ASSESSMENT OF ENVIRONMENTAL FACTORS ASSOCIATED WITH ENDEMIC CHOLERA IN LUKANGA SWAMPS OF KAPIRI MPOSHI DISTRICT IN CENTRAL PROVINCE OF ZAMBIA

BY

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A Dissertation submitted to the University of Zambia in partial fulfilment of the requirements of the degree of Master of Public Health in Environmental Health.

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DECLARATION

I, SIKWIIYA SIMEON, do hereby declare that this dissertation is my original work and has been produced in accordance with the guidelines for the Master of Public Health in Environmental Health for the University of Zambia. I also further pronounce that it has never been presented or submitted elsewhere in part or whole for the award of a degree or any qualification from any University.

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We confirm that the candidate, under our supervision, carried out the work reported in this thesis.

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DEDICATION

This dissertation is dedicated to my wife Chomba Nanyangwe and my four children; Mildah. M. Sikwiiya, Martha. M. Sikwiiya, Simeon. L. Sikwiiya and Mary. C. Sikwiiya for supporting and encouraging me throughout the duration of my Masters programme.

Above all, I express thanks to God for providing wisdom, sustenance, direction and audacity to undertake this task.

APPROVAL

This dissertation of Simeon Sikwiiya has been approved as fulfilling the requirements or partial fulfilment of the requirements for the award of a Master degree in Public Health – Environmental Health by the University of Zambia.

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ABSTRACT

Cholera, an acute diarrheal disease, caused by the bacteria *Vibrio cholera*e and is usually transmitted through water or food contaminated with faecal matter. This study assessed the environmental factors associated with endemic cholera in Lukanga Swamps from 2010 to January, 2018. This was a Time Series Correlational Study design. The data on cholera cases was collected from Kapiri Mposhi DHIS and outbreak line listing database system. Air temperature and precipitation data was collected from 19 lagoons using satellite remote sensing from 2010 to 2016. Water samples for analysis were collected from 19 Lagoons of the Lukanga Swamps. Data was analysed in Stata Version 14 and the results from standard poison regression (PR) model were compared with those obtained from Zero-inflated negative binomial regression (ZINBR) using Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) to determine the best fit model and ZINBR fitted the data well.

The study revealed that there were 133 cholera cases in the period under study and that cholera broke out mainly during the dry season between the months of May and October. Air temperature from January, 2010 to December, 2016 in the Lukanga Swamps ranged from 16°c and 28°c and the lowest was in the month of June, 2010 while the highest was in October, 2016. Precipitation had a wider range from as low as 0.05mm in April, 2016 to as high as 248mm in December, 2013. The study also found that increased rainfall was associated with decreased risk of cholera cases (IRR = 0.992, 95% CI: 0.988 – 0.997, P=0.002). The study found that one degree increase in temperature increases the risk of cholera cases by 27% and was statistically significant with (IRR = 1.265, 95% CI: 1.142 – 1.402, P= 0.0001). Water quality monitoring tests from 19 lagoons revealed that 19/36 (53%) of water samples had faecal coliforms. The study further revealed pH range of 7.1 to above 8.5. No V. *cholerae* from the 164 water samples was isolated.

The results have shown that air temperature was strongly associated with cholera outbreaks in the Lukanga Swamps and it showed ability in predicting these outbreaks with a lag time of two months prior to temperature rise. The study also revealed that increased amount of rainfall was associated with decreased risk of cholera cases. The microhabitat conditions were promotive of the *V. cholerae* growth in the swamps. Therefore, a multifaceted approach and combination of surveillance, water, sanitation and hygiene, social mobilization and water treatment should be used to prevent and control cholera in Lukanga Swamps.

