with diarrhea-associated infant death, particularly among those with normal birth weight (235). On the other hand, the same study indicated that older maternal age led to a higher chance of diarrhea death among infants with low birth weight. A number of studies have also found that lower maternal age tend to have higher risk of mortality in children (236,237). This circumstance might be attributed to social and reproductive immaturity. The further likely explanation to this observation is that older mothers are more experienced in childcare and hence there is a possibility in reducing diarrhea-related incidence and death. The lower chance of diarrhea death in infant with younger mother in our study might be the influence of infant biological characteristics such as birth weight or other factors in this maternal age category, which needs further study.

Infants in households with unsafe drinking water storage died from diarrihea 2.6 times more often than those strored their water in safe way. This could be attributed to the unhygienic handling and storage of drinking water that existed in the cases group due to lack of proper and sufficient

information on water handling. A study in Benin found that safe water storage had significantly associated with the reduction of diarrhea (238), which in turn lower the risk of death. The national study highlighted that storage water quality issues are a great public health concern in rural Ethiopia (239). Our study also revealed that death of infant from diarrhea were significant association with households without point-of-use water treatment practices, about 4.7 times higher when contrasting with those practiced regular use of any form of water treatment. This tends to contrary with that of earlier study conducted in southern Brazil (49). This might be explained by the very small differences among the comparison groups in the household's water treatment practices. However, other studies suggested as strong significant impact of household water treatment on child survival (107,240,241). It is evident that point-of-use water treatment improves the quality of drinking-water by avoiding cross-contamination and prevent diarrheal disease (242,243), which could considerably reduce risk of mortality (241). It can be seen from the report that point-of-use water treatment is not widely practiced in Ethiopia (38), posing a health risk of children.

Access to unimproved sanitation has been found to be significantly associated with diarrhea-related infant death, which had 2.7 times higher than those households with improved sanitation facility. This finding has similarity with another study (104), which designated as improved sanitation significantly associated with lower mortality. Regardless of the association strength, our finding underpins the conclusion made by different studies (50,104,244), which indicated as improved sanitation significantly higher association with the reduction of infant mortality. Evidence from the risks quantification study indicated that those countries responsible for the largest declined (13.3%) in the diarrhea mortality rate were reduction in exposure to unsafe sanitation in children (98). Another study in Egypt also found sanitation to have a more pronounced impact on infants and childhood risk of death from all causes (245). It can be enlightening that; improved sanitation is fully saved, as it effectively prevents exposure to fecal matters which possibly decrease a major cause of child morbidity and mortality.

Our analyses have been also found that infant with households presented unsafe disposing of child feces were nearly three times higher to be died from diarrhea than those households disposing their child feces safely. This might be due to small differences among the two comparison groups on having information and practices of mother/caregivers on save disposal of child feces. Several studies have reported the significant effect of child feces disposal and childhood diarrhea morbidity in Ethiopia (246–248) and elsewhere (249), which could contribute to childhood mortality. Reports indicated that most of the households in high-mortality countries dispose of children's feces in unsafe manner (250). It is recognized that, children feces are more dangerous sources of fecal contamination in the household environment, and many cultures however consider the stools of infants are harmless (200,251). The findings further indicated that households with improper management of solid wastes were 3.3 times higher to be infant dying from diarrhea than proper management. A number of studies showed that children in households without proper waste collection practices suffer significantly higher rates of diarrhea (36,252,253) , which are among the main causes of childhood deaths (254). This is due to the fact that improper disposing of domestic solid waste could be one of the suitable sites for spread of pathogens that can leads to children's morbidity and followed by mortality.

This study provides notable evidence that improper management of household's liquid wastes had a positive influence with 3.4 greater odds in infant's death from diarrhea than proper liquid waste management. Reliance on scientific explanation, this might be attributed to the potential source of breading sites for flies which can carry enteric pathogens and mediate a route to contaminate children's water and food. This result agrees with the general sense that unsafe environment places puts children at risk of death (255). The strong significant differences observed in this study might be attribute to households were not well aware with the effect of domestic liquid waste disposal on health outcome of children.

Despite the fact of human hands is one of the main vehicles for transmitting diarrhea disease, the role of poor handwashing practices at critical time as a risk factor had strong relationship with infant death from diarrhea in the study area. Our study demonstrated that those self-reported household's not practiced handwashing in any critical times were 4.7 higher to infant death from diarrhea than those washed their hands at three and more critical times. Infant with households practicing hand washing in lesser occasions (One to two critical times') increases infant deaths from diarrhea almost 3-fold greater compared with those washed their hands at three and more critical times. Regardless of its magnitude, different studies indicated the significant link between handwashing practices and diarrhea morbidity and mortality (256–258). The handwashing practices at critical time mainly in mothers of children is poor according to inland study (259). Evidences indicated that handwashing at critical times reduce diarrhea rated by almost 40 percent (260), which could be resulted significant reduction in mortality.

5.3 Molecular detection of Pathogens "Causing most of infants' Sever and fatal diarrhea" in infant drinking water and their relationship with water quality determinants

The detection of *Cryptosporidium* oocysts, rotavirus, *Shigella* species and a toxin-producing strain of *Escherichia coli* has significant public health implications because world-wide these waterborne pathogens are most frequently associated with severe infantile diarrhea (57). Molecular analytical techniques are useful tools for evaluating the microbial quality of water (261). Among a wide range of molecular techniques, nucleic acid-based amplification methods comprised of PCR have been developed to detect microbial pathogenic species directly from drinking water samples (134). Subsequently, a technology termed loop-mediated isothermal amplification (LAMP) has been developed, which is highly sensitive and accurate for the replication of DNA in isothermal conditions (140). The present study was focused on the detection of the aforementioned pathogens in infants drinking water at the point-of-use in the household and the water sources from which they were collected using the LAMP technique.

Rotaviruses were detected in drinking-water supplies used for infants in about 32.2% of households, which is higher than previously reported for Shandong, China, at 16.7% (179), southeast France at 7.1% (180), Karachi, Pakistan, at 5% (181), and Colombia at 27.3% and 20.5% (182,183). The presence of rotavirus in the 37.8% of drinking water source samples observed in this study was much higher than previously reported results in Benin at 2.1% (184), Peshawar, Pakistan, at 9.47% (192), Karachi, Pakistan, at 23% (262), Beijing, China, at 20.3% (191), Costa

Rica at 8.1% (190), Faisalabad, Pakistan, at 26.6% (193), Southern Africa at 2.0% (185), and Egypt at 15.6%, 8.3%, and 23.3% (186–188), while lower than the study in Ghana (189), where 48.1% of water samples tested by multiplex RT-PCR were positive for rotaviruses. This disparity in the prevalence status might be explained by differences between geographic areas with various contributing factors such as socio-economic and cultural factors, access coverage of water, sanitation, and hygiene behavioral practices, and also by the use of different techniques for rotavirus detection having dissimilar sensitivity and specificity. Remarkably, the rotavirus prevalence in our study appeared to be relatively higher than the other detected microbial pathogens, both in water samples at the point-of consumption and from water sources. This might be attributed to the highest spreading phenomena of rotaviruses in the environment of the study area.

Rotavirus is excreted in enormous quantities in the feces of infected person, at a rate of up to 10^{11} virus particles per gram (180). Rotavirus is very resistant to diverse environmental conditions and physicochemical treatment processes that are able to stabilize the virus and make it present in large amounts in environmental water (179). Studies indicated that rotavirus survive well enough in chlorine-based conventionally treated drinking water to make it a possible vehicle for their transmission (263). In addition, rotavirus has high infectivity and an increased risk of transmission in comparison with protozoa and bacteria (264).

Furthermore, our study revealed no significant association between households using drinking water from an unimproved water source and the presence of rotavirus in a water sample, as observed with other detected protozoa (*Cryptosporidium*) and bacteria (*Shigella* and a toxin-producing strain of *E. coli*). Thus, it is reasonable to assume that household's access to an improved water source is not a guarantee that it is always safe (265). The only significant relationship was observed in retention time, implying household's fetching water one day prior to the survey has a protective effect for the presence of rotavirus in the water sample. This might be due to the influence of temperature and water movement, such as large, slow-moving, or stagnant sources, on rotavirus survival and transmission (266).

Our study also detected *Cryptosporidium* oocysts in 28.5% of the household's drinking water provided to infants. This may have implications for the poor drinking water quality status in the study area as the World Health Organization (WHO, 2009) categorizes *Cryptosporidium* as a reference pathogen for the assessment of drinking water quality (263). Regardless of the population group that consumed the water, the result of our study is much higher than previous findings of the studies conducted in Shanghai, China, at 0% (142) and Mongolia at 2.0% (143). In the corresponding water sources, about 27% of the samples were found positive for *Cryptosporidium* oocyst, which is higher than the prevalence study reported in Tigray at 5% (144), a 2008 study in Addis Ababa at 21% (145), and less than the study in Dire-Dawa at 58.9% (147), and the 2012 study in Addis Ababa found that all (100%) water samples were positive (146). Our result appeared to be lower than the studies conducted elsewhere, such as in Egypt at 52.6% (148) and Switzerland at 40% (149).

On the contrary, our result is higher than the study conducted in four countries in Southeast Asia, which came in at 24.4% (150). The authors who reviewed varying studies showed the prevalence of *Cryptosporidium* species in drinking water ranged from 1.4% to 100% (151). Numerous reasons can cause the observed differences. One of the reasons for this rate discrepancy might be attributed to the practices of animal grazing in the area, which may be contributing to the excretion of oocysts from infected hosts in the environment, which can have a chance of contaminating water sources. Another reason could be that the LAMP technique for the detection of pathogens might also influence the magnitude of the result due to its high sensitivity.

Cryptosporidium is a protozoan parasite that exhibits various characteristics that support its extended survival in the environment (152). It can be transmitted through water in the oocyst form and is more resistant to environmental conditions and disinfection (267,268). The infective dose is low (10-100 oocysts) (269), and evidence suggests *Cryptosporidium* requires as few as 10–30 oocysts per 100 liters of water for the possible existence of an outbreak (270). It is evident that the households whose water was drawn from an unimproved water source for their infant use had a significantly higher association with the presence of *Cryptosporidium* oocysts by four-fold than those households sourcing water from an improved source for their infant consumption. This is indirectly in line with a study that suggested household's use of an improved water source had a protective effect on the presence of *Cryptosporidium* in drinking water (143). Studies suggested that *Cryptosporidium* oocysts were found more in surface water than in other water sources (271), and can survive for months (272), indicating that there could be a chance of having several moments to ingest. This is certainly attributable to the fact that unimproved water sources are more exposed to contamination by human and animal waste. Our study also further investigated the fact that households that fetched drinking water other than on the day of our survey had a significant relationship with the presence of *Cryptosporidium* oocysts in their drinking water. The possible reason for this finding might be the presence of unsafe water sources in the study area.

In terms of *Shigella* species detection, our results showed that 30% of the infant drinking water sample at the household tested positive for *Shigella* species. Apart from the specific study subject in the households, this result is higher than the previous studies conducted in Shashemene rural districts at 0% (153) and Jigjiga city at 3.33% (154), Ethiopia, and somewhere else such as Quetta, Pakistan, at 22% (156), Peshawar, Pakistan, at 6.47% (157), Yaounde, Cameroon, at 0.24% (158), and the Vanda region of South Africa at 5% (159), while slightly lower than a study in Egypt at 34.7% (160). These indicate that the drinking water in the study area has a high chance of contamination due to poor water quality. On the other hand, *Shigella* species were detected in 32.4% of the water samples tested from the corresponding water sources. This finding is higher than the study conducted in Jijiga, which found 8% (154), 4.8% in Ziway (155), and 6.5% in the rural districts of Shashemene (153) in Ethiopia. Several studies elsewhere, such as Kampala, Uganda (10%) (161), Sri Lanka (0%) (164), Bangladesh (30%) (165), Nigeria (9.4%) (162), and Kenya (6.9%) (163), have also shown lower contamination prevalence of *Shigella* species in drinking water sources than the present study. The highest prevalence of *Shigella* species in drinking water either at the point-of-use or from water sources in our study might be due to the use of an unimproved water source, a lack of water source protection, and unhygienic practices such as poor handling of water, no hand- washing, and unsafe water storage in the study area. In addition,

the differences in testing methods among the studies might be able to influence the prevalence of pathogens detected. Most of the earlier studies detected this pathogen using the biochemical test and conventional PCR, which differ greatly from the LAMP technique in sensitivity (207–209,273).

Furthermore, households that collected water from unimproved water sources were strongly associated with the presence of *Shigella* species by four times more than those that collected from improved sources. This finding is supported by the view expressed in other studies that unimproved water sources are more likely to be exposed to fecal contamination than improved water sources (274). This fecal contamination poses a greater risk due to the potential source of pathogens.

The other detected bacterial type, known as a toxin-producing strain of *E. coli*, was found positive in 26.3% of the drinking water sample from infant point-of-consumption in the households. This prevalence appeared to be slightly higher than South Wollo, Ethiopia at 17% (166), and much higher prevalent than the studies in Ouagadougou, Burkina Faso at 1% (167), Trinidad at 2% (168), and Malaysian villages at 19% (169). However, it is much lower than the result reported from a study conducted in Bangladesh, which was 87.5% (170). With regard to the water sources sampled, about 30% were contaminated with a toxin-producing strain of *E. coli***.** This finding is higher than the studies in Ziway at 5.5% (155) and Modjo at 4.2% (171) in Ethiopia, South Africa at 25.6% (172), and Brazil at 6.2%, 1.0%, and 0.65% (173,175,176), and slightly lower than the study conducted in Uganda at 33% (161) and India at 33.3% (177), while much lower than studies in northern Ghana at 75% (174) and southeast of the island of Puerto Rico at 52% (178). Similar reasons that were provided for other pathogens could explain the prevalence variance among studies. Evidently, the application of the LAMP technique may result in a high prevalence of pathogens in drinking water due to its higher sensitivity than other methods such as PCR (207–209,273).

Our study also confirmed that households getting water from unimproved sources for infant consumption were more likely to be exposed to a toxin producing strain of *E. coli* by two-fold than improved sources. This finding is indirectly consistent with the result reported in the previous studies, which suggested that improved water sources significantly reduce the detection of *E. coli* virulence genes in stored drinking water (194). This is because getting water from improved sources could reduce environmental contamination.

A positive significant correlation was observed between the water samples tested from infant point-of-use at household level and those of the corresponding water sources for the presence of *Cryptosporidium***,** *Shigella***,** and a toxin-producing strain of *E. coli*. This could be explained by the fact that the water sources might be subjected to fecal contaminations, which increase opportunities for the presence of pathogens in household drinking water. However, rotavirus shows a negligible positive correlation between the water source and drinking water at the household level. This circumstance might be explained by the fact that this pathogen holds additional favorable conditions to be present in drinking water at the household level through home-based contamination.

Generally, our results showed that 13.2% of the drinking water of infants at the household level was positive for all four pathogens. The simultaneous presence of these pathogens in drinking water is closely linked with the status of the water source. This is implying that households collecting water from unimproved sources for infant consumption were nearly four times more likely to have the presence of these pathogens as compared to those sourcing water from improved supplies. Unimproved water sources may have a chance to be contaminated by feces as well as other matters that could allow different pathogens to exist in drinking water. Studies indicated that the quality of water is much more reduced at the point-of-use in the household where source water is largely contaminated (275). In addition, a lack of practices for any form of water treatment at the household level in the study area may also contribute to the presence of these pathogens in drinking water (198).

5.4 Validity and Generalizability

5.4.1 Internal Validity

Multiple measures were taken to keep internal validity of the study by controlling selection bias, information bias, recall bias and confounding. The selection bias that could arise in the process of selecting the study subject was managed. Selection bias is unlikely to occur in this study due to the procedures used to identify study participants.

The primary steps adopted to minimize information bias during measurement were employing skilled data collectors, ensuring that study participants understood the questions being asked in the local language, and using a pre-tested questionnaire prior to data collection. With shorter recollection spans, the VA interviews were done following the period of grief. All infant death causes that could be determined, with reasonable accuracy, from a VA interview were conducted according to WHO guidelines.

5.4.2 External Validity

The term "external validity" describes how well the research findings apply to individuals living beyond the study location. The research protocols were strictly followed in this investigation. The study participants were selected using a probability sampling technique, and the sample size was increased to a sufficient degree to account for anticipated non-responses. Any of these two approaches could increase the study's generalizability to the source population (all households with mother- live borne infant’s pair in study area).

5.5 Strengths and Limitations

5.5.1 Strengths

The strengths of this study include that it used validated verbal autopsy tool and model to determine all cause of infant death with quantified degrees of certainty. Longitudinal survey covering with large geographic area, routinely enumeration of reported and notified deceased infants as well as data quality procedure is also strong side of the study. Despite a very few studies available in the analysis of cause of infant death at population level, this study provided useful

information for building evidence based health policy, better insights in planning the best solutions, and input for policy-makers in taking appropriate measures.

The strengths of this study include as it used nested case-control study design which is valid and efficient design and can minimized both selection and recall bias. Despite such studies is available insufficiently at population level, in particular, this study could provide useful information for building evidence based health policy, better insights in planning the best solutions, and generate ideas for further research.

One of the strengths of this study is that we used LAMP techniques, which is the most powerful nucleic acid amplification method that could be used to detect pathogenic agents from drinking water with high sensitivity (140). This is the first study to demonstrate an individual assay for detecting the four selected waterborne pathogens from an infant's drinking water sample. Our study result could provide new insights for building evidence based health policies and strategies by defining the magnitude of selected pathogens that cause severe and fatal diarrhea in infants drinking water and strong contributing factors such as an unimproved water source. In addition, it helps with a better outlook in planning for the best solutions and generates ideas for further research.

5.5.2 Limitations

Despite the verbal autopsy used as an alternative method in determining the cause of death, there was recognized limitation on the tools and methods which might have influenced in the determination of cause of death. The InterVA model assign cause of death only based on the present of sign and symptoms, disregarding them entirely when there are absent. InterVA marginalize over that symptom if the respondent does not report a symptom. The respondent who replied the VA questionnaire about the deceased infant might not be provided full data for the reason of sorrow and grief. Drawback due to inability of the respondents to recognize, recall and trace signs and symptoms of diseases that leads to death prior to the deceased. In order to minimize this, linguistic and ethnographic work as well as skilled interviewers were recruited and adequately trained to capture data.

This study has limitations as respondents might not give their exact observable fact towards some given questions, which leads to social desirability biases. This could be minimized by providing training to data collectors and conducted pre-test before actual data collection launched. A very shortage of previous similar studies was made comparison difficult.

This study has certain limitations that include the study design that we used, which demonstrated a snapshot of the water sample test result. Although one-time sampling information is very useful, it does not allow us to capture the burden of their presence in drinking water at multiple points in time. Our study is based only on the presence-absence test of organisms in drinking water, and there is no indication of the quantitative number of organisms. Our study was limited to detecting only four pathogens, and no total coliforms and E. coli were measured to recognize the degree of drinking water pollution. In addition, since no studies have been conducted on the molecular

detection of pathogenic agents from infant's drinking water samples, comparisons between studies were difficult.

CHAPTER SIX: CONCLUSIONS

6.1 CONCLUSIONS

The study concludes that the majority of infant deaths existed in the present study were as a result of diseases and conditions that are readily preventable or treatable cause. Majority of infant deaths were occurring in the first month of life and the leading causes of infant mortality appeared to be acute respiratory infection including pneumonia, birth asphyxia and diarrheal diseases. The age categories have a broad effect across many causes of death, as having been revealed that Neonatal pneumonia, birth asphyxia and prematurity were the major causes of deaths during the neonatal period, while acute respiratory infection including pneumonia, Diarrheal diseases and Malaria appeared to be in post-neonate period. The patterns of significant associated factors across cause-specific mortality against all-cause of death were dissimilar.

The factors that were statistically significant associated with the higher odds of infant death due to diarrhea with their reference group include; age of mother with <20 years old, unsafe drinking water storage, infants in households without point-of-use water treatment practices, households with unimproved sanitation status, unsafe disposing of child feces and improper management of solid waste, households with improper management of liquid waste and household's practiced handwashing not at all as well as in fewer occasions (One to two critical times'). The significant link of such factors could be one of the reasons for the contribution of uppermost death level of infants as the result of diarrhea in the study area.

This study also demonstrated a high level of exposure of infants to contaminated drinking water by those recognized pathogens that cause the most severe and fatal diarrhea. The high prevalence of these pathogens in the water samples in our study seems to be robustly influenced by the technique used to detect the pathogens and various contributing factors, such as unimproved water sources, poor water source protection, and a lack of point-of-use water treatment practices. With the exception of rotavirus, unimproved water sources remained the only strong significant determinants for contamination of drinking water by these pathogens at the point of consumption. The presence of pathogens in drinking water used for infants at the household level is positively correlated with the water sources, which implies that the presence of pathogens at the point-of-use mainly depends on the water source used for collection.

6.2 RECOMMENDATIONS

For Project/ programme Implementer

- More efforts require on maternal and child health program with proven preventive interventions emphasizing on the most common cause of infant death. Attention should also be given to those factors that make the specific infant death more likely to occur.

- Proper screening for infections in pregnant women, encouraging institutional delivery, Immunization, provision of safe water and sanitation with adoption of hygiene practices, promotes cross and through ventilation system into dwelling and ensuring appropriate preventive and curative care for infants should be strengthen.
- Due attention should be given to the reduction of diarrhea-related infant deaths through WASH intervention, taking into account the strong associated risk factors typically during the infantile period which eventually can turn-down the consequence of the highest reported number of diarrhea-related infant death.
- Efforts should be made on the new development and rehabilitation of improved water sources and water treatment at the household level. Water safety protection and other sanitary measures should be implemented to mitigate contamination from human and animal wastes.
- Health education must be undertaken to increase awareness among mothers or caretakers of infants on the prevention of waterborne pathogens in drinking water aimed at keeping the water safe for infants.

For Policymakers

- It is strongly advised that when policymakers are trying to create an efficient and successful reduction of causes of infant mortality intervention strategies, they take into account the identified causes of infant death and related factors that raise the most common cause of mortality risk in the study areas.
- Policymakers should give emphasis in using computer-based algorism models in local health setting to yield nationwide cause of death data.
- The policy markers should develop approaprate preventive strategy that can help infant survuival against diarrhea considering water, sanitation and hygiene components factors that are found to be significantly associated with an increased risk of death from diarrhea.
- It is advised that policymakers develop appropriate strategies to enhance the quality of drinking water for infants both at home and at water sources. This will help to lower the number of water-borne pathogens that are responsible for the most severe and deadly diarrhea that infants experience.

For Researcher

- Undertaking feasibility studies for the routine application of the InterVA model for interpreting cause of death through the district health system could prove advantageous for public health planners.
- It is important to investigate how infant mortality from diarrhea-related causes differs from infant mortality from all other causes, and how additional factors not covered by the current study contribute to this difference.
- Detecting hotspots through mapping for infant deaths linked to diarrhea should be assessed, and an in-depth study is recommended to be conducted.
- Analysing of potential contributing factors to infant mortality associated with diarrhea that the current study may not have addressed is warranted.

- Detecting waterborne pathogens other than the current studied in infant drinking water using molecular techniques can provide useful information for public health planners.

REFERENCES

1. World Health Organization. Global Health Observatory: Infant Mortality Rate [Internet]. World Health Organization; 2017 [cited 2020 May 5]. Available from: https://www.who.int/data/gho/ indicator-metadata-registry/imr-details/1

2. Centers for Disease Control and Prevention. Reproductive Health: Infant Mortality [Internet]. Centers for Disease Control and Prevention; 2019 [cited 2020 May 5]. Available from: https://www.cdc.gov/reproductivehealth/maternalinfanthealth/ infantmortality.htm

3. Association of Maternal and Child Health Program. Infant Mortality Toolkit: A standard Approach for Examining Infant Mortality. Association of Maternal and Child Health Program. 2013.

4. UNICEF. 2013 Statistical snapshot Child Mortality. UNICEF. 2013.

5. World Health Organization. Infant mortality [Internet].WHO; 2018 [cited 2020 May 5]. Available from: https://www.who.int/gho/child_health/mortality/neonatal_infant_text/en/

6. You D, Hug L, Ejdemyr S, Beise J, Idele P. Levels & Trends in Child Mortality: Estimates Developed by the UN Inter-Agency Group for Child Mortality Estimation (UN- IGME). United Nations Children's Fund;3 UN Plaza, New York, New York, 10017 USA;2015.

7. Ester PV, Torres A, Freire JM, Hernández V, Gil Á. Factors associated to infant mortality in Sub-Saharan Africa. Journal of Public Health in Africa [Internet]. 2011 Sep 5 [cited 2020 May 6];2(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5345500/

8. Assefa N, Gebeyehu A, Terefe B, Tesfayi G, Pearson R. An Analysis of the Trends, Differentials and Key Proximate Determinants of Infant and Under-five Mortality in Ethiopia: Further analysis of the 2000, 2005 and 2011 Demographic and Health Surveys data. DHS Further Analysis Reports No. 79.Calverton, Maryland, USA: ICF International; 2013.

9. Central Statistical Agency [Ethiopia] and ICF International. Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: Central Statistical Agency and ICF International.2012.

10. Child Survival Partnership. Child Survival Situational Analysis. Background Document for the National Child Survival Conference. Addis Ababa, Ethiopia.2004.

11. Eunice Kennedy Shriver National Institute of Child Health and Human Development. What causes infant mortality? [Internet].NICHHD Reviewed; 2016 [cited 2020 May 6] Available from https://www.nichd.nih.gov/health/topics/infant-mortality/topicinfo/causes

12. Shah MS, Khaliue N, Khan Z, Amir A. A community based study of Infant Mortality from rural Aligarh.AMJ.2011; 4(1):22-25.doi:10.4066/AMJ.2011.470

13. Habib H, Lohani M, Khan H, Hussain KM. Infant morbidity leading to infant mortality. Gomal Journal of Medical Sciences July-December 2009;7:2.

14. World Health Organization. Civil registration: why counting births and deaths is important [Internet]. [cited 2020 May 6]. Available from: https://www.who.int/news-room/fact-sheets/detail/civil-registration-why-counting-births-and-deaths-is-important

15. Lozano R, Freeman MK, James SL. et al. Performance of InterVA for assigning causes of death to verbal autopsies: Multisite validation study using clinical diagnostic gold standards. Popul Health Metrics.2011;9(50).doi:10.1186/1478-7954-9-50

16. Institute for Health Metrics and Evaluation. About Verbal Autopsy [Internet]. Institute for Health Metrics and Evaluation; 2014 [cited 2020 May 17]. Available from: http://www.healthdata.org/verbal-autopsy/about

17. Umeå Center for Global Health Research. InterVA - software for verbal autopsy[Internet]. CGHR;2016. [cited 2020 May 6]. Available from: http://www.byass.uk/interva/

18. World Health Organization. InterVA-4 User Guide. World Health Organization.2012. https://www.who.int/healthinfo/statistics/ verbalautopsystandards/en/

19. World Health Organization. Verbal Autopsy Standard: The 2014 WHO Verbal Autopsy Instrument. Geneva: World Health Organization.2015.

20. World Health Organization. Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. Geneva, World Health Organization;2020.

21. Humphreys EH, Smith NA, Azman H, McLeod D, Rutherford GW. Prevention of diarrhoea in children with HIV infection or exposure to maternal HIV infection. Cochrane Database Systematic Review. 2010;(6):CD008563.

22. Nelson R. Diarrhea Remains Leading Cause of Global Mortality and Morbidity. Infectious Disease Advisor. 2018.Available from: https://www.infectiousdiseaseadvisor.com

23. Will S. Diarrhea Still One of the World's Leading Cause of Death. PassportHealth.2018. Available from: https://www.passporthealthusa.com

24. Troeger C, Colombara DV, Rao PC, et al. Global disability-adjusted life-year estimates of long-term health burden and undernutrition attributable to diarrhoeal diseases in children younger than 5 years. Lancet Global Health.2018;6(3):e255-e269.doi:10.1016/S2214-109X(18)30045-7.

25. Oria R., Pinkerton R., Lima A.A.M., Guerrant R.L. Handbook of Disease Burdens and Quality of Life Measures. Preedy V.R., Watson R.R. (eds): Springer New York; 2010. doi:10.1007/978-0-387-78665-0_69.

26. World Health Organization. Updates fact sheet on Diarrhoeal diseases. Geneva, World Health Organization; 2017. Available from: https://communitymedicine4asses.com

27. Dadonaite B, Ritchie H, Roser M.Diarrheal Diseases. Our World in Data. 2018. Available from: https://ourworldindata.org/childhood-diarrheal-diseases.

28. Karambu S, Matiru V, Kiptoo M, Oundo J. Characterization and factors associated with diarrhoeal diseases caused by enteric bacterial pathogens among children aged five years and below attending Igembe District Hospital, Kenya. The Pan African Medical Journal 2013;16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3932116/

29. World Health Organization. The world health report. Geneva, World Health Organization; 2002. Available from: https://www.who.int/whr/2002/ chapter4/en/index7.html

30. Roth GA, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018;392(10159):1736–88.

31. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao S, Zimsen SR, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Infectious Diseases.2018;18(11):1211–28.

32. Troeger C, Forouzanfar M, Rao PC, Khalil I, Brown A, Reiner RC, et al. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet Infectious Diseases.2017;17(9):909–48.

33. Parashar UD, Kilgore PE, Holman RC, Clarke MJ, Bresee JS, Glass RI. Diarrheal Mortality in US Infants: Influence of Birth Weight on Risk Factors for Death. Archives of Pediatrics and Adolescent Medicine.1998;152(1):47–51.doi:10.1001/archpedi.152.1.47.

34. World Health Organization and Maternal and Child Epidemiology Estimation Group (MCEE). Child causes of death, by Country and by Region, 2000-2016. Geneva, World Health Organization; 2018. Available from: http://apps.who.int/gho/data/node.main. ChildMort?lang =en

35. Takele K, Zewotir T, Ndanguza D. Risk factors of morbidity among children under age five in Ethiopia. BMC Public Health.2019;19(1):942.

36. Melese B, Paulos W, Astawesegn FH, Gelgelu TB. Prevalence of diarrheal diseases and associated factors among under-five children in Dale District, Sidama zone, Southern Ethiopia: a cross-sectional study. BMC Public Health.2019;19(1):1235.

37. Deribew A, Tessema GA, Deribe K, Melaku YA, Lakew Y, Amare AT, et al. Trends, causes, and risk factors of mortality among children under 5 in Ethiopia, 1990–2013: findings from the Global Burden of Disease Study 2013. Population Health Metrics. 2016;14(1):42.

38. Central Statistical Agency (CSA) [Ethiopia] and ICF. Ethiopia Demographic and Health Survey 2016. Addis Ababa, Ethiopia, and Rockville, Maryland, USA: CSA and ICF; 2016.

39. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year?. The Lancet. 2003; 361(9376):2226-34. doi:10.1016/S0140-6736(03)13779-8.

40. United Nation Children's Fund, World Health Organization. Diarrhoea: Why children are still dying and what can be done. New york: United Nations Children's Fund; 2009.

41. Centers for Disease Control and Prevention. Global Diarrhea Burden: Global Water, Sanitation and Hygiene. US department of Health and Human services, Centers for Disease Control and Prevention; 2015.Available from: https://www.cdc.gov/healthywater/global/ diarrhea-burden.html

42. United Nation Children's Fund. Diarrhoeal disease:UNICEF DATA; 2018.Available from: https://data.unicef.org/topic/ child-health/diarrhoeal-disease/.

43. United Nation Children's Fund, World Health Organization. The basics - Diarrhoea: Why children are still dying and what can be done - prevention and treatment measures. The United Nations Children's Fund/WHO; 2009; 1-68.

44. Peter H.Gleick. Dirty Water: Estimated Deaths from Water-Related Diseases 2000-2020. Pacific Institute for studies in development, Environment, and security; 2002.

45. Levine MM, Nasrin D, Acacio S, et al. Diarrhoeal disease and subsequent risk of death in infants and children residing in low-income and middle-income countries: analysis of the GEMS case-control study and 12-month GEMS-1A follow-on study. The Lancet Global Health. 2019. doi:10.1016/S2214-109X(19)30541-8.

46. Centers for Disease Control and Prevention. The Management of Acute Diarrhea in Children: Oral Rehydration, Maintenance, and Nutritional Therapy. MMWR.1992;41 (No.RR-16). Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/00018677 .htm

47. Bowen A, Agboatwalla M, Luby S, Tobery T, Ayers T, Hoekstra RM. Association between intensive handwashing promotion and child development in Karachi, Pakistan: a cluster randomized controlled trial. Arch Pediatr Adolesc Med. 2012;166(11):1037–44.

48. Bartram J, Cairncross S. Hygiene, Sanitation, and Water: Forgotten Foundations of Health. PLOS Medicine.2010;7(11):e1000367.

49. Victora C G et al. Water Supply, Sanitation and Housing in Relation to the Risk of Infant Mortality from Diarrhoea. International Journal of Epidemiology.1988;17:651-654

50. Cheng et al.: An ecological quantification of the relationships between water, sanitation and infant, child, and maternal mortality. Environmental Health.2012;11:4.

51. Gashaw D et al. What factors aggravate prevalence of diarrhea among infants of 7-12 months in southern Ethiopia?. Insight Medical Publishing Group.2017;25(5):321-325

52. Mokomane M, Kasvosve I, Melo E de, Pernica JM, Goldfarb DM. The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. Therapeutic Advances in Infection.2018;5(1):29–43.

53. Gerba C.P. Environmentally Transmitted Pathogens. Environmental Microbiology (Third edition), Academic press. 2015;509-550.

54. Peterson H.G. Rural Drinking Water and Waterborne illness. 2006. Available from: https://www.safewater.org.

55. Kotloff KL et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet.2013;382(9888):209-22.doi:10.1016/S0140-6736(13) 60844-2.

56. Mary E. Wilson MD. Global Burden from Diarrhea: Decreasing but Still a Leading Cause of Death. NEJM Journal Watch.2017; doi:10.1056/nejm-jw.NA44326

57. McNeil Jr D.G. 4 Germs Cause Most of Infants' Severe Diarrhea. The New York Times-Global Health.2013. Available from: https://www.nytimes.com

58. United Nations Educational, Scientific and Cultural Organization. The global water quality challenge & SDGs. International Initiative on Water Quality. 2021. Available from: https://en.unesco.org/waterquality-iiwq/wq-challenge

59. World Health Organization. Drinking-water [Internet]. [cited 2022 Jul 5]. Available from: https://www.who.int/news-room/ fact-sheets/detail/drinking-water

60. Magana-Arachchi DN, Wanigatunge RP. Ubiquitous waterborne pathogens. Waterborne Pathogens. 2020:15–42. doi:10.1016/ B978-0-12-818783-8.00002-5.

61. Saharan P, Dhingolia S, Khatri P, Duhan S.J, Gahlawat K.S. Loop-mediated isothermal amplification (LAMP) based detection of bacteria: A Review. African Journal of Biotechnology, Academic journal.2014;13(19):1920-1928.doi:10.5897/AJB2013.13459

62. Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. Journal of Infectious Chemother. 2009;15(2):62–9.

63. Siju M. Varghese, Jeena Augustine and Sarita G. Bhat 2012 . Rapid and Sensitivity Detection of Low Number of Salmonella in Water by Loop-Mediated Isothermal Amplification. Advanced Biotech.2012;11(12):28-30

64. Becherer L, Borst N, Bakheit M, Frischmann S, Zengerle R, Stetten F von. Loop-mediated isothermal amplification (LAMP) – review and classification of methods for sequence-specific detection. Anal Methods.2020;12(6):717–46.

65. Martzy R, Kolm C, Brunner K, Mach RL, Krska R et al. A loop-mediated isothermal amplification (LAMP) assay for the rapid detection of Enterococcus spp. in water. Water Resource. 2017;122:62-69.doi:10.1016/j.watres.2017.05.023.

66. Usman MA, Gerber N, Pangaribowo EH. Determinants of Household Drinking Water Quality in Rural Ethiopia. SSRN Electronic Journal [Internet]. 2016 [cited 2022 Jul 7]; Available from: https://www.ssrn.com/abstract=2809564

67. Garenne M, Kahn K, Tollman S, Gear J. Causes of Death in a Rural Area of South Africa: An International Perspective. Journal of Tropical Pediatrics.2000;46:183-190.

68. Weldearegawi B, Melaku YA, Spigt M, Dinant GJ. Applying the InterVA-4 model to determine causes of death in rural Ethiopia. Global Health Action [Internet]. 2014 [cited 2022 Jan 16];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4220136/

69. Hagos G, Atakelti A, John K, Anna M, Peter B: Undertaking cause-specific mortality measurement in an unregistered population: an example from Tigray Region, Ethiopia. Global Health Action.2014;7(1).doi:10.3402/gha.v7.25264

70. Anteneh A, Araya T, Misganaw A. Factors associated with place of death in Addis Ababa, Ethiopia. BMC Palliative Care. 2013;12(1):14. doi:10.1186/1472-684x-12-14

71. Adair T. Who dies where? Estimating the percentage of deaths that occur at home. BMJ Global Health.2021;6(9):e006766.doi:10.1136/bmjgh-2021-006766

72. Weldearegawi B, Ashebir Y, Gebeye E, Gebregziabiher T, Yohannes M, Mussa S, et al. Emerging chronic non-communicable diseases in rural communities of Northern Ethiopia: evidence using population-based verbal autopsy method in Kilite Awlaelo surveillance site. Health Policy Plan.2013;28(8):891–8.

73. Jha P. Reliable direct measurement of causes of death in low- and middle-income countries. BMC Med. 2014;12(1):19.doi:10.1186/1741-7015-12-19

74. National Plan Commission. The 2017 Voluntary National Reviews on SDGs of Ethiopia: Government Commitments, National Ownership and Performance Trends. Addis Ababa, Ethiopia. 2017.

75. World Health Organization. Cause of death [Internet]. [cited 2022 Aug 28]. Available from: https://www.who.int/standards/classifications/classification-of-diseases/cause-of-death

76. Hoffman M. Infant mortality rates declining, but still high. Netw Res Triangle Park N C. 1992;13(2):20–3.

77. Andrews KM, Brouillette DB, Brouillette RT. Encyclopedia of Infant and Early Childhood Development. Academic Press. 2008; 343–359.doi:10.1016/B978-012370877-9.00084-0

78. Ewbank DC, Gribble JN. Trends in Mortality and Causes of Death in Africa: Effects of Health Programs on Child Mortality in Sub-Saharan Africa. National Academies Press (US); 1993 [cited 2020 May 16]. Available from: https://www.ncbi.nlm.nih.gov/books/ NBK236385/

79. Weldearegawi B, Melaku YA, Abera SF, Ashebir Y, Haile F, Mulugeta A, et al. Infant mortality and causes of infant deaths in rural Ethiopia: a population-based cohort of 3684 births. BMC Public Health.2015;15(1):770.

80. Debelew GT, Afework MF, Yalew AW. Determinants and causes of neonatal mortality in Jimma Zone, Southwest Ethiopia: a multilevel analysis of prospective follow up study. PLoS One. 2014;9(9):e107184.

81. Mengesha H.G, Sahle B.W. Cause of neonatal deaths in Northern Ethiopia: a prospective cohort study. BMC Public Health.2017;17:62.doi:10.1186/s12889-016-3979-8

82. Roro EM, Tumtu MI, Gebre DS. Predictors, causes, and trends of neonatal mortality at Nekemte Referral Hospital, East Wollega Zone, Western Ethiopia (2010–2014): Retrospective cohort study. PLoS ONE. 2019;14(10):e0221513.doi.10.1371/journal.pone. 0221513

83. Desalew A, Sintayehu Y, Teferi N, et al. Cause and predictors of neonatal mortality among neonates admitted to neonatal intensive care units of public hospitals in Eastern Ethiopia: A facility-based prospective follow-up study. BioMed Central Pediatrics.2020;20(160). doi.10.1186/s12887-020-02051-7

84. Lulu K, Berhane Y. The use of simplified verbal autopsy in identifying causes of adult death in a predominantly rural population in Ethiopia. BMC Public Health.2005;5(1):58.

85. World Health Organization. Potential and limits of verbal autopsies. Bulletin of the World Health Organization.2006;84(3):161-256.

86. Bauni E, Ndila C, Mochamah G, et al. Validating physician-certified verbal autopsy and probabilistic modeling (InterVA) approaches to verbal autopsy interpretation using hospital causes of adult deaths. Population Health Metrics.2011; 9:49.doi:10.1186/1478-7954-9-49

87. Institute for Health Metrics and Evaluation. Verbal autopsy tool [Internet]. [cited 2022 Sep 9]. Available from: https://www.healthdata.org/data-tools-practices/verbal-autopsy

88. Caballero MT, Bianchi AM, Nuno A, Ferretti AJP, et al. Mortality Associated With Acute Respiratory Infections Among Children at Home. Journal of Infectious Disease. 2019; 219(3):358-364. doi:10.1093/infdis/jiy517

89. Verfurden ML, Fitzpatrick T, Holder L, Zylbersztejn A, et al. Deprivation and mortality related to pediatric respiratory tract infection: a cohort study in 3 high-income jurisdictions. CMAJ Open. 2020;8(2):E273-E281.doi:10.9778/cmajo.20190074.

90. Ferdous F, Ahmed S, Das SK, Chisti MJ, Nasrin D, Kotloff KL, et al. Pneumonia mortality and healthcare utilization in young children in rural Bangladesh: a prospective verbal autopsy study. Tropical Medicine and Health.2018;46(1):17.

91. Mehal J. Infant and Maternal Risk Factors for Diarrhea-Associated Infant Mortality in the United States, 2005-2006. Master's theses, Emory University. 2011.

92. Villa S, Guiscafre H, Martinez H, Munoz O, Gutierrex G. Seasonal diarrhoeal mortality among Mexican children. Bullet of World Health Organization.1999;77(5):375-80.

93. Onyiriuka AN. Birth asphyxia in a mission hospital in Benin City, Nigeria. Tropical Journal of Obstetrics and Gynecology.2006;23(1):34-39.doi:10.4314/tjog.v23i1.14563

94. Meshram R, Bokade C. Risk factors for mortality in birth asphyxia of outborn neonates: A prospective observational study. Sri Lanka Journal of Child Health.2019;48(1):26-32. doi:10.4038/sljch.v48i1.8684

95. Kazembe LN, Appleton CC, Kleinschmidt I. Spatial analysis of the relationship between early childhood mortality and malaria endemicity in Malawi. Geospat Health. 2007; 2(1):41-50. doi:10.4081/gh.2007.253

96. Ramroth H, Ndugwa R, Müller O, Ye Y, Sie A, Kouyaté B, et al. Decreasing Childhood Mortality and Increasing Proportion of Malaria Deaths in Rural Burkina Faso. Glob Health Action.2009;2.doi:10.3402/gha.v2i0.1909.

97. Becher H, Kynast-Wolf G, Sié A, Ndugwa R, Ramroth H, Kouyaté B, et al. Patterns of Malaria: Cause-Specific and All-Cause Mortality in a Malaria-Endemic Area of West Africa. The American Journal of Tropical Medicine and Hygiene.2008;78(1):106-113.doi:10.4269/ajtmh.2008.78.106

98. Troeger CE, Khalil IA, Blacker BF, Biehl MH, Albertson SB, Zimsen SRM, et al. Quantifying risks and interventions that have affected the burden of diarrhoea among children younger than 5 years: an analysis of the Global Burden of Disease Study 2017. The Lancet Infectious Diseases.2020;20(1):37–59.

99. World Health Organization. World health statistics 2019: monitoring health for the SDGs, sustainable development goals. Geneva: World Health Organization; 2019.

100. Prüss-Ustün A, Bartram J, Clasen T, Colford JM, Cumming O, Curtis V, et al. Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. Tropical Medicine and International Health.2014;19(8):894–905.

101. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. International Journal of STD and AIDS.2012;380(9859):2095–128.

102. De Souza AC, Petersont KE, Cufino E, do Amaral MI, Gardner J. Underlying and proximate determinants of diarrhoea-specific infant mortality rates among municipalities in the state of Céara, north-east Brazil: an ecological study. Journal of Biosocial Science. 2001;33(2):227–44.

103. Ezeh OK, Agho KE, Dibley MJ, Hall J, Page AN. The Impact of Water and Sanitation on Childhood Mortality in Nigeria: Evidence from Demographic and Health Surveys, 2003–2013. International Journal of Environmental Research and Public Health.2014;11(9):9256–72.

104. Günther Fink, Isabel Günther, Kenneth Hill, The effect of water and sanitation on child health: evidence from the demographic and health surveys 1986–2007. International Journal of Epidemiology.2011;40(5):1196-1204.

105. Abou-Ali H. The effect of water and sanitation on child mortality in Egypt. University of Gothenburg, Department of Economics (Working Papers in Economics).2003.

106. Esrey SA, Potash JB, Roberts L, Shiff C. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. Bullet of World Health Organization.1991;69(5):609–21.

107. Haushofer J, Kremer M, Maertens R, Tan JB. Free Provision of Drinking Water Treatment and Child Survival: Evidence from Kenya [Internet]. American Economic Association; [cited 2021 Jun 13]. Available from: https://www.aeaweb.org/doi/10.1257/rct.5969-1.0

108. Mekonnen D, Bengtsson T. Infant and Child Mortality in Ethiopia: The role of Socioeconomic, Demographic and Biological factors In the previous five years period of 2000 and 2005. Lund University. Master Thesis Spring 2011.

109. Kotloff KL. The Burden and Etiology of Diarrheal Illness in Developing Countries. Pediatric Clinics of North America.2017;64(4):799–814.

110. World Health Organization. Rotavirus [Internet]. [cited 2022 Dec 27]. Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccines-quality/rotavirus

111. Rodríguez-Díaz J, Monedero V. Probiotics against Digestive Tract Viral Infections. Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease. 2013;271–84. doi:10.1016/B978-0-12-397154-8.00031-2

112. Hasso-Agopsowicz M, Ladva CN, Lopman B, Sanderson C, Cohen AL, Tate JE, Riveros X, Henao-Restrepo AM, Clark A; Global Rotavirus Surveillance Network and Rotavirus Age Study Collaborators. Global Review of the Age Distribution of Rotavirus Disease in Children Aged <5 Years Before the Introduction of Rotavirus Vaccination. Clinical Infectious Diseases. 2019;69(6):1071-1078.doi:10.1093/cid/ciz060.

113. Du Y, Chen C, Zhang X, Yan D, Jiang D, Liu X, et al. Global burden and trends of rotavirus infection-associated deaths from 1990 to 2019: an observational trend study. Virology Journal.2022;19(1):166.

114. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infectious Disease.2012;12(2):136–41.

115. PATH. Rotavirus Disease and Vaccines in Ethiopia. Vaccine access and delivery global program.2013.

116. Khan A, Shams S, Khan S, Khan MI, Khan S, Ali A. Evaluation of prevalence and risk factors associated with Cryptosporidium infection in rural population of district Buner, Pakistan. PLoS One.2019;14(1):e0209188.

117. Tombang AN, Ambe NF, Bobga TP, Nkfusai CN, Collins NM, Ngwa SB, Diengou NH, Cumber SN. Prevalence and risk factors associated with cryptosporidiosis among children within the ages 0-5 years attending the Limbe regional hospital, southwest region, Cameroon. BMC Public Health.2019;19(1):1144.doi:10.1186/s12889-019-7484-8.

118. Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG, et al. Morbidity, mortality, and long-term consequences associated with diarrhoea from Cryptosporidium infection in children younger than 5 years: a meta-analyses study. Lancet Global Health. 2018;6(7):e758–68.

119. Mølbak K, Højlyng N, Gottschau A, Sá JC, Ingholt L, da Silva AP, et al. Cryptosporidiosis in infancy and childhood mortality in Guinea Bissau, west Africa. Biomedical Journal. 1993;307(6901):417–20.

120. Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, et al. Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: the Global Burden of Disease Study 1990–2016. The Lancet Infectious Diseases.2018;18(11):1229–40.

121. World Health Organization. Enterotoxigenic Escherichia coli (ETEC) [Internet]. [cited 2022 Dec 28]. Available from: https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/enterotoxigenic-escherichia-coli-(etec)

122. Bagamian KH, Anderson JD, Muhib F, Cumming O, Laytner LA, Wierzba TF, et al. Heterogeneity in enterotoxigenic Escherichia coli and shigella infections in children under 5 years of age from 11 African countries: a subnational approach quantifying risk, mortality, morbidity, and stunting. Lancet Global Health.2019;8(1):e101–12.

123. Jeffrey K. Griffiths. Waterborne Diseases. International Encyclopidia of public Health, 2nd edition. 2017; 7:388-401.doi:10.1016/B978-0-12-803678-5.00490-2.

124. Bhagwat VR. Safety of Water Used in Food Production. Food Safety and Human Health. 2019;219–47.

125. Getahun W, Adane M. Prevalence of acute diarrhea and water, sanitation, and hygiene (WASH) associated factors among children under five in Woldia Town, Amhara Region, northeastern Ethiopia. BMC Pediatrics.2021;21(1):227.

126. Motlagh AM, Yang Z. Detection and occurrence of indicator organisms and pathogens. Water Environment Research.2019;91(10):1402–8.

127. Saxena G, Bharagava RN, Kaithwas G, Raj A. Microbial indicators, pathogens and methods for their monitoring in water environment. Journal of Water Health. 2015;13(2):319-39.doi:10.2166/wh.2014.275

128. Leclerc H, Mossel DA, Edberg SC, Struijk CB. Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. Annual Review of Microbiology.2001;55:201-34.doi:10.1146/annurev.micro.55.1.201.

129. Abramowitz S. Towards inexpensive DNA diagnostics. Trends Biotechnology. 1996;14(10):397-401.doi:10.1016/0167-7799(96)10051-2.

130. Lemarchand K, Masson L, Brousseau R. Molecular biology and DNA microarray technology for microbial quality monitoring of water. Critical Reviews in Microbiology. 2004;30(3):145-72.doi:10.1080/10408410490435142.

131. Nocker A, Burr M, Campe A.K. Synthesis Document on Molecular Techniques for the Drinking Water Industry. Water Research Foundation.2009.

132. Hellard ME, Sinclair MI, Streeton CL, Stretton CL, Fairley CK. Commentary: Drinking water and microbiological pathogens - issues and challenges for the year 2000. Journal of Public Health Medicine.1997;19(2):129–31.

133. Bej AK, Steffan RJ, DiCesare J, Haff L, Atlas RM. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Applied Environmental Microbiology. 1990;56(2):307–14.

134. Botes M, de Kwaadsteniet M, Cloete TE. Application of quantitative PCR for the detection of microorganisms in water. Analytical and Bioanalytical Chemistry.2013;405(1):91–108.

135. Girones R, Ferrús MA, Alonso JL, Rodriguez-Manzano J, Calgua B, Corrêa Ade A, Hundesa A, Carratala A, Bofill-Mas S. Molecular detection of pathogens in water--the pros and cons of molecular techniques. Water Resources. 2010;44(15):4325-39.doi:10.1016/j. watres.2010.06.030.

136. Ramírez-Castillo FY, Loera-Muro A, Jacques M, Garneau P, Avelar-González FJ, Harel J, Guerrero-Barrera AL. Waterborne pathogens: detection methods and challenges. Pathogens.2015;4(2):307-34.doi:10.3390/pathogens4020307.

137. Deshmukh RA, Joshi K, Bhand S, Roy U. Recent developments in detection and enumeration of waterborne bacteria: a retrospective minireview. Microbiologyopen. 2016;5(6):901-922.doi:10.1002/mbo3.383.

138. Toze S. PCR and the detection of microbial pathogens in water and wastewater. Water Research.1999;33(17):3545–56.doi:10.1016/S0043-1354(99)00071-8.

139. Aghababaee H, Jebelli AM, Izanloo H, Tekyeh S.Comparison of PCR Technique with MPN Method in Identification of Coliform Bacteria in Water. Archives Des Sciences. 2012. 65;29-33.

140. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28(12):E63. doi:10.1093/nar/28.12.e63.

141. Girones R, Ferrús MA, Alonso JL, Rodriguez-Manzano J, Calgua B, Corrêa Ade A, Hundesa A, Carratala A, Bofill-Mas S. Molecular detection of pathogens in water--the pros and cons of molecular techniques. Water Resources. 2010;44(15):4325-39. doi: 10.1016/j. watres.2010.06.030.

142. Zhang XP, He YY, Zhu Q, Ma XJ, Cai L. [Investigation on contamination of Cryptosporidium and Giardia in drinking water and environmental water in Shanghai]. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi.2010;28(6):435–8.

143. Barnes AN, Davaasuren A, Baasandavga U, Lantos PM, Gonchigoo B, Gray GC. Zoonotic enteric parasites in Mongolian people, animals, and the environment: Using One Health to address shared pathogens. PLOS Neglected Tropical Diseases. 2021;15(7):e0009543.

144. Kifleyohannes T, Robertson LJ. Preliminary insights regarding water as a transmission vehicle for Cryptosporidium and Giardia in Tigray, Ethiopia. Food and Waterborne Parasitology.2020;19:e00073.

145. Atnafu T, Kassa H, Keil C, Fikrie N, Leta S, Keil I. Presence, Viability and Determinants of Cryptosporidium Oocysts and Giardia Cysts in the Addis Ababa Water Supply and Distribution System. Exposure and Health.2012;1(4):55–65.

146. Fikrie N, Hailu A, Belete H. Determination and enumeration of Cryptosporidium oocysts and Giardia cysts in Legedadi (Addis Ababa) municipal drinking water system). The Ethiopian Journal of Health Development. 2008 ;22(1).

147. Amenu D, Menkir S, Gobena T. Bacteriological quality and occurrence of cryptosporidium and giardia from sources to household water samples of the rural communities of Dire Dawa

Administrative Council, Eastern Ethiopia. International Journal of Bacteriology Research. 2013; 1: 001-018.

148. Sakran, T., El-Shahawy, G., Shalaby, M., Sabry, H., Matooq, P., Elmallah, A. Detection rates of waterborne protozoa in water sources from Fayoum Governorate. Parasitologists United Journal, 2017; 10(1-2): 30-38. doi: 10.21608/PUJ.2017.4734

149. Füchslin HP, Kötzsch S, Egli T. *Cryptosporidium* spp. in drinking water. Samples from rural sites in Switzerland. Swiss Med Wkly.2012;142:w13683.doi:10.4414/smw.2012. 13683.

150. Kumar T, Majid M.A.A, Onichandran S et al. Presence of *Cryptosporidium* parvum and Giardia lamblia in water samples from Southeast Asia: towards an integrated water detection system. Infectious Diseases of Poverty. 2016;5:3.doi.10.1186/s40249-016-0095-z

151. Johnson A.M, Rochelle P.A, Di Giovanni G.D. The Risk of *Cryptosporidiosis* from Drinking Water. American Water Works Association. 2008.

152. Armon R, Gold D, Zuckerman U, Kurzbaum E. Environmental Aspects of *Cryptosporidium*. Journal of Veterinary Medicine and Research. 2016; 3(2): 1048.

153. Negera, E., Nuro G., Kebede M. Microbiological assessment of drinking water with reference to diarrheagenic bacterial pathogens in Shashemane Rural District, Ethiopia. African Journal of Microbiology Research. 2017;11(6), 254-263.

154. Asfaw HS, Reta MA, Yimer FG. High enteric bacterial contamination of drinking water in Jigjiga city, Eastern Ethiopia. Ethiopian Journal of Health Development. 2016;30(3):103-176.

155. Mekonnen M, Assefa F, Lemma B, Haaren CV, Casper P. Assessing the occurrence of waterborne pathogens in Lake Ziway and drinking water system of Batu (Ziway) Town, Ethiopia. Ethiopian Journal of Health Development.2014;28(2).

156. Saima, Samad A, Roomeela, Rizwan M, Abbas F et al. Isolation & identification of *Shigella* species from food and water samples of Quetta, Pakistan. Pure and Applied Biology. 2018;7(1):227-235.doi.10.19045/bspab.2018.70027.

157. Nisa I, Qasim M, Driessen A, Nijland J, Rafiullah, et al. Prevalence and associated risk factors of *Shigella* flexneri isolated from drinking water and retail raw foods in Peshawar, Pakistan. Journal of food science, 86(6),2579-2589.doi:10.1111/1750-3841.15777.

158. Nguendo-Yongsi H.B. Microbiological Evaluation of Drinking Water in a Sub-Saharan Urban Community (Yaounde). American Journal of Biochemistry and Molecular Biology.2011;1:68-81.doi:10.3923/ajbmb.2011.68.81

159. Potgieter N, Obi CL, Bessong PO, Igumbor EO, Samie A, Nengobela R. Bacterial contamination of Vhuswa-a local weaning food and stored drinking-water in impoverished households in the Venda region of South Africa. Journal of Health Population and Nutrition.2005;23(2):150–5.

160. Nouran H. Assar PD, Mohamed PD. Bacterial Sanitary Survey of Drinking Water Quality in Some Areas in Giza Governorate. The Medical Journal of Cairo University. 2019;87:2539–46.

161. Sadik NJ, Uprety S, Nalweyiso A, Kiggundu N, Banadda NE, Shisler JL, et al. Quantification of multiple waterborne pathogens in drinking water, drainage channels, and surface water in Kampala, Uganda, during seasonal variation. GeoHealth. 2017;1(6):258–69.

162. Okpasuo OJ, Aguzie IO, Joy AT, Okafor FC, Okpasuo OJ, Aguzie IO, et al. Risk assessment of waterborne infections in Enugu State, Nigeria: Implications of household water choices, knowledge, and practices. AIMSPH. 2020;7(3):634–49.

163. Wahome CN, Okemo PO, Nyamache AK. Microbial quality and antibiotic resistant bacterial pathogens isolated from groundwater used by residents of Ongata Rongai, Kajiado North County, Kenya. International Journal of Biological and Chemical Sciences. 2014;8(1):134–43.

164. Mahagamage MGYL, Pathirage MVSC, Manage PM. Contamination Status of *Salmonella spp*., *Shigella spp*. and *Campylobacter spp*. in Surface and Groundwater of the Kelani River Basin, Sri Lanka. Water.2020;12(8):2187.

165. Sarker S, Mahmud S, Sultana R, Biswas R, Sarkar PP, Munayem MA, et al. Quality Assessment of Surface and Drinking Water of Nakla Paurosova, Sherpur, Bangladesh. Advances in Microbiology.2019;9(8):703–27.

166. Gemeda ST, Desta AF, Gari SR, Jass J, Woldemedhin WM, Haileyesus MN. Enterotoxins as a molecular marker of water quality. Water Practice and Technology. 2022;17(2):623–636. doi:10.2166/wpt.2022.016.

167. Bonkoungou IJO, Somda NS, Traoré O, Zoma BS, Garba Z, Drabo KM, Barro N. Detection of diarrheagenic *Escherichia coli* in human diarrheic stool and drinking water samples in Ouagadougou, Burkina faso. African Journal of Infectious Diseases. 2020;15(1):53-58. doi:10.21010/ajid.v15i1.7.

168. Welch P, David J, Clarke W, Trinidade A, Penner D, et al. Microbial quality of water in rural communities of Trinidad. Revista Panamericana de Salud Publica. 2000;8(3):172-180. doi:10.1590/s1020-49892000000800004.

169. Vadivelu J, Feachem RG, Drasar BS, Harrison TJ, Parasakthi N, Thambypillai V, et al. Enterotoxigenic Escherichia coli in the domestic environment of a Malaysian village. Epidemiology and Infection.1989;103(3):497–511.doi:10.1017/s0950268800030909.

170. Lothigius A, Janzon A, Begum Y, Sjöling A, Qadri F, Svennerholm AM, et al. Enterotoxigenic Escherichia coli is detectable in water samples from an endemic area by real-time PCR. Journal of Applied Microbiology.2008;104(4):1128–36.doi:10.1111/j. 1365-2672.2007.03628.x.

171. Mersha G, Asrat D, Zewde BM, Kyule M. Occurrence of *Escherichia coli* O157:H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. Letters to Applied Microbiology.2010;50(1):71-76.doi:10.1111.j.1472-765X.2009.02757.x.

172. Momba MNB, Abong'o BO, Mwambakana JN. Prevalence of enterohaemorrhagic *Escherichia coli* O157:H7 in drinking water and its predicted impact on diarrhoeic HIV/AIDS patients in the Amathole District, Eastern Cape Province, South Africa. Water SA. 2008;34(3):365–72.

173. Caroline RS, Matheus SS, Kawana HM, et al. Shiga toxin-producing Escherichia coli isolation and characterization in drinking water. In: proceedings of the III Parana Congress of Microbiology.2018;2. Available from: https://proceedings.science/acpm/cpm-2018/papers/shiga-toxin-producing-escherichia-coli-isolation-and-characterization-in-drinking-water?lang=pt-br

174. Kichana E, Addy F, Dufailu OA. Genetic characterization and antimicrobial susceptibility of *Escherichia coli* isolated from household water sources in northern Ghana. Journal of Water and Health.2022;20(5):770–780.doi:10.2166/wh.2022.197.

175. Moreira M, Ferreira JFG, Salles LAF, Carneiro TGS, Fongaro G, Farias LM, et al. Isolation, Molecular Characterization and Geoprocessing of Enteropathogenic, Enterotoxigenic, and Shiga Toxin-Producing *Escherichia coli* in Drinking Water Sources from Southeast Brazil. Water Air Soil Pollution.2020;231(2):39.

176. Lascowski KM, Guth BE, Martins FH, Rocha SP, Irino K, Pelayo JS. Shiga toxin-producing *Escherichia coli* in drinking water supplies of north Paraná State, Brazil. Journal of Applied Microbiology.2013;114(4):1230-9.doi:10.1111/jam.12113.

177. Ram S, Vajpayee P, Shanker R. Contamination of Potable Water Distribution Systems by Multiantimicrobial-Resistant Enterohemorrhagic *Escherichia coli*. Environmental Health Perspective. 2008;116(4):448-452. doi:10.1289/ehp.10809.

178. Crespo-Medina M, Greaves I, Hunter PR, Minnigh H, Ramírez-Toro G. Detection of Shiga toxin-encoding genes in small community water supplies. Journal of Water and Health. 2020;18(6):937–945. doi:10.2166/wh.2020.236.

179. YANG Bo-yun,WEI Yu-mei,YANG Li,SONG Han,YANG Hai-xia, et al. LAMP based method for detecting human rotavirus and its application in water samples. Journal of Safety and Environment. 2013;01.

180. Gratacap-Cavallier B, Genoulaz O, Brengel-Pesce K, Soule H, Innocenti-Francillard P, Bost M, et al. Detection of Human and Animal Rotavirus Sequences in Drinking Water. Applied and Environmental Microbiology. 2000;66(6):2690–2. doi:10.1128/ AEM.66.6. 2690-2692.

181. Yousuf FA, Siddiqui R, Khan NA. Presence of rotavirus and free-living *amoebae* in the water supplies of Karachi, Pakistan. Revista do Instituto de Medicina Tropical de São Paulo. 2017;59:e32. doi:10.1590/S1678-9946201759032.

182. Toranzos GA, Hanssen H, Gerba CP. Occurrence of enteroviruses and rotaviruses in drinking water in Colombia: Health-Relat Water Microbiol 1986. Water Science and Technology.1986;18:109–14.

183. Pelaez-Carvajal D, Guzman B, Rodríguez J, Acero F, Nava G. Presence of enteric viruses in water samples for consumption in Colombia: Challenges for supply systems. Biomedica Revista del Instituto Nacional de Salud. 2016;36:169–78. doi:10.7705/ biomedica.v36i0. 2987.

184. Verheyen J, Timmen-Wego M, Laudien R, Boussaad I, Sen S, et al. Detection of Adenoviruses and Rotaviruses in Drinking Water Sources Used In Rural Areas of Benin, West Africa. Applied and Environmental Microbiology.2009;75(9):2798-801.doi:10.1128/ AEM.01807-08.

185. Van Zyl WB, Page NA, Grabow WO, Steele AD, Taylor MB. Molecular Epidemiology of Group A Rotaviruses in Water Sources and Selected Raw Vegetables in Southern Africa. Applied and Environmental Microbiology.2006;72(7):4554-60. doi:10.1128/ AEM.02119-05.

186. Mahmoud A.G, Abdou K.A, Elmahdy M.E, Mohamed N.F, Neveen M.R, et al. Genotyping and interaction-reality of *Acanthamoeba*, enteric adenovirus and rotavirus in drinking water, Egypt. Egyptian Journal of Aquatic Biology and Fisheries.2019;23(2):65–79.

187. Rizk NM, Allayeh AK. Multiplex Semi-Nested RT-PCR for Genotyping of Rotaviruses Group A in Giza Tap Water, Egypt. Asian Journal of Water, Environment and Pollution. 2018;15(2):217-221.

188. Shaheen MNF. Prevalence, Genotyping, and Seasonality of Rotavirus in Environmental Water Samples and Stool Samples of Gastroenteritis Children in Egypt (2010-2016). Journal of Infectious Disease and Travel Medicine.2019;3(1):000125.

189. Dongdem J, Adjimani J, Armah G. Detection and characterization of human rotavirus in tap water by multiplex RT-PCR. Journal of Medicine and Medical Sciences.2010;1:223–30.

190. Barrantes K, Chacón L, Morales E, Rivera-Montero L, Pino M, Jiménez AG, et al. Occurrence of pathogenic microorganisms in small drinking-water systems in Costa Rica. Journal of Water and Health.2022;20(2):344–55.doi:10.2166/wh.2022.230.

191. He XQ, Cheng L, Zhang DY, Li W, Xie XM, et al.First Molecular Detection of Group A Rotaviruses in Drinking Water Sources in Beijing, China. Bulletin of Environmental Contamination and Toxicology.2009;83(1):120-4.doi:10.1007/s00128-009-9708-6.

192. Ahmad T, Arshad N, Adnan F, Zaidi N us SS, Shahid MT, Zahoor U, et al. Prevalence of rotavirus, adenovirus, hepatitis A virus and enterovirus in water samples collected from different region of Peshawar, Pakistan. Annals of Agricultural and Environmental Medicine. 2016;23(4):576–80.

193. Shoaib M, Furqan K, Rahman S ur, Naveed A, Islam Aqib A, Fakhar-e-Alam Kulyar M, et al. Detection of rotavirus in sewage and drinking water by Latex agglutination test. International Journal of Science and Research (IJSR).2019;9(2):8694.

194. Mattioli MC, Boehm AB, Davis J, Harris AR, Mrisho M, Pickering AJ. Enteric Pathogens in Stored Drinking Water and on Caregiver's Hands in Tanzanian Households with and without Reported Cases of Child Diarrhea.PLoS One.2014;9(1):e84939.

195. Mosley WH, Chen LC. An analytical framework for the study of child survival in developing countries. Bulletin of the World Health Organization.2003;81(2):140–150.

196. Federal Democratic Republic of Ethiopia- Population Census Commission. Summary and Statistical Report of the 2007 Population and Housing Census. Addis Ababa, Ethiopia; 2008.

197. Anouk Zijlma. The Weather and Climate in Ethiopia . TripSavvy; 2020. Available from: https:// www.tripsavvy.com/ethiopia-weather-and-average-temperatures-4071422

198. Mengistie B, Berhane Y, Worku A. Household Water Chlorination Reduces Incidence of Diarrhea among Under-Five Children in Rural Ethiopia: A Cluster Randomized Controlled Trial. PLoS ONE. 2013;8(10):e77887.

199. Ministry of Water, Irrigation and Electric. Second Growth and Transformation National Plan for the Water Supply and Sanitation Sub-sector (2015/16 – 2019/20), Addis Ababa, Ethiopia;2015.

200. World Health Organization, United Nation Children's Fund. Core questions on drinking-water and sanitation for household surveys. Joint Monitoring Programme (JMP); 2006. Available from: http://awareness.who.int/water_sanitation_health/publications/core_questions/en/

201. World Health Organization and Maternal and Child Epidemiology Estimate (MCEE) group. Estimates of child cause of death. WHO and MCEE. 2018.

202. World Health Organization. Global Health Observatory: Proportion of deaths by country - HIV/AIDS. World Health Organization.2016.

203. Song T, Toma C, Nakasone N, Iwanaga M. Sensitive and rapid detection of *Shigella* and enteroinvasive *Escherichia coli* by a loop-mediated isothermal amplification method. FEMS Microbiology Letters. 2005;243(1):259-263.doi:10.1016/j.femsle.2004.12.014.

204. Fout S.G, Schaefer W.F, Messer W.J, Dahling R.D, Stetler E.R. ICR Microbial Laboratory Manual. Environmental Protection Agency.1996.

205. Polaczyk AL, Roberts JM, Hill VR. Evaluation of 1MDS electropositive microfilters for simultaneous recovery of multiple microbe classes from tap water. Journal of Microbiology Methods.2007;68(2):260-266.doi:10.1016/j.mimet.2006.08.007.

206. New England BioLabs Inc. First Strand cDNA Synthesis Protocols (E6300). New England BioLabs Inc. Available from: https://international.neb.com

207. Shi, Y. D, Dong H.J, Wang R, Zhang L, Ning C, Jian F.C. Exploration of Loop-mediated Isothermal Amplification (LAMP) for detection of *Cryptosporidium* parvum. Chinese Journal of Zoonoses.2012;28(12):1195–1201.

208. Ranjbar R, Erfanmanesh M, Afshar D, Mohammadi M, Ghaderi O, Haghnazari A. Visual Detection of Enterohemorrhagic *Escherichia coli* O157:H7 Using Loop-Mediated Isothermal Amplification. Electron Physician.2016;8(6):2576-2585.doi:10.19082/2576.