Keywords: Endemic cholera, Environmental factors, Lukanga Swamps

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ABBREVIATIONS AND ACRONYNS

ARI:	Acute Respiratory Infections
CAMS:	Climate Anomaly Monitoring System
CIDRZ:	Center for Infectious Disease Research in Zambia
CSO:	Central Statistics Office
DFID:	Department for International Development
DMO:	District Health Office
DRC:	Democratic Republic of Congo
GHCN:	Global Historical Climatology Network
HMIS:	Health Management Information System
ICF:	International Children's Fund
IHR:	International Health Regulations
IDSR:	Integrated Disease Surveillance and Response
MOH:	Ministry of Health
MOWDSEP	Ministry of Water Development Sanitation and environmental Protection
OVC:	Oral Cholera Vaccine
TRMM:	Tropical Rainfal Measuring Mission
UNICEF:	United Nations International Children Emergency Fund
UNZABREC:	University of Zambia Biomedical Research Ethics Committee
USA:	United States of America
WHO:	World Health Organisation

OPERATIONAL DEFINITIONS

Cholera: Is an acute bacterial diarrheal disease, caused by the bacteria *Vibrio cholerae* which is usually transmitted through water or food contaminated with faecal matter.

Outbreak: The "unusual" occurrence in a community or region of disease, specific health related behaviour, or other health related events clearly in excess of "expected occurrences".

Environmental Factors: These factors include Environmental Factors as including climatological conditions (air temperature, precipitation, humidity); Sociological conditions (sanitation and water quality) and Microhabitat Conditions (pH, and water conductivity).

Hydro-climatological conditions: These include not limited to air temperature, precipitation and humidity.

Temperature: The degree of hotness and coldness of an object. It is an indication of the thermal energy content of matter.

Precipitation: Is water that falls in any form from the sky to the earth or its any product of the condensation of water vapour that fall under gravity.

Water conductivity: Conductivity is a measure of the ability of water to pass an electrical current.

Sanitation: Sanitation literally means measures necessary for improving and protecting health and wellbeing of the people. Sanitation is any system that promotes proper disposal of human and animal wastes, proper use of toilet and avoiding open space defecation.

Epidemic Cholera: Is a sudden outbreak in a previously relatively disease-free region and primarily inland.

Endemic Cholera: Is a recurring outbreak essentially annually and predominantly along coastal regions

CHAPTER ONE

INTRODUCTION

1.0 Background Information

Cholera, an acute diarrheal disease, caused by the bacteria *Vibrio cholera* is usually transmitted through water or food contaminated with faecal matter Levy (2015). The organism is capable of producing a potent toxin known as cholera toxin (CT). The *V. cholerae* infection displays a clinical spectrum that ranges from asymptomatic infection to severe cholera known as cholera gravis (Lantagne et al., 2014). The diarrhoea becomes very severe in about 10–20 % of these individuals and may be accompanied by vomiting. Without prompt and adequate treatment, these patients lose large amounts of fluid and salts leading to severe dehydration and death within hours. With appropriate treatment which largely hinges on fluid replacement, case fatality rate is less than 1%. Annually about 2.8 million cholera cases are reported globally and deaths from cholera are estimated to range from 28,000 to 142,000 (Ali, 2012). Cholera has become endemic in Africa with large epidemics occurring in different parts of the continent Figure 2. Over the course of 2007 to 2011, more than 100,000 cases of cholera were reported to WHO annually from at least 20 African countries with case fatality rates reaching about 3% (WHO, 2014).

Cholera is preventable and treatable disease however, it remains a major public health problem in many Sub-Saharan African countries causing deaths and retarding development (Naidoo and Patric, 2002). Though, knowledge on stopping cholera transmission and deaths is well documented and was used successfully by countries in South America to eliminate cholera during the previous decades, the environmental factors associated with epidemic cholera have not been widely documented and understood in Africa (Glass et al., 1991).

Interventions for cholera prevention and control include provision of good sanitation, safe water, hygiene, health education, surveillance, treatment of the patients, and recently vaccination with the World Health Organization (WHO) recommended vaccines

(Bhattacharya et al., 2009). However, there is little documentation in trying to predict when and where such outbreaks would occur and this can only be achieved by assessing the environmental conditions under which the Vibrio bacteria proliferate. The consequences of this gap are protracted epidemics, unnecessary suffering of the populations, economic lose and social disruption. On the other hand, for a long time, the Asian sub-continent was the home of cholera. However, in recent years most of the reported cholera cases have been from Sub-Saharan Africa which contribute 60% of all reported cases and deaths globally (Ali, 2012). Majority of cholera affected countries in Africa subscribe to WHO and use Integrated Disease Surveillance and Response (IDSR) strategy to prevent and control cholera outbreaks (Kasolo et al., 2013). Zambia is also one of the countries that are obliged to report any cholera outbreaks to WHO as they occur.