209. Mahony J, Chong S, Stone C, Chui L (2016) Evaluation of Four Loop-Mediated Isothermal Amplification (LAMP) Assays for Identification of Shiga Toxin Producing *E.Coli* O157 (STEC) and Non-O157 Strains. Advances in Molecular Diagnostics.2016;1:104. doi:10.4172/amd.1000104.

210. Malik YS, Sharma K, Kumar N, Shivachandra SB, Rawat V, Rakholia R, et al. Rapid detection of human rotavirus using NSP4 gene specific reverse transcription loop-mediated isothermal amplification assay. Indian Journal of Virology.2013;24(2):265–271.

211. Bio-Synthesis Inc. What is an oligo- or oligonucleotide?. Bio-Synthesis Inc.2014. Available from: https://www.biosyn.com

212. National Human Genome Research Institute. Primer [Internet]. Genome.gov. [cited 2022 Jul 25]. Available from: https://www.genome.gov/genetics-glossary/Primer

213. Das A, Chowdhury R. Searching cause of death through different autopsy methods: A new initiative. Journal of Family Medicine and Primary Care. 2017;6(2):191 doi:10.4103/jfmpc.jfmpc_194_16.

214. Tunga M, Lungo J, Chambua J, Kateule R. Verbal autopsy models in determining causes of death.Tropical Medicine and International Health.2021;26(12):1560–7.doi:10.1111/tmi.13678

215. Robert M, Zahra R, Loraine W, Nobuko M. Comparison of Cause-of-Death Classification Methods for Verbal Autopsies in Mozambique. Chapel Hill, NC, USA: Measure Evaluation.2020.

216. Baqui AH, Black RE, Arifeen SE, Hill K, Mitra SN, al Sabir A. Causes of childhood deaths in Bangladesh: results of a nationwide verbal autopsy study. Bulletin of the World Health Organization,1998;76(2):161-171.

217. Rai SK, Kant S, Srivastava R, et al. Causes of and contributors to infant mortality in a rural community of North India: Evidence from verbal and social autopsy. BMJ Open.2017;7:e012856. doi:10.1136/bmjopen-2016-012856.

218. UNICEF. Neonatal mortality:UNICEF DATA.2017.Available from: https://data.unicef.org/topic/child-survival/neonatal-mortality/.

219. World Health Organization. Newborn death and illness [Internet]. World Health Organization; 2011 [cited 2020 May 17]. Available from: https://www.who.int/pmnch/media/press_materials/fs/fs_newborndealth_illness/en/

220. UNICEF. Maternal and Newborn Health Disparities in Ethiopia.UNICEF.2016.

221. Green R, Kolberg JM. Neonatal pneumonia in sub-Saharan Africa. BMC.2016;8(3). doi:10.1186/s41479-016-0003-0

222. Deribew A, Tessema F, Girma B. Determinants of under-five mortality in Gilgel Gibe Field Research Center, Southwest Ethiopia. Ethiopian Journal of Health Development. 2007;21(2):117–124.doi:10.4314/ejhd.v21i2.10038

223. Tessema F, et al. Vital Events Indicator and Causes of Death: From Longitudinal Datasets of Health and Demographic Surveillance System and Addis Ababa Mortality Surveillance Program in six Ethiopian Public Universities. Jimma University.2013.

224. Shamebo D, Sandstrom A, Muhe L, Freij L, Krantz I, et al. The Butajira project in Ethiopia: A nested case referent study of under-five mortality and its public health determinants. Bulletin of the World Health Organization.1993;71(3-4):389-396.

225. Shamebo D. Mothers' Perceived Cause of Death: A survey of Infant Mortality in Butajira, Ethiopia. The Ethiopian Journal of Health Development.1990;4(1).

226. Fikree FF, Azam SI, Berendes HW. Time to focus child survival programmes on the newborn: Assessment of levels and causes of infant mortality in rural Pakistan. Bulletin of the World Health Organization.2002;80(4):271-276.

227. Liang L, Kotadia N, English L, Kissoon N, Ansermino JM, Kabakyenga J, et al. Predictors of Mortality in Neonates and Infants Hospitalized With Sepsis or Serious Infections in Developing Countries: A Systematic Review. Front Pediatric.2018;6(277).doi:10.3389/fped.2018.00277

228. Kaduuli S. Infant and Child Mortality in Eastern Africa: Causes and Differentials. A Review of the Literature. National Centre for Development Studies. Australian National University.1988.

229. Wimalasena NN, Chang-Richards A, Wang KI, Dirks KN.Housing Risk Factors Associated with Respiratory Disease:A Systematic Review. International Journal of Environmental Research and Public Health.2021;18(6):2815.doi:10.3390/ijerph18062815

230. Mizgerd JP. Acute lower respiratory tract infection. The New England Journal of Medicine. 2008;358(7):716-727.doi:10.1056/NEJMra074111

231. Centers for Disease Control and Prevention. Disease Threats and Global WASH Killers: Cholera, Typhoid, and Other Waterborne Infections [Internet]. CDC;2020. [cited 2022 Jan 28]. Available from: https://www.cdc.gov/healthywater/global/WASH.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fhealthywater%2Fglobal%2Fdiarrhea-burden.html.

232. Asefa A, Qanche Q, Asaye Z, Abebe L. Determinants of Delayed Treatment-Seeking for Childhood Diarrheal Diseases in Southwest Ethiopia: A Case–Control Study. Pediatric Health, Medicine and Therapeutics.2020;11:171-178.doi:10.2147/PHMT.S257804

233. Amha M, Frew L, Tesfaye S. Child Health Problems in Ethiopia. Review Article. Ethiopian Public Health Association Expert Group.1996.

234. Farrar DS, Awasthi S, Fadel SA, Kumar R, Sinha A, et al. Seasonal variation and etiologic inferences of childhood pneumonia and diarrhea mortality in India. eLife.2019;8:e46202.

235. Jm M, Dh E, Rc H, Je T, Ls C, Ud P. Risk factors for diarrhea-associated infant mortality in the United States, 2005-2007.The Pediatric infectious disease journal. 2012;31. Available from: https://pubmed.ncbi.nlm.nih.gov/22411052/

236. Mugo NS, Agho KE, Zwi AB, Damundu EY, Dibley MJ. Determinants of neonatal, infant and under-five mortality in a war-affected country: analysis of the 2010 Household Health Survey in South Sudan. British Medical Journal Global Health.2018;3(1).

237. Ezeh OK, Agho KE, Dibley MJ, Hall J, Page AN. The Impact of Water and Sanitation on Childhood Mortality in Nigeria: Evidence from Demographic and Health Surveys, 2003–2013. International Journal of Environmental Research and Public Health.2014;11(9) :9256–72.

238. Günther I, Schipper Y. Pumps, germs and storage: the impact of improved water containers on water quality and health.Health Economics.2013;22(7):757-74.doi:10.1002/hec.2852.

239. Muhammed AU, Nikolaus G, Joachim VB. The impact of drinking water quality and sanitation on child health: Evidence from rural Ethiopia. The Journal of Development Studies.2016;55:10(2193-221).

240. Crump JA, Otieno PO, Slutsker L, Keswick BH, Rosen DH, Hoekstra RM, et al. Household based treatment of drinking water with flocculant-disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomised controlled trial. British Medical Journal.2005;331(7515):478.

241. Kelly A. Reynolds. Household Water Treatment: Saving Lives in the Developing World. WCP;2006.Available from: https://wcponline.com/2006/04/14/household-water-treatment-saving-lives-developing-world/

242. World Health Organization. Household water treatment and safe storage. Geneva, World Health Organization; 2019. Available from: http://www.who.int/water_sanitation_health/water-quality/household/en/

243. Quick RE, Kimura A, Thevos A, et al. Diarrhea prevention through household-level water disinfection and safe storage in Zambia. American Journal of Tropical Medicine and Hygiene.2002;66(5):584-9.doi:10.4269/ajtmh.2002.66.584

244. Alemu AM. To what extent does access to improved sanitation explain the observed differences in infant mortality in Africa?. African Journal of Primary Health Care and Family Medicine.2017;9(1).

245. Roushdy R, Sieverding M, Radwan H. The impact of water supply and sanitation on child health: evidence from Egypt. 3ie Grantee Final Report. 2013.

246. Getahun W, Adane M. Prevalence of acute diarrhea and water, sanitation, and hygiene (WASH) associated factors among children under five in Woldia Town, Amhara Region, northeastern Ethiopia.BMC Pediatrics.2021;21(1):227.

247. Soboksa NE. Associations between Improved Water Supply and Sanitation Usage and Childhood Diarrhea in Ethiopia: An Analysis of the 2016 Demographic and Health Survey. Environmental Health Insights.2021;15.doi:10.1177/11786302211002552

248. Delelegn MW, Endalamaw A, Belay GM. Determinants of Acute Diarrhea among Children Under-Five in Northeast Ethiopia: Unmatched Case-Control Study. Pediatrics Health, Medicine and Therapeutics.2020;11:323–33.

249. Bawankule R, Singh A, Kumar K, Pedgaonkar S. Disposal of children's stools and its association with childhood diarrhea in India. BioMed Central Public Health.2017;17.

250. Fayehun OA. Household Environmental Health Hazards and Child Survival in Sub-Saharan Africa. DHS Working Papers No. 74. Calverton, Maryland, USA: ICF Macro;2010.

251. Brown J, Cairncross S, Ensink JH. Water, sanitation, hygiene and enteric infections in children. Archives of Disease in Childhood.2013;98(8):629-34.doi:10.1136/archdischild-2011-301528.

252. Asfaha K, Alemseged F, Fisseha G, Haile K, Weldu M, Welehaweria N, et al. Determinants of childhood diarrhea in Medebay Zana District, Northwest Tigray, Ethiopia: A community based unmatched case-control study. BMC Pediatrics.2018;18:120.

253. Solomon ET, Gari SR, Kloos H, Mengistie B. Diarrheal morbidity and predisposing factors among children under 5 years of age in rural East Ethiopia. Tropical Medicine and Health. 2020;48(1):66.

254. United Nation-HABITAT. Solid Waste Management in the World's Cities. United Nations Human Settlements Programme (UN-HABITAT), UNON Print Shop; 2009.

255. Ezzati M, et al. Selected major risk factors and global and regional burden of disease. The Lancet. 2002; doi:10.1016/S0140-6736(02)11403-6

256. Curtis V, Cairncross S. Effect of washing hands with soap on diarrhoea risk in the community: A systematic review. The Lancet Infectious Diseases.2003;3(5):275-81. doi:10.1016/S1473-3099(03)00606-6

257. Cairncross S, Hunt C, Boisson S, Bostoen K, Curtis V, et al. Water, sanitation and hygiene for the prevention of diarrhoea. International Journal of Epidemiology.2010;39(1):i193-i205

258. Oloruntoba O. E. Hygiene and sanitation risk factors of diarrhoeal disease among under-five children in Ibadan, Nigeria.African Health Sciences.2014;14(4).doi:10.4314/ahs. v14i4.32

259. Dagne H, Bogale L, Borcha M, et al. Hand washing practice at critical times and its associated factors among mothers of under five children in Debark town, northwest Ethiopia. Italian Journal of Pediatrics.2019;45:120.

260. Waddington H, Snilstveit B, White H, Fewtrell L. Water, Sanitation and Hygiene interventions to combat Childhood diarrhoea in developing countries: A systematic Review. International Initiative for Impact evaluation.2009.

261. Li P, Yang SF, Lv BB, Zhao K, Lin MF, et al. Comparison of extraction methods of total microbial DNA from freshwater. Genetics and Molecular Research.2015;14(1):730-738. doi: http://dx.doi.org/10.4238/

262. Rashid M, Khan MN, Jalbani N. Detection of Human Adenovirus, Rotavirus, and Enterovirus in Tap Water and Their Association with the Overall Quality of Water in Karachi, Pakistan. Food and Environmental Virology.2021;13(1):44–52.

263. Sattar SA, Raphael RA, Springthorpe VS. Rotavirus survival in conventionally treated drinking water. Canadian Journal of Microbiology.1984;30(5):653–6.

264. Francy D.S, Stelzer E.A, Bushon R.N, Brady A.M.G, Mailot B.E, et al. Quantifying viruses and bacteria in wastewater—Results, interpretation methods, and quality control: U.S Geological Survey Scientific Investigations Report 2011-5150. 2011;44.

265. Shaheed A, Orgill J, Montgomery MA, Jeuland MA, Brown J. Why "improved" water sources are not always safe. Bulletin of World Health Organization.2014;92(4):283–9.

266. Kraay ANM, Brouwer AF, Lin N, Collender PA, Remais JV, Eisenberg JNS. Modeling environmentally mediated rotavirus transmission: The role of temperature and hydrologic factors. Proceedings of the National Academy of Sciences of the USA. 2018; 115(12):E2782-E2790. doi:10.1073/pnas.1719579115.

267. Plutzer J, Torokne A, Karanis P. Combination of ARAD microfibre filtration and LAMP methodology for simple, rapid and cost-effective detection of human pathogenic Giardia duodenalis and Cryptosporidium spp. in drinking water. Letters in Applied Microbiology. 2010; 50:82-88. doi:10.1111/j.1472-765X.2009.02758.x

268. Kothavade R.J. Potential molecular tools for assessing the public health risk associated with waterborne Cryptosporidium oocysts. Journal of Medical Microbiology.2012;61:1039–1051.doi:10.1099/jmm.0.043158-0.

269. Lowery CJ, Moore JE, Millar BC, Burke DP, McCorry KA, et al. Detection and speciation of Cryptosporidium spp. in environmental water samples by immunomagnetic separation, PCR and endonuclease restriction. Journal of Medical Microbiology.2000;49(9):779-85.doi:10.1099/0022-1317-49-9-779.

270. Haas C.N, Rose J.B. Developing an action level for Cryptosporidium. American Water Works Association.1995;87(9):81-84.doi:10.1002/J.1551-8833.1995.TB06424.X

271. Kolören Z, Delioğlu B.K, Taş B. Detection of cryptosporidium oocysts by loop mediated isothermal amplification (lamp) in surface water from river yeşilirmak and stream tersakan (samsun-amasya). Anadolu University Journal of Science and Technology C- Life Sciences and Biotechnology.2017;6(1):31-37.doi:10.18036/aubtdc.269434

272. World Health Organization. Risk Assessment of Cryptosporidium in Drinking Water. World Health Organization, Geneva, Switzerland.2009.

273. Yamazaki W. Sensitive and rapid detection of cholera toxin-producing Vibrio cholerae using loop-mediated isothermal amplification. Methods of Molecular Biology.2011;739:13-22.doi:10.1007/978-1-61779-102-4_2.

274. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis. PLoS Med.2014;11(5):e1001644.

275. Wright J, Gundry S, Conroy R. Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. Tropical Medicine and International Health.2004;9(1):106-117.

SUPPLEMENTARY/APPENDICES

LIST OF PUBLICATIONS

I. Mebrahtom S, Worku A, Gage DJ. Causes of infant deaths and patterns of associated factors in Eastern Ethiopia: Result of verbal autopsy (InterVA-4) study. PLos ONE. 2022;17(8):e0270245. https://doi.org/10.1371/journal.pone.0270245

II. Mebrahtom S, Worku A, Gage DJ. The risk of water, sanitation and Hygiene on diarrhea-related infant mortality in eastern Ethiopia: a population-based nested case-control. BMC Public Health.2022;22:343. https://:doi.org/10.1186/s12889022 12735-7

III. Mebrahtom S, Worku A, Gage DJ, Sime H, Abera A. Molecular detection of waterborne pathogens in infant drinking water and their relationship with water quality determinants: Loop-medicated Isothermal Amplification (LAMP) based study. Journal of Water and Health. 2023. http://:doi.org/10.2166/wh.2023.201

APPENDICES
ANNEX I: Data Collection Instruments

HOUSEHOLD LEVEL QUESTIONNAIRE

Identification Date of Interview ___/___/_____

Region:			Household code No		**Result**
Zone:			Team Number		1. Complete 2. Partially complete 3. Refused to take the interview
Woreda:			Interviewer Name		
Cluster number:					
Informed consent:	☐ Yes	☐ No		Supervisors name	
				Signature	

INFORMATION SHEET AND CONSENT FORM

Good Morning/Afternoon!

My name is…… I'm a data collector for dissertation study, which is going to be organized by Addis Ababa University, Institute of water Resource. I would like to collect data about your newborn child and household health status. The objective of this study is to assess the causes of Infant mortality and its associated factors in the community. Your name will not be written in this form. All information that you give will be kept strictly confidential. Your participation is voluntary and you are not obliged to answer any question you do not wish to answer. If you are not comfortable with the interview, you have full right to refuse or participate in the study at any time you want. But your honest response will contribute to generate information, which can be used to improve the status of children under one year of age in the community.

We would greatly appreciate your help in responding to this interview. The interview will take about ***15-20 minutes***. Would you be willing to participate?"

1. If yes, continue to interview
2. If no, skip to the other households

SNo	**QUESTIONS**	**RESPONSES**	**Skip**

1. Socio-demographic characteristics of Respondents			
1.1.	How old are you?	_____ Years	
1.2.	How many children do you have?		
1.3.	How many are all members of the household (all ages)	_____ number of people in HH	
1.4.	What is your current marital status?	1. Single 2. Married 3. Divorced 4. Widowed	
1.5.	What is your ethnicity?	1. Oromo 2. Afar 3. Amhara 4. Tigre 5. Somali 6. Other/Specifiy……	
1.6.	What is your religion?	1. Orthodox 2. Muslim 3. Catholic 4. Protestant 5. Others/specify……	
1.7.	What is the educational status of the respondent?	1. Unable to read and write 2. Able to read and write 3. Primary (Grade 1-8) 4. Secondary (Grade 9-12) 5. Vocational/Technique 6. College and above	
1.8.	What is your spouses' level of education?	1. Unable to read and write 2. Able to read and write 3. Primary (Grade 1-8) 4. Secondary (Grade 9-12) 5. Vocational/Technique 6. College and above 7. Don't know	
1.9.	What is your current occupation?	1. Housewife 2. Livestock herding 3. Farmer/own farm labor 4. Employed (salaried) 5. Daily labor/Wage labor 6. Small business/Petty trade like shop 7. Others/specify…….	
1.10.	What is your spouse's employment status?	1. Livestock herding 2. Farmer/own farm labor 3. Employed (salaried) 4. Daily labor/Wage labor 5. Small business/Petty trade like shop 6. Others/specify…….	
1.11	What is the main source of income in your household?	1. Employer (salary) 2. Livestock 3. own farm 4. Daily labor/Wage 5. Small business/Petty trade like shop 6. Other/specify……	
1.12	What is the average monthly income of your household	_____ birr	
2. Drinkig Water Access and utilization			

2.1.	What is the MAIN source of drinking water for your household?	1. Piped water into dwelling or compound 2. Public tap/ stand alone pipe 3. Tube Well or borehole 4. Dug well protected 5. Dug well unprotected 6. Spring protected 7. Spring unprotected 8. Private water vendor 9. Rain water 10. Unprotected source (River, pond, etc) 11. Others/Specify……	
2.2.	How far the water source from your household?	1. On the premises 2. Below 250meter 3. Between 250-500meter 4. Between 500-1000meter 5. Above 1000meter 6. Don't know	
2.3.	How long does it take to go to your main water source to get water and come back?	1. On the premises 2. Below 10 minutes 3. Between 10-30 minutes 4. Between 30-60 minutes 5. Above 60 minutes 6. Don't know	
2.4.	In your opinin, do yo get water all the time?	1. Yes 2. No	
2.5.	In the past 2 weeks, Is drinking water available?	1. Yes 2. No	
2.6.	How much water did you collect or fetch per day? (***Multiply the number of water collection container by their capacities by the number of trips (frequency) taken to get the total water collected)***	1. Number of jerry cans (5 Liter)_____) 2. Number of jerry cans (10 Liter)_____) 3. Number of jerry cans (20 Liter)_____) 4. Number of jerry cans (25 Liter)_____) 5. Number of buckets (10 Liter) _____) 6. Number of buckets (20 Liter)_____) 7. Other water vessels capacity……. Liters and number of water collected per day……. Total water collected_____ liters	
2.7.	What is the container being used to store water in the household?	1. Pot 2. Traditional water bag made of animal skin 3. Bucket 4. Jerry can 5. Barrel 6. Other/specify……	
2.8.	Can you show me where you store drinking water? **[CHECK WITH VISUAL OBSERVATION]** (Mark "**Y**es" for safe drinking water storage – if the drinking water storage container is narrow neck, clean and kept covered. Otherwise Mark "**N**o"	1. Yes 2. No	
2.9.	In your opinion, what are methods of making water safe for drinking at household level?	1. Filtration (Sand, ceramic etc) 2. Boiling 3. Water chemicals (Water guared, Pur etc)	

	Note: DO NOT READ THE ANSWERS OUT! Prompt the respondent for additional times by saying "do you know any other ways?"	4. Sun light 5. Filter with cloth 6. Leave to stand and settle 7. Don't know 8. Other/ Specify……	
2.10.	Do you treat drinking water at household level?	1. Yes 2. No……→	S.N 3.1
2.11.	If yes, how do you treat water at household level?	1. Filtration (Sand, ceramic etc) 2. Boiling 3. Water chemicals (Water guared, Pur etc) 4. Sun light 5. Filter with cloth 6. Leave to stand and settle 7. Other/ Specify……	
3. **Sanitation**			
3.1.	Do your household have/own a latrine	Yes No ..... →	S.N 3.6
3.2.	What type of latrine/toilet facility does your household have? **[CHECK WITH VISUAL OBSERVATION]**	1. Water/pour flush 2. Ventilated improved pit latrine (VIP) 3. Traditional Pit latrine with slab 4. Traditional Pit latrine without slab/open pit 5. Composting toilet 6. Other/ Specify	
3.3.	Do the households use latrine? **[CHECK WITH VISUAL OBSERVATION]** [Proxy evidence of use is described as "faeces in pit, visible access, absence of spider webs, and absence of faeces around household or pit latrine, and well maintained superstructure]	1. Yes 2. No	
3.4.	Is the latrine sanitary? [**CHECK WITH VISUAL OBSERVATION]** (**Y**es - includes a clean squatting hole and slab, few or no flies) (No - includes filled up pits, dirty hole or slab, severe fly problem)	1. Yes 2. No	
3.5.	Is there hand washing facility with soap and/or water at the latrine? [**CHECK WITH VISUAL OBSERVATION]**	Only hand washing facility available Hand washing facility filled with water and soap or ash available Hand washing facility filled with water available Empty Hand washing facility and soap or ash available No hand washing facility Others/ Specify……	

3.6.	If no latrine, Where do you and your household members defecate regularly?	1. Open field 2. Sharing with neighborhood 3. Communal latrine 4. Public latrine 5. Bucket/popo/plastic use 6. Others/Specify…… 7. Not applicable	
3.7.	How do you dispose of feces of children in the household?	1. Put/rinsed into toilet or latrine 2. Disposed in the waste disposal 3. Buried 4. Left it open 5. Provided to animals feed 6. Other/Specify…….	
3.8.	How do you dispose liquid wastes of the household?	1. Dispose/Spiel on an open field 2. Dispose to latrine 3. Infiltrate to the ground 4. Linked with storm water canal 5. Disposed by water and liquid waste disposal authority 6. Others/Specify……	
3.9.	How do you dispose solid wastes of the household?	1. Dispose to waste collection pit 2. Buried 3. Burn it 4. Provide to privet waste collection groups 5. Composting 6. Dispose to open field 7. Other/Specify…….....	
4. Hygiene			
4.1.	In your opinion, when is a good time to wash your hands? Note: DO NOT READ OUT THE ANSWERS! Prompt the person for additional times by saying "do you know any other times?"	1. After using the toilet 2. Before eating 3. Before preparing food 4. Before feeding infants/baby 5. After cleaning baby's bottom/handling baby's excreta 6. Before breastfeeding the baby 7. Don't know	
4.2.	When usually wash your hands? [Multiple answers are allowed]	1. After using the toilet 2. Before eating 3. Before preparing food 4. Before feeding infants/baby 5. After cleaning baby's bottom/handling baby's excreta 6. Before breastfeeding the baby 7. I do not wash my hands	
4.3.	What do you use usually when you wash your hands?	1. Using water only 2. Using water & soap 3. Using water and ash/sand 4. Others/ Specify……	
4.4.	Where do you received any water, sanitation and hygiene education/messages?	1. Health Extension Workers 2. Health Professionals (Doctors, Nurse, HO,..) 3. Radio/Television 4. I didn't receive any education	

		5. Other/Specify……	

VERBAL AUTOPSY QUESTIONNAIRE

INFORMATION SHEET AND CONSENT FORM

Good Morning/Afternoon!

My name is…… I'm a data collector for the study associated with cause of infant mortality. This study is organized by Addis Ababa University, Institute of Water Resources. I would like to ask you about the recent illness of your child that led to his/her death.

All the information that you provide will be kept confidential, and will only be used to understand the causes of infant deaths with their contributing factors in your area. This information will be used to improve the health program for our community to decrease future deaths. Your participation is voluntary and you are not obliged to answer any question you do not wish to answer. If you are not comfortable with the interview, you have full right to refuse or participate in the study at any time you want. But your honest response will contribute to generate information, which can be used to improve the health problems associated with mortality. Your name will be written on our forms so we can recognize what you said and contact you in the future if necessary. However, when the data are put together with those from other people, nobody else will have access to your name, if you prefer so.

We would greatly appreciate your help in responding to this interview. The interview will take about ***25-30 minutes***. Would you be willing to participate?"

1. If yes, continue to interview
2. If no, skip to the other households

Instruction to the Interviewers:

- For the deceased neonate (<1month of age), use Verbal Autopsy Questionnaire (I)
- For the deceased post-neonate (1-11months of age), use Verbal Autopsy Questionnaire (II)

ETHIOPIAN INSTITUTE OF WATER RESOURCES

ADDIS ABABA UNIVERSITY

VERBAL AUTOPSY QUESTIONNAIRE (I)

Death of a child aged <1month

NO.	QUESTIONS AND FILTERS	ANSWER		SKIP
0A100a	Is this a region of high HIV/AIDS prevalence?	YES	☐	
		NO	☐	
0A100b	Is this a region of high malaria prevalence?	YES	☐	
		NO	☐	
SECTION 1. INFORMATION ON THE DECEASED				
1A100a	What was the first or given name(s) of the deceased? _____			
1A100b	What was the surname (or family name) of the deceased? _____			
1A110	What was the sex of the deceased?	MALE	☐	
		FEMALE	☐	
1A200	Is the date of birth known?	YES	☐	
		NO	☐	➡ 1A220
		Refuse to answer	☐	➡ 1A220
1A210	When was the deceased born?	DAY	☐	
		MONTH	☐	
		YEAR	☐	
1A220	Is the date of death known?	YES	☐	
		NO	☐	➡ AAAA
		Refuse to answer	☐	➡ AAAA
1A230	When did (s)he die?	DAY	☐☐	
		MONTH	☐☐	
		YEAR	☐☐	
AAAA	Enter neonate's age in days	DAYS	☐☐	
AAAA	Enter neonate's age in hours	HOURS	☐☐	
AAAA	Enter neonate's age in minutes	MINUTES	☐☐	
1A500	What was her/his citizenship / nationality?	Citizen at birth	☐	
		Naturalized citizen	☐	

		Foreign national	☐	
		Don't know	☐	
1A510	What was her/his ethnicity? _____			
1A520	What was her/his place of birth? _____			
1A530	What was her/his place of residence? (– if applicable) _____			
1A550	Where did death occur?(specify country, province, district, village) _____			
1A560	Where did the deceased die?	Hospital	☐	
		Other health facility	☐	
		Home	☐	
		On route to facility or hospital	☐	
		Other	☐	
		Don't know	☐	
		Refuse to answer	☐	
1A620	What was the name of the father? Surname: _____ Name: _____			
1A630	What was the name of the mother? Surname: _____ Name: _____			

SECTION 2. VITAL REGISTRATION AND CERTIFICATION				
1A700	Death registration number/certificate _____			
1A710	Date of registration	DAY MONTH YEAR	☐☐ ☐☐ ☐☐	
1A720	Place of registration _____			
1A730	National identification number of deceased _____			
SECTION 3. INFORMATION ON THE RESPONDENT AND BACKGROUND ABOUT INTERVIEW				
2A100	What is the name of VA respondent? Surname: _____ Name: _____			
2A110	What is the respondent's relationship to the deceased?	Parent Child Other family member Friend Health worker Public official Another relationship	☐ ☐ ☐ ☐ ☐ ☐ ☐	
2A115	Did the respondent live with the deceased in the period leading to her/his death?	YES NO Don't know Refuse to answer	☐ ☐ ☐ ☐	
2A120	Name of VA interviewer Surname: _____ Name:_____			
2A130	Time at start of interview	hh:mm 24h ___:___		
2A130	Time at end of interview	hh:mm 24h ___:___		
2A140	Date of interview	DAY MONTH YEAR	☐☐ ☐☐ ☐☐	
2A150	Did the respondent give consent?	YES NO	☐ ☐	
3A280	During which season did (s)he die?	Wet Dry	☐ ☐	
3A310	Did (s)he die suddenly?	YES NO Don't know Refuse to answer	☐ ☐ ☐ ☐	
3A3100	What age of the respondent (in full years)			
3A3101	What was her/his marital status?	Single Married Life partner Divorced Widowed Too young to be married Don't know Refuse to answer	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	

3A 3101	What was the date of marriage?	DAY ☐☐ MONTH ☐☐ YEAR ☐☐		
3A3102	What was her/his highest level of schooling?	No formal education	☐	
		Primary school(1-8 Grade)	☐	
		Secondary school (9-12 Grade)	☐	
		Higher then secondary school	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A3103	Was (s)he able to read and write? (select 'yes' also if only one of either reading or writing is know to the respondent)	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A3104	What was her/his economic activity status in year prior to death?	Mainly unemployed	☐	
		Mainly employed	☐	
		Home-maker	☐	
		Pensioner	☐	
		Student	☐	
		Other	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A3105	What was her/his occupation, that is, what kind of work does (s)he mainly do? _____			

SECTION 4. GENERAL SIGNS AND SYMPTOMS ASSOCIATED WITH FINAL ILLNESS				
3B100	Did (s)he have a fever?	YES	☐	
		NO	☐	➡ 3B130
		Don't know	☐	➡ 3B130
		Refuse to answer	☐	➡ 3B130
3B110	How many days did the fever last?	DAYS	☐☐	
3B130	Did (s)he have a cough?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B180	Did (s)he have any breathing problem?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B190	During the illness that led to death, did (s)he have fast breathing?	YES	☐	
		NO	☐	➡ 3B210
		Don't know	☐	➡ 3B210
		Refuse to answer	☐	➡ 3B210
3B200	For how many days did the fast breathing last?	DAYS	☐☐	
3B210	Did (s)he have breathlessness?	YES	☐	
		NO	☐	➡ 3B242
		Don't know	☐	➡ 3B242
		Refuse to answer	☐	➡ 3B242
3B220	For how many weeks did (s)he have breathlessness?	WEEKS	☐☐	
3B242	During the illness that led to death, did (s)he have difficulty breathing?	YES	☐	
		NO	☐	➡ 3B250
		Don't know	☐	➡ 3B250
		Refuse to answer	☐	➡ 3B250
3B244	For how many days did the difficulty breathing last?	DAYS	☐☐	
3B250	Did you see the lower chest wall/ribs being pulled in as the child breathed?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B260	During the illness that led to death did his/her breathing sound like any of the following:	Stridor	☐	
		Grunting	☐	
		Wheezing	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B280	Did (s)he have diarrhoea?	YES	☐	
		NO	☐	➡ 3B310
		Don't know	☐	➡ 3B310
		Refuse to answer	☐	➡ 3B310
3B300	At any time during the final illness was there blood in the stools?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B310	Did (s)he vomit?	YES	☐	
		NO	☐	➡ 3B330
		Don't know	☐	➡ 3B330
		Refuse to answer	☐	➡ 3B330
3B315	For how many days before death did (s)he vomit?	DAYS	☐☐	
3B320	Did (s)he vomit blood?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3B330	Did (s)he have any abdominal problem?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B360	Did (s)he have a more than usually protruding abdomen?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B440	Was (s)he unconscious for more than 24 hours before death?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B460	Did (s)he have convulsions?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B530	Did (s)he have any skin problems?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B560	During the illness that led to death, did (s)he have any skin rash?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B594	During the illness that led to death, did he/she have areas of the skin that turned black?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B596	During the illness that led to death, did (s)he bleed from anywhere?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B750	Did (s)he have yellow discoloration of the eyes?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 5. NEONATAL AND CHILD HISTORY, SIGNS AND SYMPTOMS				
3D070	How old was the baby when the fatal illness started?	DAYS	☐☐	
3D100	Was the child part of a multiple birth?	YES	☐	
		NO	☐	➡ 3D104
		Don't know	☐	➡ 3D104
		Refuse to answer	☐	➡ 3D104
3D102	Was the child the first, second, or later in the birth order?	First	☐	
		Second or later	☐	
3D104	Is the mother still alive?	YES	☐	➡ 3D155
		NO	☐	
		Don't know	☐	➡ 3D155
		Refuse to answer	☐	➡ 3D155
3D106	Did the mother die during or after the delivery?	During delivery	☐	➡ 3D155
		After delivery	☐	
3D108a	How many months after the delivery did the mother die?	MONTHS	☐☐	
3D108b	How many days after the delivery did the mother die?	DAYS	☐☐	
3D155	Where was the deceased born?	Hospital	☐	
		Other health facility	☐	
		Home	☐	
		On route to hospital or facility	☐	
		Other	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D165	Did the mother receive professional assistance during the delivery?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D180	At birth, was the baby of usual size?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D190	At birth, was the baby smaller than usual, (weighing less than 2.5 kg)?	YES	☐	➡ 3D201
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D200	At birth, was the baby larger than usual, weighing over 4.5 kg)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D201	What was the weight (in grams) of the deceased at birth?	GRAMS	☐☐☐☐	
3D210	How many months long was the pregnancy before the child was born?	MONTHS	☐☐	
3D215	Were there any complications in the late part of the pregnancy (defined as the last 3 months, before labour)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D221	Were there any complications during labour or delivery?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D230	Was any part of the baby physically abnormal at time of delivery? (for example: body part too large or too small, additional growth on body)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3D240	Did the baby/ child have a swelling or defect on the back?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D241	Did the baby/ child have a very large head?	YES	☐	➡ 3D251
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D242	Did the baby/ child have a very small head?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D251	Did the baby stop moving in the womb before labour started?	YES	☐	
		NO	☐	➡ 3D255
		Don't know	☐	➡ 3D255
		Refuse to answer	☐	➡ 3D255
3D251a	How many days before labour did you or the mother last feel the baby move? (maybe the respondent or health worker had examined the mother)	DAYS	☐☐	
3D251b	How many hours before labour did you or the mother last feel the baby move? (maybe the respondent or health worker had examined the mother)	HOURS	☐☐	
3D253	Was the baby born 24 hours or more after the water broke?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D254	Was the liquor foul smelling?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
How was the baby delivered?				
3D258	Was the delivery normal vaginal, without forceps or vacuum?	YES	☐	➡ 3D261
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D259	Was the delivery vaginal, with forceps or vacuum?	YES	☐	➡ 3D261
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D260	Was the delivery a caesarean section?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D261	Did you/the mother receive any vaccinations since reaching adulthood including during this pregnancy?	YES	☐	
		NO	☐	➡ 3D267
		Don't know	☐	➡ 3D267
		Refuse to answer	☐	➡ 3D267
3D263	How many doses?	DOSES	☐☐	
3D265	Did the mother receive tetanus toxoid (TT) vaccine?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D267	How many births, including stillbirths, did the baby's mother have before this baby?	BIRTHS	☐☐	

3D269	During the last 3 months of pregnancy, labour or delivery, did the baby's mother suffer from high blood pressure?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D271	Did the baby's mother have foul smelling vaginal discharge during pregnancy or after delivery?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D273	During the last 3 months of pregnancy, labour or delivery, did the baby's mother suffer from convulsions?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D275	During the last 3 months of pregnancy did the baby's mother suffer from blurred vision?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D276	Did the baby's mother have vaginal bleeding during the last 3 months of pregnancy but before labour started?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D277	Did the baby's bottom, feet, arm or hand come out of the vagina before its head?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D278	Was the umbilical cord wrapped more than once around the neck of the child at birth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D280	Was the baby blue in colour at birth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D285	Did the baby ever cry?	YES	☐	
		NO	☐	➡ 3D298
		Don't know	☐	
		Refuse to answer	☐	➡ 3D298
3D290	Did the baby cry immediately after birth, even if only a little bit?	YES	☐	
		NO	☐	➡ 3D294
		Don't know	☐	➡ 3D294
		Refuse to answer	☐	➡ 3D294
3D292	How many minutes after birth did the baby first cry?	MINUTES	☐☐	
3D294	Did the baby stop being able to cry?	YES	☐	
		NO	☐	➡ 3D298
		Don't know	☐	➡ 3D298
		Refuse to answer	☐	➡ 3D298
3D296	How many hours before death did the baby stop crying?	HOURS	☐☐	
3D298	Did the baby ever move?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D299	Did the baby ever breathe?	YES	☐	
		NO	☐	➡ 3D310
		Don't know	☐	
		Refuse to answer	☐	
3D300	Did the baby breathe immediately after birth, even a little?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3D310	Was the baby given assistance to breathe at birth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D320	If the baby didn't show any sign of life, was it born dead? Ask this question only if you answer three questions(3D285, 3D298, 3D299) as 'NO'	YES	☐	
		NO	☐	➡ 3D345
		Don't know	☐	➡ 3D345
		Refuse to answer	☐	➡ 3D345
3D325	Were there any bruises or signs of injury on child's body after the birth? Ask this question only if the baby was born dead	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D330	Was the dead baby macerated, that is, showed signs of decay? Ask this question only if the baby was born dead	YES	☐	➡ 3E100
		NO	☐	➡ 3E100
		Don't know	☐	➡ 3E100
		Refuse to answer	☐	➡ 3E100
3D345	Did the baby stop suckling?	YES	☐	
		NO	☐	➡ 3D360
		Don't know	☐	➡ 3D360
		Refuse to answer	☐	➡ 3D360
3D350	How many days after birth did the baby stop suckling?	DAYS	☐☐	
3D360	Did the baby have convulsions starting within the first 24 hours of life?	YES	☐	➡ 3D380
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D370	Did the baby have convulsions starting more than 24 hours after birth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D380	Did the baby's body become stiff, with the back arched backwards?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D390	During the illness that led to death, did the baby have a bulging or raised fontanelle?	YES	☐	➡ 3D410
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D400	During the illness that led to death, did the baby have a sunken fontanelle?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D410	Did the baby become unresponsive or unconscious soon after birth, within less than 24 hours?	YES	☐	➡ 3D430
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D420	Did the baby become unresponsive or unconscious more than 24 hours after birth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D430	During the illness that led to death, did the baby become cold to touch?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D435	During the illness that led to death, did the baby become lethargic, after a period of normal activity?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3D440	Did the baby have redness or discharge from the umbilical cord stump?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D445	During the illness that led to death, did the baby have skin ulcer(s) or pits?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D450	During the illness that led to death, did the baby have yellow skin, palms (hand) or soles (foot)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3D455	Did the baby or infant appear to be healthy and then just die suddenly?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 6. HISTORY OF INJURIES/ACCIDENTS

3E100	Did (s)he suffer from any injury or accident that led to her/his death?	YES	☐	
		NO	☐	➡ 3G110
		Don't know	☐	➡ 3G110
		Refuse to answer	☐	➡ 3G110
3E102	Was the injury intentionally inflicted by someone else?	YES	☐	
		NO	☐	➡ 3E115
		Don't know	☐	➡ 3E115
		Refuse to answer	☐	➡ 3E115
3E104	Was (s)he injured by a firearm?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E106	Was (s)he stabbed, cut or pierced?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E108	Was (s)he strangled?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E111	Was (s)he injured by a blunt force?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E112	Was (s)he injured by burns?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E115	Was it a road traffic accident?	YES	☐	
		NO	☐	➡ 3E310
		Don't know	☐	➡ 3E310
		Refuse to answer	☐	➡ 3E310
3E120	What was her/his role in the road traffic accident?	Pedestrian	☐	
		In car or light vehicle	☐	
		In bus or heavy vehicle	☐	
		On a motorcycle	☐	
		On a pedal cycle	☐	
		Other	☐	

3E170	What was the counterpart that was hit during the road traffic accident?	Pedestrian	☐	
		Stationary object	☐	
		Car or light vehicle	☐	
		Bus or heavy vehicle	☐	
		Motorcycle	☐	
		Pedal cycle	☐	
		Other	☐	
3E310	Was (s)he injured in a fall?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E320	Did (s)he die of drowning?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E330	Did (s)he suffer from accidental burns?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E335	Was (s)he accidentally injured by a blunt force?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E340	Was (s)he accidentally injured by a plant/animal/insect that led to her/his death?	YES	☐	
		NO	☐	➡ 3E500
		Don't know	☐	➡ 3E500
		Refuse to answer	☐	➡ 3E500
3E400	What was the plant/animal/insect?	Dog	☐	
		Snake	☐	
		Insect or scorpion	☐	
		Other	☐	
		Don't know	☐	
3E500	Was (s)he injured by a force of nature?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E510	Was there any poisoning?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E520	Was (s)he subject to violence/assault?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E530	Was it electrocution?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 7. HEALTH SERVICE UTILISATION				
3G110	Did (s)he receive any treatment for the illness that led to death?	YES	☐	
		NO	☐	➡ 3H100
		Don't know	☐	➡ 3H100
		Refuse to answer	☐	➡ 3H100
3G120	Did (s)he receive oral rehydration salts?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G130	Did (s)he receive (or need) intravenous fluids (drip) treatment?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G140	Did (s)he receive (or need) a blood transfusion?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G150	Did (s)he receive (or need) treatment/food through a tube passed through the nose?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G160	Did (s)he receive (or need) injectable antibiotics?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G165	Did (s)he receive (or need) antiretroviral therapy (ART)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G170	Did (s)he receive (or need) an operation for the illness?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G190	Was (s)he discharge from hospital very ill?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3H100	Has (s)he received immunization?	YES	☐	
		NO	☐	➡ 3H130
		Don't know	☐	➡ 3H130
		Refuse to answer	☐	➡ 3H130
3H110	Do you have the child's vaccination card?	YES	☐	
		NO	☐	➡ 3H130
		Don't know	☐	➡ 3H130
		Refuse to answer	☐	➡ 3H130
3H120	Can I see the vaccination card (note the vaccines the child received)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3H125	Note vaccines here: _____ _____ _____ _____			
3H130	Was care sought outside the home while (s)he had this illness?	YES	☐	
		NO	☐	➡ 3H160
		Don't know	☐	➡ 3H160
		Refuse to answer	☐	➡ 3H160

3H140	Where or from whom did you seek care?	Traditional healer	☐	
		Homeopath	☐	
		Religious leader	☐	
		Private hospital	☐	
		Government hospital	☐	
		Government health centre or clinic	☐	
		Community-based practitioner associated with Health system	☐	
		Trained birth attendant	☐	
		Private physician	☐	
		Pharmacy	☐	
		Don't know	☐	
		Refuse to answer	☐	
3H150	Record the name and address of any hospital, health centre or clinic where care was sought _____ _____			
3H160	Did a health care worker tell you the cause of death?	YES	☐	
		NO	☐	➡ 3H180
		Don't know	☐	➡ 3H180
		Refuse to answer	☐	➡ 3H180
3H170	What did the health care worker say? _____ _____			
3H180	Do you have any health records that belonged to the deceased?	YES	☐	
		NO	☐	➡ 3H330
		Don't know	☐	➡ 3H330
		Refuse to answer	☐	➡ 3H330
3H190	Can I see the health records?	YES	☐	
		NO	☐	➡ 3H330
		Don't know	☐	➡ 3H330
		Refuse to answer	☐	➡ 3H330
3H200	Record the date of the most recent (last) visit	DAY	☐☐	
		MONTH	☐☐	
		YEAR	☐☐	
3H210	Record the date of the last but one (second last) visit	DAY	☐☐	
		MONTH	☐☐	
		YEAR	☐☐	
3H220	Record the date of the last note on the health records	DAY	☐☐	
		MONTH	☐☐	
		YEAR	☐☐	
3H230	Record the weight (in kilogrammes) written at the most recent (last) visit	[KG]	☐☐.☐	
3H240	Record the weight (in kilogrammes) written at the last but one (second last) visit	[KG]	☐☐.☐	
3H250	Transcribe the last note on the health records _____ _____			

3H330	Has the deceased's (biological) mother ever been tested for HIV?	YES	☐	
		NO	☐	➡ 3H350
		Don't know	☐	➡ 3H350
		Refuse to answer	☐	➡ 3H350
3H340	Was the HIV test ever positive?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3H350	Has the deceased's (biological) mother ever been told she had HIV/AIDS by a health worker?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
SECTION 8. BACKGROUND AND CONTEXT				
4A100	In the final days before death, did s/he travel to a hospital or health facility?	YES	☐	
		NO	☐	➡ 4A150
		Don't know	☐	➡ 4A150
		Refuse to answer	☐	➡ 4A150
4A110	Did (s)he use motorised transport to get to the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A120	Were there any problems during admission to the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A130	Were there any problems with the way (s)he was treated (medical treatment, procedures, interpersonal attitudes, respect, dignity) in the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A140	Were there any problems getting medications, or diagnostic tests in the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A150	Does it take more than 2 hours to get to the nearest hospital or health facility from the deceased's household?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A160	In the final days before death, were there any doubts about whether medical care was needed?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A170	In the final days before death, was traditional medicine used?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A180	In the final days before death, did anyone use a telephone or cell phone to call for help?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A190	Over the course of illness, did the total costs of care and treatment prohibit other household payments?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 9. OPTIONAL OPEN NARRATIVE				
5A100	Narrative Description _____ _____ _____ _____ _____ _____			
SECTION 10. DEATH CERTIFICATE				
6H260	Was a death certificate issued?	YES	☐	
		NO	☐	➡ End
		Don't know	☐	➡ End
		Refuse to answer	☐	➡ End
6H270	Can I see the death certificate?	YES	☐	
		NO	☐	➡ End
		Don't know	☐	➡ End
		Refuse to answer	☐	➡ End
6H280	Record the immediate cause of death from the certificate (line 1a) * _____			Duration 1(a)
6H290	Record the first antecedent cause of death from the certificate (line 1b) _____			Duration 1(b)
6H300	Record the second antecedent cause of death from the certificate (line 1c) _____			Duration 1(c)
6H310	Record the third antecedent cause of death from the certificate (line 1d) _____			Duration 1(d)
6H320	Record the contributing cause(s) of death from the certificate (part 2) _____			