In 2013, a total of 25,762 cholera cases and 490 deaths were reported from WHO African region with the three countries namely DRC, Angola and Mozambique contributing 79.4% (20,455) of cases and 89% (436) of reported deaths (Schaetti et al., 2013). To reverse the status quo, the current approach has to be reviewed and lessons of the good actions in South America to prevent cholera outbreaks copied. In addition to its severe toll on public health, cholera causes serious social and economic disruption. Outbreaks may cause panic, and this may generate reactions such as quarantine, excessive isolation, and the use of mass chemoprophylaxis. These inappropriate responses can be avoided by providing adequate and timely information to policy-makers, decision-makers, the media and the public. The introduction of the new Oral Cholera Vaccines (OCV) which works synergistically with the other known vaccines has added value in the prevention and control of the disease (WHO, 2010a).

However, since the production and the stock of these new vaccines is limited, efficient and effective use of the available vaccine stocks is highly recommended by WHO. Therefore, for the Sub-Saharan cholera endemic countries to qualify to use these vaccines, accurate data and documentation on the affected population is a necessity. Furthermore, there is need to understand the variability of the environmental hydro-climatological conditions that influence epidemic cholera in different countries and locations.

In Zambia, cholera is endemic and cases appear almost every year. Certain regions of the country are more prone to epidemics, among which is Lusaka, the capital of Zambia. In 2003 it was estimated that 36% of the total country population lived in urban areas and only 55% had access to drinking water (Fernández et al., 2009). *Vibrio cholerae* is a natural inhabitant of the aquatic environment (Colwell, 1996), which with evidence of new biotypes emerging from its environmental reservoir, indicates that cholera bacteria cannot be eradicated (Gaudart et al., 2013). This proves the endemicity of the *V. cholerae* in the Lukanga swamps which cause frequent outbreaks of cholera in the area. It is believed that the *V. cholerae* thrives in the aquatic environment of the swamps and only causes the outbreak when the environment is conducive for its proliferation.

Because cholera outbreaks will continue to occur over time, the most effective means of controlling or preventing the disease is to minimize exposure to pathogenic strains and/or high concentrations of cholera bacteria. Observational records show that the vast majority of cholera outbreaks originate in coastal regions, indicating a strong association between environmental hydro-climatological factors including air temperature, precipitation, humidity, pH and water conductivity and the disease (Akanda et al., 2009). Despite significant advances in our knowledge of the metabolism, pathogenesis, and genomics of *V. cholerae*, we still cannot predict precisely when the next cholera epidemic will occur or the probability, timing, and/or location of an outbreak, all of which is essential if an effective intervention strategy is to be designed and implemented (Gaudart et al., 2013).

According to Gaudart and others, *V. cholerae* is autochthonous to the aquatic environment globally and that outbreaks of cholera in endemic regions show strong hydroclimatological influence, which is encouraging because those conditions can be monitored in epidemic regions. They also contextualized in their study, seasonality as a mechanism of occurrence of cholera outbreaks. This tallies with the seasonal occurrence of cholera in Lukanga swamps which usually occurs between June and October (MOH, 2016). Their study revealed also that about 50% or more cholera outbreaks occurred when the air temperature was > 31°C, approximately one standard deviation from average air temperature over the previous 2 months. However, air temperature alone cannot cause an epidemic, unless accompanied by appropriate transmission mechanisms such as poor water quality and lack of sanitation infrastructure, as well as rainfall.

Satellite derived hydrological processes can be used to capture environmental conditions related to epidemic or endemic, as occurred in Zimbabwe, thereby providing an early warning system. Since cholera cannot be eradicated because the causative agent, *V. cholerae,* is autochthonous to the aquatic environment, prediction of conditions favourable for its growth and estimation of risks of triggering the disease in a given population can be used to alert responders, potentially decreasing infection and saving lives (Jutla et al., 2015). In Lukanga swamps we investigated the conditions that provide conducive environment for the proliferation of the *V. cholerae* and trigger the cholera outbreaks.