ETHIOPIAN INSTITUTE OF WATER RESOURCES

ADDIS ABABA UNIVERSITY

VERBAL AUTOPSY QUESTIONNAIRE (II)

Death of an infant aged 1-11 months

NO.	QUESTIONS AND FILTERS	ANSWER		SKIP
0A100a	Is this a region of high HIV/AIDS prevalence?	YES	☐	
		NO	☐	
0A100b	Is this a region of high malaria prevalence?	YES	☐	
		NO	☐	
SECTION 1. INFORMATION ON THE DECEASED INFANT				
1A100a	What was the first or given name(s) of the deceased? _____			
1A100b	What was the **surname (or family name)** of the deceased? _____			
1A110	What was the sex of the deceased?	MALE	☐	➡ 1A200
		FEMALE	☐	
1A400	Was this a woman who died more than 42 days but less than 1 year after being pregnant or delivering a baby?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
1A401	Was this a woman who died more than 42days after being pregnant or delivering a baby?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
1A200	Is the date of birth known?	YES	☐	
		NO	☐	➡ 1A220
		Refuse to answer	☐	➡ 1A220
1A210	When was the deceased born?	DAY	☐	
		MONTH	☐	
		YEAR	☐	
1A220	Is the date of death known?	YES	☐	
		NO	☐	➡ AAAA
		Refuse to answer	☐	➡ AAAA
1A230	When did (s)he die?	DAY	☐☐	
		MONTH	☐☐	
		YEAR	☐☐	
AAAA	Put infant's age in months	MONTHS	☐☐	
1A500	What was her/his citizenship/nationality?	Citizen at birth	☐	
		Naturalized citizen	☐	
		Foreign national	☐	
		Don't know	☐	
1A510	What was her/his ethnicity? _____			
1A520	What was her/his place of birth? _____			
1A530	What was her/his place of usual residence? (The place where the person lived most of the year) _____			
1A540	What was her/his place of normal residence 1 to 5 years before death? _____			
1A550	Where did death occur? (specify country, province, district, village) _____			
1A560	Where did the deceased die?	Hospital	☐	
		Other health facility	☐	
		Home	☐	
		On route to facility or hospital	☐	
		Other	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 2. Vital Registration and Certification			
1A700	Death registration number/ certificate _____		
1A710	Date of registration	DAY ☐☐ MONTH ☐☐ YEAR ☐☐	
1A720	Place of registration _____		
1A730	National identification number of deceased _____		
SECTION 3. Information on the respondent and background about interview			
2A100	What is the name of VA respondent? _____		
2A110	What is the respondent's relationship to the deceased?	Parent ☐ Child ☐ Other family member ☐ Friend ☐ Health worker ☐ Public official ☐ Another relationship ☐	
2A115	Did the respondent live with the deceased in the period leading to her/his death?	YES ☐ NO ☐ Don't know ☐ Refuse to answer ☐	
2A120	Name of VA interviewer _____		
2A130	Time at start of interview	hh:mm 24h ___:___	
2A135	Time at end of interview	hh:mm 24h ___:___	
2A140	Date of interview	DAY ☐☐ MONTH ☐☐ YEAR ☐☐	
2A150	Did the respondent give consent?	YES ☐ NO ☐	
3A280	During which season did (s)he die?	WET ☐ DRY ☐	
3A300	For how many days was (s)he ill before (s)he died?	DAYS ☐☐	
3A310	Did (s)he die suddenly?	YES ☐ NO ☐ Don't know ☐ Refuse to answer ☐	
3A3100	What age of the respondent (in full years)		
3A3101	What was her/his marital status?	Single ☐ Married ☐ Life partner ☐ Divorced ☐ Widowed ☐ Too young to be married ☐ Don't know ☐ Refuse to answer ☐	

3A 3101	What was the date of marriage?	DAY MONTH YEAR	☐☐ ☐☐ ☐☐	
3A3102	What was her/his highest level of schooling?	No formal education	☐	
		Primary school(1-8 Grade)	☐	
		Secondary school (9-12 Grade)	☐	
		Higher then secondary school	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A3103	Was (s)he able to read and write? (select 'yes' also if only one of either reading or writing is know to the respondent)	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A3104	What was her/his economic activity status in year prior to death?	Mainly unemployed	☐	
		Mainly employed	☐	
		Home-maker	☐	
		Pensioner	☐	
		Student	☐	
		Other	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A3105	What was her/his occupation, that is, what kind of work does (s)he mainly do? _____			

SECTION 4: MEDICAL HISTORY ASSOCIATED WITH FINAL ILLNESS.

3A100	Was there any diagnosis by a physician or health worker of tuberculosis?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A110	Was there any diagnosis by a physician or health worker of HIV/AIDS?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A120	Did (s)he have a recent positive test by a physician or health worker for malaria?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A130	Did (s)he have a recent negative test by a physician or health worker for malaria?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A135	Was there any diagnosis by a physician or health worker of dengue fever?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A140	Was there any diagnosis by a physician or health worker of measles?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A150	Was there any diagnosis by a physician or health worker of high blood pressure?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3A160	Was there any diagnosis by a physician or health worker of heart disease?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A170	Was there any diagnosis by a physician or health worker of diabetes?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A180	Was there any diagnosis by a physician or health worker of asthma?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A190	Was there any diagnosis by a physician or health worker of epilepsy?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A200	Was there any diagnosis by a physician or health worker of cancer?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A210	Was there any diagnosis by a physician or health worker of Chronic Obstructive Pulmonary Disease (COPD)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A220	Was there any diagnosis by a physician or health worker of dementia?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A230	Was there any diagnosis by a physician or health worker of depression?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A240	Was there any diagnosis by a physician or health worker of stroke?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A250	Was there any diagnosis by a physician or health worker of sickle cell disease?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A260	Was there any diagnosis by a physician or health worker of kidney disease?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3A270	Was there any diagnosis by a physician or health worker of liver disease?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 5: GENERAL SIGNS AND SYMPTOMS ASSOCIATED WITH FINAL ILLNESS				
3B100	Did (s)he have a fever?	YES	☐	
		NO	☐	➡ 3B130
		Don't know	☐	➡ 3B130
		Refuse to answer	☐	➡ 3B130
3B110	How many days did the fever last?	DAYS	☐☐	
3B115	How severe was the fever?	Mild	☐	
		Moderate	☐	
		Severe	☐	
3B120	Did (s)he have night sweats?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B130	Did (s)he have a cough?	YES	☐	
		NO	☐	➡ 3B180
		Don't know	☐	➡ 3B180
		Refuse to answer	☐	➡ 3B180
3B140	For how many days did (s)he have a cough?	DAYS	☐☐	
3B150	Was the cough productive, with sputum?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B155	Was the cough very severe?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B160	Did (s)he cough up blood?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B180	Did (s)he have any breathing problem?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B190	During the illness that led to death, did (s)he have fast breathing?	YES	☐	
		NO	☐	➡ 3B210
		Don't know	☐	➡ 3B210
		Refuse to answer	☐	➡ 3B210
3B200	For how many days did the fast breathing last?	DAYS	☐☐	
3B210	Did (s)he have breathlessness?	YES	☐	
		NO	☐	➡ 3B242
		Don't know	☐	➡ 3B242
		Refuse to answer	☐	➡ 3B242
3B220	For how many weeks did (s)he have breathlessness?	WEEKS	☐☐	
3B230	Was (s)he unable to carry out daily routines due to breathlessness?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B240	Was (s)he breathless while lying flat?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B242	During the illness that led to death, did (s)he have difficulty breathing?	YES	☐	
		NO	☐	➡ 3B260
		Don't know	☐	➡ 3B260
		Refuse to answer	☐	➡ 3B260
3B246	Was the difficulty continuous or on and off?	Continuous	☐	
		On and off	☐	

3B260	During the illness that led to death did his/her breathing sound like any of the following: Stridor, Grunting, Wheezing	Stridor	☐	
		Grunting	☐	
		Wheezing	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B270	Did (s)he have severe chest pain?	YES	☐	
		NO	☐	➡ 3B280
		Don't know	☐	➡ 3B280
		Refuse to answer	☐	➡ 3B280
3B272	How many days before death did (s)he have severe chest pain?	DAYS	☐☐	
3B274	How many minutes did the pain last?	MINUTES	☐☐	
3B280	Did (s)he have diarrhoea?	YES	☐	
		NO	☐	➡ 3B310
		Don't know	☐	➡ 3B310
		Refuse to answer	☐	➡ 3B310
3B290	For how many days did (s)he have diarrhoea?	DAYS	☐☐	
3B300	At any time during the final illness was there blood in the stools?	YES	☐	
		NO	☐	➡ 3B310
		Don't know	☐	➡ 3B310
		Refuse to answer	☐	➡ 3B310
3B305	Was there blood in the stool up until death?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B310	Did (s)he vomit?	YES	☐	
		NO	☐	➡ 3B330
		Don't know	☐	➡ 3B330
		Refuse to answer	☐	➡ 3B330
3B315	For how many days before death did (s)he vomit?	DAYS	☐☐	
3B320	Did (s)he vomit blood?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B325	Was the vomit black?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B330	Did (s)he have any abdominal problem?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B340	Did (s)he have severe abdominal pain?	YES	☐	
		NO	☐	➡ 3B360
		Don't know	☐	➡ 3B360
		Refuse to answer	☐	➡ 3B360
3B350	For how many days before death did (s)he have severe abdominal pain?	DAYS	☐☐	
3B355	Was the pain in the upper or lower abdomen?	Upper abdomen	☐	
		Lower abdomen	☐	
3B360	Did (s)he have a more than usually protruding abdomen?	YES	☐	
		NO	☐	➡ 3B380
		Don't know	☐	➡ 3B380
		Refuse to answer	☐	➡ 3B380
3B370	For how many days did (s)he have a more than usually protruding abdomen?	DAYS	☐☐	
3B375	How rapidly did (s)he develop the protruding abdomen?	Rapidly	☐	
		Slowly	☐	

3B380	Did (s)he have any mass in the abdomen?	YES	☐	
		NO	☐	➡ 3B400
		Don't know	☐	➡ 3B400
		Refuse to answer	☐	➡ 3B400
3B390	For how many days before death did (s)he have a mass in the abdomen?	DAYS	☐☐	
3B400	Did (s)he have a severe headache?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B405	Did (s)he have a stiff neck during illness that led to death?	YES	☐	
		NO	☐	➡ 3B409
		Don't know	☐	➡ 3B409
		Refuse to answer	☐	➡ 3B409
3B407	For how many days before death did (s)he have stiff neck?	DAYS	☐☐	
3B409	Did (s)he have a painful neck during the illness that led to death?	YES	☐	
		NO	☐	➡ 3B420
		Don't know	☐	➡ 3B420
		Refuse to answer	☐	➡ 3B420
3B410	For how many days before death did (s)he have a painful neck?	DAYS	☐☐	
3B420	Did (s)he have mental confusion?	YES	☐	
		NO	☐	➡ 3B440
		Don't know	☐	➡ 3B440
		Refuse to answer	☐	➡ 3B440
3B430	For how many months did (s)he have mental confusion?	MONTHS	☐☐	
3B440	Was (s)he unconscious for more than 24 hours before death?	YES	☐	
		NO	☐	➡ 3B460
		Don't know	☐	➡ 3B460
		Refuse to answer	☐	➡ 3B460
3B450	Did the unconsciousness start suddenly, quickly (at least within a single day)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B455	Did the unconsciousness continue until death?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B460	Did (s)he have convulsions?	YES	☐	
		NO	☐	➡ 3B490
		Don't know	☐	➡ 3B490
		Refuse to answer	☐	➡ 3B490
3B465	Did (s)he experience any generalized convulsions or fits during the illness that led to death?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B470	For how many minutes did the convulsions last?	MINUTES	☐☐	
3B480	Did (s)he become unconscious immediately after the convulsion?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B490	Did (s)he have any urine problems?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B500	Did (s)he pass no urine at all?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3B510	Did (s)he go to urinate more often than usual?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B520	During the final illness did (s)he ever pass blood in the urine?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B530	Did (s)he have any skin problems?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B535	Did (s)he have sores?	YES	☐	
		NO	☐	➡ 3B540
		Don't know	☐	➡ 3B540
		Refuse to answer	☐	➡ 3B540
3B537	Did the sores have clear fluid or pus?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B540	Did (s)he have any ulcers, abscess or sores anywhere except on the feet?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B542	Did (s)he have an ulcer (pit) on the foot?	YES	☐	
		NO	☐	➡ 3B560
		Don't know	☐	➡ 3B560
		Refuse to answer	☐	➡ 3B560
3B544	Did the ulcer ooze pus?	YES	☐	
		NO	☐	➡ 3B550
		Don't know	☐	➡ 3B550
		Refuse to answer	☐	➡ 3B550
3B546	For how many days did the ulcer ooze pus?	DAYS	☐☐	
3B550	Did (s)he have any ulcers, abscess or sores on the feet that were not also on other parts of the body?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B560	During the illness that led to death, did (s)he have any skin rash?	YES	☐	
		NO	☐	➡ 3B596
		Don't know	☐	➡ 3B596
		Refuse to answer	☐	➡ 3B596
3B570	For how many days did (s)he have the skin rash?	DAYS	☐☐	
3B575	Where was the rash?	Face	☐	
		Trunk or abdomen	☐	
		Extremities	☐	
		Everywhere	☐	
3B580	Did (s)he have measles rash (use local term)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B590	Did (s)he ever have shingles or herpes zoster?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B596	During the illness that led to death, did (s)he bleed from anywhere?	YES	☐	
		NO	☐	➡ 3B610
		Don't know	☐	➡ 3B610
		Refuse to answer	☐	➡ 3B610

3B600	Did (s)he bleed from the nose, mouth or anus?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B610	Did (s)he have noticeable weight loss?	YES	☐	
		NO	☐	➡ 3B630
		Don't know	☐	➡ 3B630
		Refuse to answer	☐	➡ 3B630
3B620	Was (s)he severely thin or wasted?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B630	During the illness that led to death, did s/he have a whitish rash inside the mouth or on the tongue?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B640	Did (s)he have stiffness of the whole body or was unable to open the mouth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B650	Did (s)he have puffiness of the face?	YES	☐	
		NO	☐	➡ 3B654
		Don't know	☐	➡ 3B654
		Refuse to answer	☐	➡ 3B654
3B652	For how many days did (s)he have puffiness of the face?	DAYS	☐☐	
3B654	During the illness that led to death, did (s)he have swelling in the armpits?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B656	During the illness that led to death, did (s)he have swollen legs or feet?	YES	☐	
		NO	☐	➡ 3B660
		Don't know	☐	➡ 3B660
		Refuse to answer	☐	➡ 3B660
3B658	How many days did the swelling last?	DAYS	☐☐	
3B660	Did (s)he have both feet swollen?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B665	Did (s)he have general puffiness all over hi(s)her body?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B670	Did (s)he have any lumps?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B680	Did (s)he have any lumps or lesions in the mouth?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B690	Did (s)he have any lumps on the neck?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B700	Did (s)he have any lumps on the armpit?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3B710	Did (s)he have any lumps on the groin?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B720	Did she have any swelling or lump in the breast?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B722	Did she have any ulcers (pits) in the breast?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B724	Was (s)he in any way paralysed?	YES	☐	
		NO	☐	➡ 3B732
		Don't know	☐	➡ 3B732
		Refuse to answer	☐	➡ 3B732
3B730	Did s(he) have paralysis of only one side of the body?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B731	Which were the limbs or body parts paralysed?	Right side	☐	
		Left side	☐	
		Lower part of body	☐	
		Upper part of body	☐	
		One leg only	☐	
		One arm only	☐	
		Whole body	☐	
		Other	☐	
3B732	Did (s)he have difficulty swallowing?	YES	☐	
		NO	☐	➡ 3B745
		Don't know	☐	➡ 3B745
		Refuse to answer	☐	➡ 3B745
3B734	For how many days before death did (s)he have difficulty swallowing?	DAYS	☐☐	
3B740	Was the difficulty with swallowing with solids, liquids, or both?	Solids	☐	
		Liquids	☐	
		Both	☐	
3B745	Did (s)he have pain upon swallowing?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B750	Did (s)he have yellow discoloration of the eyes?	YES	☐	
		NO	☐	➡ 3B760
		Don't know	☐	➡ 3B760
		Refuse to answer	☐	➡ 3B760
3B755	For how many days did (s)he have the yellow discoloration?	DAYS	☐☐	
3B760	Did her/his hair change in colour to a reddish or yellowish colour?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B770	Did (s)he look pale (thinning/lack of blood) or have pale palms, eyes or nail beds?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3B780	Did (s)he have sunken eyes?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3B790	Did (s)he drink a lot more water than usual?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
SECTION 6: HISTORY OF INJURIES/ACCIDENTS				
3E100	Did (s)he suffer from any injury or accident that led to her/his death?	YES	☐	
		NO	☐	➡ 3F100
		Don't know	☐	➡ 3F100
		Refuse to answer	☐	➡ 3F100
3E102	Was the injury intentionally inflicted by someone else?	YES	☐	
		NO	☐	➡ 3E113
		Don't know	☐	➡ 3E113
		Refuse to answer	☐	➡ 3E113
3E104	Was (s)he injured by a firearm?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E106	Was (s)he stabbed, cut or pierced?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E108	Was (s)he strangled?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E111	Was (s)he injured by a blunt force?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E112	Was (s)he injured by burns?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E115	Was it a road traffic accident?	YES	☐	
		NO	☐	➡ 3E310
		Don't know	☐	➡ 3E310
		Refuse to answer	☐	➡ 3E310
3E120	What was her/his role in the road traffic accident?	Pedestrian	☐	
		Driver or passenger in car or light vehicle	☐	
		Driver or passenger in bus or heavy vehicle	☐	
		Driver or passenger on a motorcycle	☐	
		Driver or passenger on a pedal cycle	☐	
3E170	What was the counterpart that was hit during the road traffic accident?	Pedestrian	☐	
		Stationary object	☐	
		Car or light vehicle	☐	
		Bus or heavy vehicle	☐	
		Motorcycle	☐	
		Pedal cycle	☐	
		Other	☐	
3E310	Was (s)he injured in a fall?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3E320	Did (s)he die of drowning?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E330	Was (s)he suffering from burns?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E335	Was (s)he injured by a blunt force?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E340	Was (s)he injured by a plant/ animal/insect that led to her/his death?	YES	☐	
		NO	☐	➡ 3E500
		Don't know	☐	➡ 3E500
		Refuse to answer	☐	➡ 3E500
3E400	What was the plant/animal/insect?	Dog	☐	
		Snake	☐	
		Insect or Scorpion	☐	
		Others	☐	
		Don't know	☐	
3E500	Was (s)he injured by a force of nature?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E510	Was there any poisoning?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E520	Was (s)he subject to violence/assault?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3E530	Was it electrocution?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 7: HEALTH SERVICE UTILISATION

3G110	Did (s)he receive any treatment for the illness that led to death?	YES	☐	
		NO	☐	➡ 3H100
		Don't know	☐	➡ 3H100
		Refuse to answer	☐	➡ 3H100
3G120	Did (s)he receive oral rehydration salts?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G130	Did (s)he receive (or need) intravenous fluids (drip) treatment?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G140	Did (s)he receive (or need) a blood transfusion?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G150	Did s/he receive (or need) treatment/food through a tube passed through the nose?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

3G160	Did (s)he receive (or need) injectable antibiotics?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G165	Did (s)he receive (or need) antiretroviral therapy (ART)?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G170	Did (s)he have (or need) an operation for the illness?	YES	☐	
		NO	☐	➡ 3G190
		Don't know	☐	➡ 3G190
		Refuse to answer	☐	➡ 3G190
3G180	Did (s)he have the operation within 1 month before death?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3G190	Was (s)he discharged from hospital very ill?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
3H130	Was care sought outside the home while (s)he had this illness?	YES	☐	
		NO	☐	➡ 3H160
		Don't know	☐	➡ 3H160
		Refuse to answer	☐	➡ 3H160
3H140	Where or from whom did you seek care?	Traditional healer	☐	
		Homeopath	☐	
		Religious leader	☐	
		Private hospital	☐	
		Government hospital	☐	
		Government health centre or clinic	☐	
		Community-based practitioner associated with health system	☐	
		Trained birth attendant	☐	
		Private physician	☐	
		Pharmacy	☐	
3H150	Record the name and address of any hospital, health centre or clinic where care was sought _____			
3H160	Did a health care worker tell you the cause of death?	YES	☐	
		NO	☐	➡ 3H180
		Don't know	☐	➡ 3H180
		Refuse to answer	☐	➡ 3H180
3H170	What did the health care worker say? _____			
3H180	Do you have any health records that belonged to the deceased?	YES	☐	
		NO	☐	➡ 4A100
		Don't know	☐	➡ 4A100
		Refuse to answer	☐	➡ 4A100
3H190	Can I see the health records?	YES	☐	
		NO	☐	➡ 4A100
		Don't know	☐	➡ 4A100
		Refuse to answer	☐	➡ 4A100
3H200	Record the date of the most recent (last) visit	DAY	☐☐	
		MONTH	☐☐	
		YEAR	☐☐	

3H210	Record the date of the last but one (second last) visit	DAY ☐☐ MONTH ☐☐ YEAR ☐☐		
3H220	Record the date of the last note on the health records	DAY ☐☐ MONTH ☐☐ YEAR ☐☐		
3H230	Record the weight (in kilograms) written at the most recent (last) visit	[KG] ☐☐.☐		
3H240	Record the weight (in kilograms) written at the last but one (second last) visit	[KG] ☐☐.☐		
3H250	Transcribe the last note on the health records _____ _____			
SECTION 8: BACKGROUND AND CONTEXT				
4A100	In the final days before death, did s/he travel to a hospital or health facility?	YES	☐	
		NO	☐	➡ 4A150
		Don't know	☐	➡ 4A150
		Refuse to answer	☐	➡ 4A150
4A110	Did (s)he use motorised transport to get to the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A120	Were there any problems during admission to the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A130	Were there any problems with the way (s)he was treated (medical treatment, procedures, interpersonal attitudes, respect, dignity) in the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A140	Were there any problems getting medications, or diagnostic tests in the hospital or health facility?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A150	Does it take more than 2 hours to get to the nearest hospital or health facility from the deceased's household?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A160	In the final days before death, were there any doubts about whether medical care was needed?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A170	In the final days before death, was traditional medicine used?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A180	In the final days before death, did anyone use a telephone or cell phone to call for help?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	
4A190	Over the course of illness, did the total costs of care and treatment prohibit other household payments?	YES	☐	
		NO	☐	
		Don't know	☐	
		Refuse to answer	☐	

SECTION 9: OPTIONAL OPEN NARRATIVE		
5A100	Narrative Description	

SECTION 10: DEATH CERTIFICATE				
6H260	Was a death certificate issued?	YES	☐	
		NO	☐	➡ END
		Don't know	☐	➡ END
		Refuse to answer	☐	➡ END
6H270	Can I see the death certificate?	YES	☐	
		NO	☐	➡ END
		Don't know	☐	➡ END
		Refuse to answer	☐	➡ END
6H280	Record the immediate cause of death from the certificate (line 1a) * _____			Duration 1(a)
6H290	Record the first antecedent cause of death from the certificate (line 1b) _____			Duration 1(b)
6H300	Record the second antecedent cause of death from the certificate(line 1c) _____			Duration1(c)
6H310	Record the third antecedent cause of death from the certificate (line 1d) _____			Duration 1(d)
6H320	Record the contributing cause(s) of death from the certificate (part 2) _____			

Molecular Based Microbial Water Quality Test Questionnaire Form (Households Level)

Region: Zone: Woreda: Kebele: Name of Interviewer/Sampler:

Note (1)	Note (2)	Note (3)	Note (4)		Note (5)	Note (6)	Note (7)	Note (8)	Note (9)	Note (10)	Note (11)	Note (12)
Date	Household ID/ Water Sample ID	Village name where the HH lives	GPS Coordinates of the Dwelling (UTM Reading)		Please provide me with a glass of water that you would give to a child (under 1 year old) to drink		Did you or anyone in your household do anything to this water to make it safer to drink? (Yes/No)	If yes, What did you/household member do to make this water safe for drinking?	When did you fetch this water?	What is the source of this glass of water?	Where is the source of this glass of water?	Does the water storage safe or unsafe?
			Latitude (N)	Longitude (E)	Age in months	Sex (M/F)						

Notes:

Note (5): Age in Months (this is the age of a child "Under 1 year old" which the provided sample of a glass of water that the households would give to a child (under 1 year old) to drink. The age should be written in a complete months.

Note (6): Sex (this is the sex of a child "Under 1 year old" which the provided sample of a glass of water that the households would give to a child (under 1 year old) to drink. The sex should be recorded either in "Male" or "Female"

Note (8): Household member do to make this water safe for drinking means (Boiling, add Chlorine, use a water filter…)

Note (9): When did you fetch this water? 1-Today, 2- Yesterday, 3- Less than a week age, 4- Over 1 week ago

Note (10): What is the source of this glass of water? 1- PIPED WATER INTO DWELLING, 2- PIPED WATER INTO YARD/PLOT, 3- PIPED WATER PUBLIC TAP/STANDPIPE, 4- TUBEWELL/BOREHOLE, 5- PROTECTED DUG WELL, 6- UNPROTECTED DUGWELL, 7-PROTECTED SPRING, 8- UNPROTECTED SPRING, 9-RAINWATER COLLECTION, 10-PIPED WATER KIOSK/RETAILER, 11- BOTTLED WATER 12- SURFACE WATER (RIVER, DAM, LAKE, POND, STREAM, CANAL, IRRIGATION CHANNELS), 13- OTHERS,SPECIFY……...

Note (11): Where is the source of this glass of water? 1- IN THE DWELLING, 2- PRIVATE YARD/PLOT, 3- NEIGHBOR'S YARD/SHARED COMPOUND, 4- PUBLIC SPACE, 5- OTHERS,SPECIFY……...

Ask them: Can you please show me the source of this glass of water, so that I can also take a water sample from there? ….. Go to Next page

Molecular Based Microbial Water Quality Test Questionnaire Form
Main Water Source Level

Region: **Zone:** **Woreda:** **Name of Interviewer/Sampler:**

Q1	Q2	Q3	Q4	Q5	Q6		Q7	Q8	Q9
Date	Household ID	Village name where the HH lives	Type of Water source used?	Water source ID Number	GPS Coordinates of the Water Source (UTM Reading)		Did the Water Source is being cleaned regularly? (Yes/No)	Did the Water Source is being treated? (Yes/No)	Did the water Source fenced/ Catchment protection? (Yes/No)
					Latitude (N) /Y	Longitude (E) /X			

Notes

Q1,Q2, Q3: These all should be recorded from page 1

Q4: Type of Water Source should be recorded from Page 1 (Note 10) and TAKE PHOTO OF THE WATER SOURCE

Q5: Go to the water source that the households used and TAKE THE WATER SAMPLE

Q8: Confirm whether the water source is being treated or not

ANNEX II: Loop-based Isothermal Amplification (LAMP) Laboratory Protocol

LAMP PROTOCOL FOR THE DETECTION OF FIVE PATHOGENS IN DRINKING WATER

(Cryptosporidium, Shigella, Toxin producing strain of E.Coli and Rotavirus)

PHASE ONE: Field Work

Step 1: Water Sample Collection

Equipment and Consumables needed

- ☐ GPS (Quantity # 02)
- ☐ One liter sterile bottle (Quantity # 20)
- ☐ Ethanol (70-90%)
- ☐ Ice box (Quantity # 02)
- ☐ Ice pack (Quantity # 20)
- ☐ Gauze
- ☐ Glove
- ☐ Plaster to level the sample
- ☐ Sterilize forceps
- ☐ Lighter/Matches
- ☐ Autoclave and Sterilizer Indicator paper
- ☐ Aluminum Foil
- ☐ Field testing checklist/format

Procedure

1. Collect one liters of water sample from the households (Drinking water sample from infant's point-of-use) in sterile bottles without chemical additives and label with date, time, and place of collection.
2. Transfer to the next step 2 immediately for processing in a cooler box at 4 °C ± 1°C[1] and the next step 2 (Filtration) should be done with six hours from time of collection.

Step 2: Filtration

Equipment and Consumables needed

- ☐ Virosorb 1MDS 47-mm disc filters
- ☐ Filtration Apparatus (Plastic collar, Funnel, Bronze Disc, Silicone Rings (pair), Aluminum Base, Vacuum Connection, Black Rubber O-Ring, Vacuum Cup, Vacuum pump with Connector)
- ☐ Petri Dishes (Quantity # 70)
- ☐ Sterilized forceps/Tweezers

[1] *This is to help minimize changes in the microbial content with exposure to light and high temperatures. As recommended by WHO and UNEP (Gray, 2008)*

- ☐ Gauze
- ☐ Lighter/Matches
- ☐ Methanol (Methyle Alcohol)
- ☐ Ice bag (Quantity # 01)
- ☐ Ice pack (Quantity # 05)

Procedure

1. Assemble the filtration apparatus (loosen the filter funnel and remove from the rubber base support). Invert the filter funnei and place it down on the clean work-surface.
2. Sterilize the forceps by passing them from side to side through a flame from a lighter and allow cooling. N.B *(Take care not to heat for too long as this will cause sooty deposit to form).*
3. Remove a sterile, individually wrapped 1mds disk membrane filter using a sterile forceps.
4. Place the 1mds disk membrane filter directly onto the bronze filter support disc housed in the blue rubber base.
5. Lock the membrane filter in place by pushing the filter funnel firmly into position in the blue rubber base. Ensure the filter funnel is aligned correctly.
6. Pour the water sample into the filter funnel and commence the filtration until all (1liter) sample filtered. N.B (*Do not pump too many times so as to avoid drawing excess air through the membrane filter*)
7. When the entire sample has been filtered, detach the vaccum pump and remove the filter funnel from the rubber base and remove the 1mds membrane filter by using sterile forceps and place in a sterile petri dish and stored at 4 ºC until received by laboratory base (PHASE TWO).

PHASE TWO: Laboratory Work at EPHI

Step 3: Elusion

Equipment and Consumables needed

Laboratory Facilities

- ☐ A laboratory bench for the Elusion procedures
- ☐ A laboratory bench for the DNA/RNA Extraction procedures
- ☐ A laboratory bench for the LAMP ASSAY/Amplification procedures

Equipment and Supplies

- ☐ Pathogens adsorbed 1MDS 47-mm disc filters sample
- ☐ Filtration Apparatus (Plastic collar, Funnel, Bronze Disc, Silicone Rings (pair), Aluminum Base, Vacuum Connection, Black Rubber O-Ring, Vacuum Cup, Vacuum pump with Connector)
- ☐ Centrifuge (or equivalent assembly) capable of spinning 50 ml conical tubes and 15 ml centrifuge tubes at 4,400 x g for 30 min
- ☐ Vortex mixer
- ☐ Conical tube (50ml, 15ml) with rack
- ☐ Serological pipette
- ☐ Stir with a stir bar
- ☐ Autoclave with sterilizer Indicator paper
- ☐ dH_2O (distilled water)
- ☐ Sterilized forceps/Tweezers
- ☐ Gauze
- ☐ Lighter/Matches

- ☐ Methanol (Methyle Alcohol)

Reagents

- ☐ Sodium polyphosphate (NaPP), Sigma, Cat. No. 305553-25G
- ☐ Tween 80 (Non-Ionic Surfactant), CARELABMED, CAS NO = 9005-65-6: 500ml
- ☐ Anti-foam Agent (BIO-RAD, CA 94547; 100ml)
- ☐ Beef Extract Powder (Accumix, Microxpress #AB002, BEP-1803)
- ☐ Glycerol anhydrous (Fluka Chemie AG CH -9470 Buchs)
- ☐ NaOH (Sodium Hydroxide)
- ☐ HCl (Hydrochloric Acid)

Stage 1: Preparation of Backflush Solution: (*1.5% beef extract / 0.05M glycerin/ 0.01% Tween 80/0.1% NaPP/0.001% Antifoam Agent).*

Note: the solution should be prepared freshly on the first day and stored at 4 °C for no more than 48 hours. The pH of the eluting solutions should be adjusted to 8

a) Prepare buffered protein solution (1.5% beef extract in an aqueous glycerin solution)
   - Prepare buffered 1.5% beef extract by dissolving 30g of beef extract powder and 7.5g of glycine (final glycine concentration=0.05M) in 1.9L of dH_2O (distilled water)
   - Adjust the PH to 9.5 with 1 or 5M NaOH[2] and bring the final volume to 2L with dH_2O (distilled water).
   - Autoclave at 121^oC for 15min and use at room temperature. (Beef extract solutions may be stored for one week at 4^OC for longer period at -20^oC)

     (Source: ICR microbial lab Manual)

b) Prepare Tween 80 stock solution (0.01%)
   - Mix 0.01mL (10µl) of polyoxyethylenesorbitan monooleate 80 (Tween 80) stock solutions with 99.99 mL of distilled water.

c) Prepare Sodium Polyphosphate (NaPP) stock solution (0.1%)
   - Prepare solution by dissolving 0.1 g of Sodium polyphosphate powder in distilled water to a final volume of 100 mL.

d) Prepare Anti-foam Agent stock solution (0.001%)
   - Prepare solution by dissolving 0.001ml (1µl) of Anti-foam Agent stock solutions with 99.999 mL of distilled water.

Stage 2: Eluting/Backflush Solution for one sample:

For each 1MDS disk filter sample to be processed, make 400ml Eluting/backflush solution

- Prepare solution by mixing 100ml 1.5% beef extract with 0.05M glycerin + 100 mL 0.01% Tween 80 + 100 mL 0.1% Sodium polyphosphate + 100mL 0.001% Antifoam agent.
- Adjust the pH to 8 using a pH meter.

[2] *Prepare 1M and 5M solutions by dissolving 4g or 20g of NaOH in a final volume of 100ml of dH2O, respectively. (NaOH solution may be stored for several months at room temperature). Source: ICR M.Lab)*

Stage 3: Backflush / Elusion Procedure

Procedure:

1. Get ready a sample (Pathogens adsorbed Virosorb 1MDS 47-mm disc filter). *(Remove the selected filters from the refrigerator at least one hour prior to executing the backflush procedure)*
2. Assemble the filtration system with one filter
3. Place a 1MDS disk filter onto the bronze disc filter support. *(Replace the filter funnel and collar immediately, without allowing them to come into contact with any external objects)*
4. Screw the plastic collar down tightly to hold the membrane and to provide a solution tight seal.
5. Pour the 400ml of backflush solution into the filtration funnel up to the appropriate mark (10, 50 or 100ml) engraved on the internal surface of the funnel. (*Take care not to allow external debris to enter the funnel).* Allow the backflush solution to contact the 1MDS disc filter for 10 min.
6. Insert the plastic connector of the vacuum pump into the vacuum connection on the filtration base. Squeeze the pump bulb several times to draw a vacuum, then squeeze as required to draw all the backflush solution through the 1mds disk filter.
7. Continue pumping until no backflush solution remains in the container or the tubing and the out flow from the 1mds disk filter has slowed to a trickle. Do not pump air into the filter for more than approximately 10 seconds.
8. Measure and record back-flush volume or weight, if necessary.
9. Repeat the back-flush procedure for the remaining filters of the set.

Stage 3: Concentrating Targeted Pathogens from Backflush Effluent

1. Each filter yields approximately 400 ml of effluent. Divide the effluent collected from each filter into four 100ml conical centrifuge bottles. You will have a total of four 100 ml conical bottles for each filter.
2. Balance the four conical bottles with PBS[3] and centrifuge at 4,400 x g for 30 min with maximal acceleration and a brake setting of 6 (on a scale of 0-9) for deceleration.
3. Carefully remove and discard all but ~25-30 ml of the supernatant from each centrifuge bottle using a glass Pasteur pipet connected to a vacuum aspirator or a 50 ml serological pipette. A vacuum aspiration system will save time.
4. Re-suspend each pellet in the remaining supernatant by pipetting up/down and vortexing. Transfer approximately 13-15 ml aliquots of the re-suspended material from the four 100 ml centrifuge bottles to multiple 15 ml centrifuge tubes. Use a single 10 ml aliquot of PBS to rinse all four empty bottles one by one and add the rinse material to the 15 ml tubes. Between eight to ten 15 ml centrifuge tubes will be required to accommodate the re-suspended material and the rinse from the four 100 ml centrifuge bottles for each filter. Keep all centrifuge tubes for the same filter in a

[3] PBS (Phosphate Buffered Saline)

single Styrofoam stand or rack. At this point, if needed, samples can be stored at 4°C overnight before continuing the procedure.

5. Centrifuge the 15 ml centrifuge tubes at 4,400 x g for 30 min.
6. After the centrifugation, aspirate all but ~1.5 ml of the supernatant from each of the 15 ml tubes to waste without disturbing the pellets.
7. Re-suspend the pellets in the remaining supernatant and pool the pellets for each filter by transferring the re-suspended material into a single 15 ml tube. Use a single 2 ml aliquot of PBS to rinse all of the eight to ten tubes one by one and add the rinse material to the single 15 ml tube. Centrifuge at 4,400 x g for 30 min.
8. Carefully aspirate all but approximately 300 µl of the supernatant above the pellet in each of the 15 ml tubes to waste without disturbing the pellets. The sample in each 15ml tube represents the sample from each 1mds disk filter.
9. Store the pellets at 4°C for no more than 24 hours/ or a final 300 µl pellets should be stored at -20°C for later user of DNA extraction.
10. Autoclave the supernatant waste.

Step 4: DNA and RNA Extraction

- DNA and RNA is extracted from 1 ml eluate using a UV transilluminator (SYNGENE, Synoptics Ltd., UK), according to the manufacturer's instructions.
- The extracted DNA/RNA should be stored at -80°C until use the next step 5.

Step 5: LAMP ASSAY

Equipment and Consumables needed

- ☐ Extracted DNA/RNA Sample
- ☐ Oligonuclotide
- ☐ Master Mix
- ☐ Sterilized tubes for master mix preparation (0.5mL or 1.5mL)
- ☐ Loopamp Reaction Tube
- ☐ dH_2O (distilled water)
- ☐ Micropipette (0.5 ~ 10µL, 10 ~ 100µL, 100 ~ 1,000µL)
- ☐ Heat block (Use at 95°C)
- ☐ Aluminum rack for cooling tubes
- ☐ Crushed ice and ice box
- ☐ Loopamp Realtime Turbidimeter
- ☐ Centrifuge for microtubes
- ☐ Centrifuge for 8-strip tube
- ☐ Vortex mixer

Procedure

Stage 1: Prepare Oligonuclutide

- Briefly centrifuge each tube before opening to prevent the loss of the pellet

- Prepare stock solution of oligos (e.g 100μM=100 pmole per μl) preferably with a sterile buffered solution such as TE (10mM Tris, PH 7.5 to 8.0, 1 mM EDTA). If sterile distilled water is used, make sure that the PH is above 7.0 since acidic solutions favours oligo depurination and subsequent loss of activity. (For each oligos stock solution preparation refer Table 1)
- Working solutions might be diluted from the stock solution with sterile, nuclease-free water to prevent inhibition of enzymatic reactions (e.g PCR) by EDTA.
- Store the oligos as concentrated stock solution or lyophilized at -20^{o}C
- Avoid frequent freeze-thaw cycles by dividing the stock solution into smaller aliquots for long term storage and to prevent accidental contamination
- Dye-modified oligos are light sensitive and should always be stored in the dark.
- Re-suspend modified oligos preferably in a slightly basic solution (i.e TE at PH 8.0). However, Cy dye modified oligos are best kept at PH 7.0 at -20^{o}C
- Preferably store the modified oligos as dried aliquots at -20^{o}C.

Table 1: Stock solution for each Oligos[4]

	Name	Stock solution
	18S RRNA F3	For a 100μM stock solution add 661.15μ1 water or buffer
	18S RRNA B3	For a 100μM stock solution add 683.49μ1 water or buffer
	18S RRNA LB	For a 100μM stock solution add 747.02μ1 water or buffer
	18S RRNA FIP	For a 100μM stock solution add 173.72μ1 water or buffer
	18S RRNA BIP	For a 100μM stock solution add 129.15μ1 water or buffer
	IPAH F3	For a 100μM stock solution add 629.79μ1 water or buffer
	IPAH B3	For a 100μM stock solution add 685.67μ1 water or buffer
	IPAH LF	For a 100μM stock solution add 662.05μ1 water or buffer
	IPAH LB	For a 100μM stock solution add 489.61μ1 water or buffer
	IPAH FIP	For a 100μM stock solution add 78.26μ1 water or buffer
	IPAH BIP	For a 100μM stock solution add 209.71μ1 water or buffer
	STX2A/B F3	For a 100μM stock solution add 640.25μ1 water or buffer
	STX2A/B B3	For a 100μM stock solution add 749.5μ1 water or buffer
	STX2A/B LF	For a 100μM stock solution add 655.12μ1 water or buffer
	STX2A/B FIP	For a 100μM stock solution add 138.57μ1 water or buffer
	STX2A/B BIP	For a 100μM stock solution add 118.92μ1 water or buffer
	VP7 F3	For a 100μM stock solution add 717.33μ1 water or buffer
	VP7 B3	For a 100μM stock solution add 677.79μ1 water or buffer
	VP7 LB	For a 100μM stock solution add 684.48μ1 water or buffer
	VP7 FIP	For a 100μM stock solution add 210.36μ1 water or buffer
	VP7 BIP	For a 100μM stock solution add 74.7μ1 water or buffer

[4] *According to the manufacture Instruction (Inqaba Biotechnical Industries)*

Stage 2: Prepare reaction mixture for LAMP

The reaction mixture will be prepared on ice containing box to a final volume of 25µl.

Stage 3: Mixing Reaction Mix with sample solution

- Distribute the master mix into Loopamp Reaction Tube, depending on the number of samples
- Add 5µl of sample solution (DNA/RNA extracted) to the master mix, and the volume of the solution should be 25µl in a total reaction. Mix the solution well by pipetting or tapping the tube with the cap closed and then spin down. Be careful not to cause air-bubbles when mixing.

Stage 4: Amplification reaction

- Set the temperature, measurement time, inactivation based on the optimization result of each pathogen.
- Confirm that the temperature has reached
- Set the prepared reaction tubes and immediately start reaction/Incubate LAMP reaction in heat bath
- Detect positive by naked eye looking for turbidity compared to negative control water sample.
-

Stage 5: Detection Using U.V Trans-illuminator

- Detect using a UV transilluminator (SYNGENE, Synoptics Ltd., UK) and photographed, and finally the numbers are noted down for all positive samples.

ANNEX III: Oligonuclotide Synthesis Report

inqaba biotec™

Africa's Genomics Company

inqaba biotec East Africa Ltd. (IBE002)

Co. Reg. No: CPR / 2010 / 25338
VAT No: P051332967H

Synthesis Report

Prepared for

Addis Ababa University
Samuel Mebrahtom
NBH1, 4 Killo King George VI Street
P.O.Box. 1176
Addis Ababa
Ethiopia
Phone: +251921567389

Thank you for choosing inqaba biotec, Africa's Genomics company, for your oligonucleotide needs.We are the only commercial DNA synthesis facility in Africa and boast over 15 years of experience.Do not hesitate to contact us for technical support. We do also offer a quality portfolio of auxiliary PCR reagents and sequencing services.

Delivery addres

Addis Ababa University
Samuel Mebrahtom
NBH1, 4 Killo King George VI Street
P.O.Box. 1176
Addis Ababa
Ethiopia
Phone: +251921567389

References

Order Number: KE2018/7107 **Oligo Ref #:** 1041223 **Validated On:** **Print Date:** 2019-02-12

Name: 18S RRNA F3	**Barcode:** S2B19	**Manufacturing Date:**	
Sequence: GTATATATTCCTGTTTCGAAGGA	**Length:** 23		**PAGE QC Image**
OD: 16.66	**MW min \ max:** 7068.69	**5' Mod:** None	
nmoles: 72.44	**GC % min \ max:** 34.78	**3' Mod:** None	
Tm min \ max: 49.93	**Purification:** Standard		
For a 100 µM stock solution add 724.4 **µl water or buffer**			
Comments:			

Name: 18S RRNA B3	**Barcode:** S2B1A	**Manufacturing Date:**	
Sequence: TCCGAATAATTCACCGGATC	**Length:** 20		**PAGE QC Image**
OD: 14.72	**MW min \ max:** 6061.01	**5' Mod:** None	
nmoles: 73.58	**GC % min \ max:** 45.0	**3' Mod:** None	
Tm min \ max: 49.73	**Purification:** Standard		
For a 100 µM stock solution add 735.77 **µl water or buffer**			
Comments:			

Name: 18S RRNA LB	**Barcode:** S2B1B	**Manufacturing Date:**	
Sequence: GCTGATTACGTCCCTGCCCTTTG	**Length:** 23		**PAGE QC Image**
OD: 16.29	**MW min \ max:** 6957.55	**5' Mod:** None	
nmoles: 70.84	**GC % min \ max:** 56.52	**3' Mod:** None	
Tm min \ max: 58.84	**Purification:** Standard		
For a 100 µM stock solution add 708.37 **µl water or buffer**			
Comments:			

Name: IPAH F3	**Barcode:** S2B1C	**Manufacturing Date:**	
Sequence: GCCTTTCCGATACCGTCTCT	**Length:** 20		**PAGE QC Image**
OD: 11.71	**MW min \ max:** 5994.93	**5' Mod:** None	
nmoles: 58.54	**GC % min \ max:** 55.0	**3' Mod:** None	
Tm min \ max: 53.83	**Purification:** Standard		
For a 100 µM stock solution add 585.39 **µl water or buffer**			
Comments:			

Name: IPAH B3	**Barcode:** S2B1D	**Manufacturing Date:**	
Sequence: TGATGGACCAGGAGGGTT	**Length:** 18		**PAGE QC Image**
OD: 14.06	**MW min \ max:** 5619.72	**5' Mod:** None	
nmoles: 78.09	**GC % min \ max:** 55.56	**3' Mod:** None	
Tm min \ max: 50.32	**Purification:** Standard		
For a 100 µM stock solution add 780.9 **µl water or buffer**			
Comments:			

Name: IPAH LF	**Barcode:** S2B1E	**Manufacturing Date:**	
Sequence: TGCAGCGACCTGTTCACG	**Length:** 18		**PAGE QC Image**
OD: 12.16	**MW min \ max:** 5475.6	**5' Mod:** None	
nmoles: 67.57	**GC % min \ max:** 61.11	**3' Mod:** None	
Tm min \ max: 52.6	**Purification:** Standard		
For a 100 µM stock solution add 675.66 **µl water or buffer**			
Comments:			

Name: IPAH LB	**Barcode:** S2B1F	**Manufacturing Date:**	
Sequence: CACTGAGAGCTGTGAGGACCG	**Length:** 21		**PAGE QC Image**
OD: 11.43	**MW min \ max:** 6496.27	**5' Mod:** None	
nmoles: 54.44	**GC % min \ max:** 61.9	**3' Mod:** None	
Tm min \ max: 58.26	**Purification:** Standard		
For a 100 µM stock solution add 544.4 **µl water or buffer**			
Comments:			

Name: STX2A/B F3	**Barcode:** S2B20	**Manufacturing Date:**	
Sequence: TCGGTGTCTGTTATTAACCA	**Length:** 20		**PAGE QC Image**
OD: 13.32	**MW min \ max:** 6098.04	**5' Mod:** None	
nmoles: 66.59	**GC % min \ max:** 40.0	**3' Mod:** None	
Tm min \ max: 47.68	**Purification:** Standard		
For a 100 µM stock solution add 665.86 **µl water or buffer**			
Comments:			

Name: STX2A/B B3	**Barcode:** S2B21	**Manufacturing Date:**	
Sequence: TGGAAACCGTTGTCACAC	**Length:** 18		**PAGE QC Image**
OD: 14.65	**MW min \ max:** 5483.63	**5' Mod:** None	
nmoles: 81.4	**GC % min \ max:** 50.0	**3' Mod:** None	
Tm min \ max: 48.04	**Purification:** Standard		
For a 100 µM stock solution add 814.04 **µl water or buffer**			
Comments:			

Name: STX2A/B LF	**Barcode:** S2B22	**Manufacturing Date:**	
Sequence: TGATAGACATCAAGCCCTCGTA	**Length:** 22		**PAGE QC Image**
OD: 15.88	**MW min \ max:** 6703.43	**5' Mod:** None	
nmoles: 72.18	**GC % min \ max:** 45.45	**3' Mod:** None	
Tm min \ max: 52.97	**Purification:** Standard		
For a 100 µM stock solution add 721.83 **µl water or buffer**			
Comments:			

Name: CTXA/B F3	**Barcode:** S2B23	**Manufacturing Date:**	
Sequence: CGAGGTACTCAAATGAATATCAAC	**Length:** 24		**PAGE QC Image**
OD: 16.93	**MW min \ max:** 7353.88	**5' Mod:** None	
nmoles: 70.55	**GC % min \ max:** 37.5	**3' Mod:** None	
Tm min \ max: 52.26	**Purification:** Standard		
For a 100 µM stock solution add 705.45 **µl water or buffer**			
Comments:			

Name: CTXA/B B3	**Barcode:** S2B24	**Manufacturing Date:**	
Sequence: GCCCCTAATACATCATTAACGT	**Length:** 22		**PAGE QC Image**
OD: 15.44	**MW min \ max:** 6638.39	**5' Mod:** None	
nmoles: 70.18	**GC % min \ max:** 40.91	**3' Mod:** None	
Tm min \ max: 51.11	**Purification:** Standard		
For a 100 µM stock solution add 701.79 **µl water or buffer**			
Comments:			

Name: CTXA/B LF	**Barcode:** S2B25	**Manufacturing Date:**	
Sequence: TGCCTAACAAATCCCGTCTGA	**Length:** 21		**PAGE QC Image**
OD: 16.07	**MW min \ max:** 6350.19	**5' Mod:** None	
nmoles: 76.51	**GC % min \ max:** 47.62	**3' Mod:** None	
Tm min \ max: 52.4	**Purification:** Standard		
For a 100 µM stock solution add 765.07 **µl water or buffer**			
Comments:			

Name: VP7 F3	**Barcode:** S2B26	**Manufacturing Date:**	
Sequence: CCAATAACAGGATCAATGGATAC	**Length:** 23		**PAGE QC Image**
OD: 19.55	**MW min \ max:** 7049.68	**5' Mod:** None	
nmoles: 84.99	**GC % min \ max:** 39.13	**3' Mod:** None	
Tm min \ max: 51.71	**Purification:** Standard		
For a 100 µM stock solution add 849.88 **µl water or buffer**			
Comments:			

Name: VP7 B3	**Barcode:** S2B27	**Manufacturing Date:**	
Sequence: GGATCGACAGAAAATTCAACAA	**Length:** 22		**PAGE QC Image**
OD: 18.42	**MW min \ max:** 6769.51	**5' Mod:** None	
nmoles: 83.74	**GC % min \ max:** 36.36	**3' Mod:** None	
Tm min \ max: 49.25	**Purification:** Standard		
For a 100 µM stock solution add 837.38 **µl water or buffer**			
Comments:			

Name: VP7 LB	**Barcode:** S2B28	**Manufacturing Date:**	
Sequence: TGTTTCTTACAAAAGGCTGGCCAAC	**Length:** 25		**PAGE QC Image**
OD: 18.6	**MW min \ max:** 7641.04	**5' Mod:** None	
nmoles: 74.39	**GC % min \ max:** 44.0	**3' Mod:** None	
Tm min \ max: 56.04	**Purification:** Standard		
For a 100 µM stock solution add 743.89 **µl water or buffer**			
Comments:			

Name: 18S RRNA FIP	**Barcode:** C2B29	**Manufacturing Date:**	
Sequence: CCTCGTTCAAGATCAATAATTGCAAATGGGTAATCTTTTGAATATGCA	**Length:** 48		**PAGE QC Image**
OD: 9.37	**MW min \ max:** 14763.72	**5' Mod:** None	
nmoles: 19.51	**GC % min \ max:** 33.33	**3' Mod:** None	
Tm min \ max: 64.56	**Purification:** Cartridge		
For a 100 µM stock solution add 195.15 **µl water or buffer**			
Comments:			

Name: 18S RRNA BIP	**Barcode:** C2B2A	**Manufacturing Date:**	
Sequence: TCCTAGTAAGCGCAAGTCATCAGATTCAATCGGTAGGAGCG	**Length:** 41		**PAGE QC Image**
OD: 5.92	**MW min \ max:** 12658.29	**5' Mod:** None	
nmoles: 14.44	**GC % min \ max:** 48.78	**3' Mod:** None	
Tm min \ max: 68.5	**Purification:** Cartridge		
For a 100 µM stock solution add 144.4 **µl water or buffer**			
Comments:			

inqaba biotec™

Africa's Genomics Company

inqaba biotec East Africa Ltd. (IBE002)

Co. Reg. No: CPR / 2010 / 25338
VAT No: P051332967H

Name: IPAH FIP	**Barcode:** C2B2B	**Manufacturing Date:**	
Sequence: AAGCTCCGCAGAGGCACTGACACGCAATACCTCCGGATTC	**Length:** 40		**PAGE QC Image**
OD: 3.36	**MW min \ max:** 12219.96	**5' Mod:** None	
nmoles: 8.41	**GC % min \ max:** 57.5	**3' Mod:** None	
Tm min \ max: 71.67	**Purification:** Cartridge		
For a 100 µM stock solution add 84.08 **µl water or buffer**			
Comments:			

Name: IPAH BIP	**Barcode:** C2B2C	**Manufacturing Date:**	
Sequence: AGCAGTCTTTCGCTGTTGCTGCCCGGAGATTGTTCCATGTGA	**Length:** 42		**PAGE QC Image**
OD: 9.0	**MW min \ max:** 12918.42	**5' Mod:** None	
nmoles: 21.42	**GC % min \ max:** 52.38	**3' Mod:** None	
Tm min \ max: 70.37	**Purification:** Cartridge		
For a 100 µM stock solution add 214.2 **µl water or buffer**			
Comments:			

Name: STX2A/B FIP	**Barcode:** C2B2D	**Manufacturing Date:**	
Sequence: AGACGAAGATGGTCAAAACGCGCAGTTATTTTGCTGTGGA	**Length:** 40		**PAGE QC Image**
OD: 6.33	**MW min \ max:** 12424.16	**5' Mod:** None	
nmoles: 15.83	**GC % min \ max:** 45.0	**3' Mod:** None	
Tm min \ max: 66.54	**Purification:** Cartridge		
For a 100 µM stock solution add 158.32 **µl water or buffer**			
Comments:			

Name: STX2A/B BIP	**Barcode:** C2B2E	**Manufacturing Date:**	
Sequence: CGGGTTCGTTAATACGGCAACGGGCACTGATATATGTGT	**Length:** 39		**PAGE QC Image**
OD: 5.08	**MW min \ max:** 12077.91	**5' Mod:** None	
nmoles: 13.02	**GC % min \ max:** 48.72	**3' Mod:** None	
Tm min \ max: 67.63	**Purification:** Cartridge		
For a 100 µM stock solution add 130.17 **µl water or buffer**			
Comments:			

Name: CTXA/B FIP	**Barcode:** C2B2F	**Manufacturing Date:**	
Sequence: TTGAGGTGGAAACATATCCATCATCCTTTATGATCATGCAAGAGGAA	**Length:** 47		**PAGE QC Image**
OD: 6.86	**MW min \ max:** 14509.54	**5' Mod:** None	
nmoles: 14.59	**GC % min \ max:** 38.3	**3' Mod:** None	
Tm min \ max: 66.3	**Purification:** Cartridge		
For a 100 µM stock solution add 145.86 **µl water or buffer**			
Comments:			

Name: CTXA/B BIP	**Barcode:** C2B30	**Manufacturing Date:**	
Sequence: GTGGGTCAAACTATATTGTCTGGTAACATGTTGGGTGCAGTG	**Length:** 42		**PAGE QC Image**
OD: 2.73	**MW min \ max:** 13070.57	**5' Mod:** None	
nmoles: 6.49	**GC % min \ max:** 45.24	**3' Mod:** None	
Tm min \ max: 67.44	**Purification:** Cartridge		
For a 100 µM stock solution add 64.92 **µl water or buffer**			
Comments:			

Name: VP7 FIP	**Barcode:** C2B31	**Manufacturing Date:**	
Sequence: TTGGTTGCTAGCTTCAATTGGATAACGCATATGCTAACTCTACTCAA	**Length:** 47		**PAGE QC Image**
OD: 10.68	**MW min \ max:** 14402.45	**5' Mod:** None	
nmoles: 22.72	**GC % min \ max:** 38.3	**3' Mod:** None	
Tm min \ max: 66.3	**Purification:** Cartridge		
For a 100 µM stock solution add 227.19 **µl water or buffer**			
Comments:			

Name: VP7 BIP	**Barcode:** C2B32	**Manufacturing Date:**	
Sequence: TGGTGAATGGAAAGATACATTGTCATGTACTCTTTAAAGTAGACCGAT	**Length:** 48		**PAGE QC Image**
OD: 4.12	**MW min \ max:** 14868.79	**5' Mod:** None	
nmoles: 8.57	**GC % min \ max:** 35.42	**3' Mod:** None	
Tm min \ max: 65.41	**Purification:** Cartridge		
For a 100 µM stock solution add 85.74 **µl water or buffer**			
Comments:			

RECOMMENDATIONS FOR HANDLING AND STORAGE OF OLIGOS

- Lyophilized oligo pellets might become displaced from the bottom of the tube during shipment. Briefly centrifuge each tube before opening to prevent the loss of the pellet.
- Prepare stock solution of oligos (e.g. 100 uM = 100 pmole per ul) preferably with a sterile buffered solution such as TE (10 mM Tris, pH 7.5 to 8.0,1 mM EDTA). If sterile distilled water used, make sure that the pH is above 7.0 since acidic solutions favours oligo depurination and subsequent loss of activity.
- Working solutions might be diluted from the stock solution with sterile, nuclease-free water to prevent inhibition of enzymatic reactions (e.g. PCR) by EDTA.
- Store the oligos as concentrated stock solution or lyophilized at -20° C.
- Avoid frequent freeze-thaw cycles by dividing the stock solution into smaller aliquots for long term storage and to prevent accidental contamination.
- Dye-modified oligos are light sensitive and should always be stored in the dark.
- Resuspend modified oligos preferably in a slightly basic solution (i.e., TE at pH 8.0). However, Cy dye modified oligos are best kept at pH 7.0 at -20° C.
- Preferably store the modified oligos as dried aliquots at -20° C.

PRODUCTS FOR RESEARCH USE ONLY!

ANNEX IV: Published Papers (I-III)

PLOS ONE

RESEARCH ARTICLE

Causes of infant deaths and patterns of associated factors in Eastern Ethiopia: Results of verbal autopsy (InterVA-4) study

Samuel Mebrahtom[1]*, **Alemayehu Worku**[2], **Daniel J. Gage**[3]

1 Ethiopian Institute of Water Resources, Addis Ababa University, Addis Ababa, Ethiopia, **2** School of Public Health, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia, **3** Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, United States of America

* samrontom@gmail.com

OPEN ACCESS

Citation: Mebrahtom S, Worku A, Gage DJ (2022) Causes of infant deaths and patterns of associated factors in Eastern Ethiopia: Results of verbal autopsy (InterVA-4) study. PLoS ONE 17(8): e0270245. https://doi.org/10.1371/journal.pone.0270245

Editor: Bernardo Hernandez, Institute for Health Metrics and Evaluation, UNITED STATES

Received: November 27, 2020

Accepted: June 7, 2022

Published: August 4, 2022

Data Availability Statement: All relevant data are within the paper and its Supporting information files.

Funding: The author(s) received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: ARTI, Acute Respiratory Tract Infection; CI, Confidence Interval; CSV, Comma Separated Variable; EIWR, Ethiopian Institute of

Abstract

Background

In a range of setting, detecting and generate empirical information on the cause of infant death and contributing risk factors at population level is basically utmost essential to take evidence-based measures in reducing infant morbidity and mortality. An electronic verbal autopsy is suitable tool and best alternative solution to determine individuals' cause of death in a setting where the majority of deaths occur at home and civil registration systems do not exist. The present study was undertaken to find out cause of infant death, applying computer-based probabilistic model (InterVA-4) and analyze the patterns of association factors of mother's and the deceased infant's characteristics to the leading cause-specific infant mortality in Eastern Ethiopia.

Methods

The study employed a community-based prospective longitudinal survey, which was conducted with routinely enumeration of reported infant deaths for a period of two years (from September 2016 to August 2018) in Eastern part of Ethiopia. Using the two-stage cluster sampling technique, the study was undertaken in four randomly selected districts of West Hararghe zone and two districts of zone 3 in Oromia and Afar regional state, respectively. The study included a total of 362 infants who were deceased during the study period. Data was collected by trained enumerators by interviewing the mothers or guardians of the deceased infant using a 2014 standardize World Health Organization (WHO) Verbal Autopsy questionnaire. InterVA-4 model were used for processing and interpreting verbal autopsy data in order to arrive at the most likely causes of infant death. SPSS version 23 was also used for statistical analysis of frequency distribution and logistic regression for the association between covariates and outcomes.

Findings

Of the overall (362) deceased infants' during the study period, 53.0% of deaths occurred during neonatal time while 47.0% died in the post-neonatal period. Acute respiratory

Mebrahtom et al. BMC Public Health (2022) 22:343
https://doi.org/10.1186/s12889-022-12735-7

BMC Public Health

RESEARCH Open Access

The risk of water, sanitation and hygiene on diarrhea-related infant mortality in eastern Ethiopia: a population-based nested case-control

Samuel Mebrahtom[1*], Alemayehu Worku[2] and Daniel J. Gage[3]

Abstract

Background: Diarrhea is still appeared to be as one of the leading global killers and disability-adjusted life-years lost, particularly in the Infant and children. As per WHO, about 88% of diarrhea-related deaths are attributable to unsafe water, inadequate sanitation and insufficient hygiene, mainly in developing world. Thus, the main objective of this study was to find out the risk of such factors that contribute for diarrhea-related Infant mortality in Eastern Ethiopia.

Methods: This study employed community based unmatched nested case-control study design in Eastern Ethiopia. The cases were Infants who died from diarrheal disease while controls were those who survived their first year of life from September, 2016 to August, 2018. A total of 305 study subjects (61 cases and 244 controls) were included in the study. Infants dying from diarrhea were compared to four neighborhood controls in terms of several risk components of Water, Sanitation and Hygiene. Data were collected from mothers/care takers of Infants using pre-tested structured questionnaires, and entered onto CSpro version 5.1 and transform to SPSS version 23 to analyzed potential risk factors.

Findings: Finding of this study revealed that the risk factors that found to be significantly associated with Infant death from diarrhoea after adjustment for confounding variables included the age of mother with < 20 years old ($P = 0.009$, AOR: 0.01, 95% CI: 0.01, 0.47), unsafe drinking water storage ($P = 0.013$, AOR: 0.4, 95% CI: 0.18, 0.81), Infants in households without point-of-use water treatment practices ($P = 0.004$, AOR: 0.21, 95% CI: 0.08, 0.61), households with unimproved sanitation ($P = 0.050$, AOR: 0.36, 95% CI: 0.13, 1.00), unsafe disposing of child feces ($P = 0.014$, AOR: 0.34, 95% CI: 0.15, 0.81), and Improper management of solid waste ($P = 0.003$, AOR: 0.29, 95% CI: 0.13, 0.66). These exposure factors had lower risk for the contribution of Infants dying from diarrhoea than those with their reference group in the study area. However, Infants in households with Improper management of liquid waste management showed strongly significant association which had three times more likely to occur diarrhea-related Infant death ($P = 0.010$, AOR: 3.43, 95% CI: 1.34, 8.76). Similarly, Infants whose mother/caretaker practiced hand washing with less critical time (one-two occasions) had three times greater risk to Infant death from diarrhea than those who had practice more than three critical times of hand washing ($P = 0.027$, AOR: 3.04, 95% CI: 1.13, 8.17).

Conclusion: This study suggests that Infants in households with Improper management of liquid waste and hand washing practices with fewer occasions (one-two critical time) are a greater risk of getting a diarrhea-related Infant

*Correspondence: samsontom@gmail.com
[1] Ethiopian Institute of Water Resources, Addis Ababa University, Addis Ababa, Ethiopia
Full list of author information is available at the end of the article

Journal of Water & Health

Journal of Water and Health Vol 00 No 0, 1 doi: 10.2166/wh.2023.201

Molecular detection of waterborne pathogens in infant's drinking water and their relationship with water quality determinants in Eastern Ethiopia: loop-mediated isothermal amplification (LAMP)-based study

Samuel Gebregziabher Mebrahtom [a,*], Alemayehu Yalew Worku[b], Daniel Joseph Gage[c], Heven Sime[c] and Adugna Abera[c]

[a] Ethiopian Institute of Water Resources, Addis Ababa University, Addis Ababa, Ethiopia
[b] School of Public Health, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia
[c] Bacterial, Parasitic and Zoonotic Diseases Research Directorate, EPHI, Addis Ababa, Ethiopia
*Corresponding author. E-mail: samrontom@gmail.com

SGM, 0000-0001-5297-4091

ABSTRACT

Cryptosporidium, Shigella, toxin-producing *Escherichia coli*, and rotavirus were reported to be the most responsible for severe and fatal diarrhea among infants. This study aimed to investigate the presence of these pathogens in infant's drinking water samples and analyzing using water quality determinants in Eastern Ethiopia. A molecular (LAMP)-based cross-sectional study design was employed. A total of 410 and 37 water samples were tested from infant point-of-use at household and corresponding water source, respectively, from June 2020 to May, 2021. Cryptosporidium, Shigella, toxin-producing *E. coli*, and rotavirus were detected in 28.5, 30.0, 26.3, and 32.2%, of water samples tested from infant point-of-use, respectively. About 13.2% of the water samples were positive for all (four) pathogens together. Cryptosporidium, Shigella, toxin-producing *E. coli*, and rotavirus were detected in 27.0, 32.4, 29.7, and 37.8%, of water samples tested from water sources, respectively. Positive significant correlation was observed between infant point-of-consumption and water sources from which it is drawn toward the presence of each targeted pathogens. Unimproved water source showed a strong significant association with the presence of Cryptosporidium, Shigella and toxin-producing *E. coli*. Therefore, efforts should be made on development of improved water sources, source protection safety and health education to caretakers of infants.

Key words: *Cryptosporidium*, drinking water at point-of-use, infant, LAMP, rotavirus, *Shigella*, toxin-producing strain of *E. coli*, water source

HIGHLIGHTS

- Highest prevalent agent detected in water samples was rotavirus (32.2%), followed by Shigella (30.0%), Cryptosporadium (28.5%), and toxin-producing *E. coli* (26.5%).
- Positive correlation was observed between drinking water at point-of-use and water sources on presence of pathogens.
- Significant association was observed between unimproved water source and presence of *Cryptosporidium*, *Shigella*, and toxin-producing *E. coli*.

ABBREVIATIONS

AOR	adjusted odds ratio
cDNA	complement deoxyribonucleic acid
CI	confidence interval
COR	crude odds ratio
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
EIWR	Ethiopian Institute of Water Resources
ICR	Information Collection Rule

DECLARATION

I, the undersigned declare that this Dissertation is my original work and it has not been presented in other universities, colleges or institutions for a degree or other purpose. All sources of the materials used have been duly acknowledged.

Name: Samuel Mebrahtom Signature: _____ Date: _____

This work has been done under my supervision

Name: Prof. Alemayehu Worku Signature: _____ Date: _____

Prof. Daniel J. Gage Signature: _____ Date: _____

BIOGRAPHIC SKETCH

Samuel Mebrahtom Gebregziabher

Cell Phone:+251-921567389
E-Mail: saron1988@yahoo.com, samrontom@gmail.com
Skype: Samuel.mb80
ID Number: GSR/0060/07

Samuel Mebrahtom was born on the 29th of April year 1979, in Dupti town, Afar regional state of Ethiopia. He took his elementary school in Melika werer, Arba trap and Ourael elementary school, and completed his high school at Bole High school and Nemelefen high school in 1998.

He joined diploma program at Gondar College of Medical Sciences after finishing high school, and graduated in the field of Environmental Health in 2000. Following that, he employed as an environmental health technician at the Werer Health Center, which was enrolled under the Zone 3 Health Department of Afar National Regional Health Bureau from 2001 to 2006. Apart from his employment, he served as a partnership with Oxfam GB and CARE International in emergency and development project, respectively. After then, he joined the BSc degree program in Environmental Health in Hawassa University from 1998 to 2009 and graduated with distinction. Other than that, he attended Ethiopian Adventist College from 2008 and 2010, and holds BA degree with distinction in Community Development and Leadership minor in Management. He pursues master of public health program at Haramaya University and graduated in general public health in 2013.

From May 1, 2007, to June 30, 2007, he worked as an environmental health assistant for the emergency project at IRC. He is a founder of DADAL Consulting PLC and now acting as a general manager. He also works as a senior consultant for different governmental and nongovernmental organizations. His work has been performed in conducting baseline survey, mid and final evaluation of projects, operational researches, One WASH Consultant and different assessments in Environmental and public health aspects.

He began his PhD study in the stream of Water and Health, specialized in Water and Public Health, in September 2015 at Addis Ababa University's School of Graduate Studies, Ethiopian Institute of Water Resources. As of right now, he is the author of four publications and produced more than twenty Environmental and Public health project survey/assessment/ research documents.