1.1 Statement of the Problem

In Zambia Cholera has been endemic since its first occurrence in 1977/1978, just like in other areas of Africa (Fernández et al., 2009). Recent studies have established that cholera occurs in three forms: epidemic (a sudden outbreak in a previously relatively disease-free region and primarily inland), endemic (recurring essentially annually and predominantly along coastal regions), and mixed-mode (a combination of epidemic and endemic disease occurrence). Epidemic and endemic cholera show significantly different mortality rates, with epidemic cholera having higher mortality, over 6% in some cases. The mortality rates reported for recent epidemics include Haiti 6.4% in 2010, Madagascar 6% in 2000, Zimbabwe 4.3% in 2008–09, and Nigeria 3.8% in 2010 (Jutla et al., 2015). In Lukanga swamps however, endemic cholera has been experienced with recurrence of almost every year (MOH, 2016). Through the surveillance system that has been established by the Ministry of Health, Central province recorded a number of cholera cases in three consecutive years; In 2014 there were 47 cholera cases, 2015 seven (7) cases and in 2016, 68 cases were recorded (MOH, 2016).

In Kapiri Mposhi district there has been a problem of cholera for a number of years now emanating from the Lukanga swamps which is located in Kapiri Mposhi district of Central Province. In 2014 the district recorded 47 cases of cholera, 7 cases in 2015 and 35 cases in 2016. Most of the recorded cases were attributed to Lukanga swamps alone which recorded 47 cases in 2014, 6 cases in 2015 and 30 cases in 2016 respectively (MOH, 2016) and this confirms the endemicity of the *V. cholerae* in this aquatic environment.

The disease outbreaks in Lukanga swamps commonly occur in the dry season from June through to October, unlike other areas in the country where cholera breaks out in the rainy season (MOH, 2016). Despite the yearly recurrence of cholera outbreaks in the Lukanga swamps, little is known about the conditions of the environmental factors associated with endemic cholera within the swamps. The disease affects mostly Fishermen and fish traders who frequently visit the swamps to trade in fish. A number of interventions have been carried out such as community sensitization, distribution of chlorine and many more but outbreaks have frequently continued in the Lukanga swamps without understanding when and where they would occur. This study therefore, aims to assess environmental factors associated with endemic cholera in Lukanga swamps.

1.2 **Study Justification**

There are few studies conducted in Zambia linking environmental factors with health, this has made it very difficult for the health sector to have evidence based interventions targeting specific climate sensitive diseases. This research therefore, serves to assess the environmental factors associated with endemic cholera in Lukanga Swamps to create the knowledge base of the conditions under which these factors make the environment conducive for the proliferation of the *V. cholerae* in this aquatic environment and eventually trigger the outbreak.

This research will also help to inform stakeholders; such as (the community, programme implementers); partners (UNICEF, CIDRZ, etc); Policy makers such as (MOH, MOWDSEP, etc) and the Academia that have the interest in solving the problem of endemic cholera in Lukanga Swamps. The study will also contribute to the body of knowledge by establishing the conditions under which environmental factors influence the endemic cholera. Little has been done to assess the hydroclimatological conditions namely temperature, precipitation and humidity in conjunction with environmental conditions including sanitation and water quality together with Microhabitat conditions such as, pH and water conductivity in cholera endemic areas like the Lukanga swamps Figure . Understanding the environmental drivers of cholera outbreaks could facilitate some degree of outbreak prediction, allowing governments to prepare and respond to potential outbreaks. Following an outbreak it is recommended that an assessment of the outbreak response is undertaken to identify strengths and weaknesses to inform planning for improved preparedness and response towards future outbreaks. Generally therefore, when such conditions are properly understood, effective interventions can be implemented which will culminate into cholera outbreak reduction in Lukanga Swamps.

The Figure 1 presents a theoretical pathway connecting large-scale hydro-climatological processes with cholera occurrence in epidemic regions. Two rectangles are depicted, with the inner rectangle representing conditions associated with cholera in an epidemic region. If air temperature is above the climatological average for 2 months, and is followed by above climatological average rainfall, in combination with poor or damaged water and sanitation access, it is probable that the region will experience a cholera epidemic. On the other hand, the outer rectangle, encapsulates conditions under which cholera generally does not occur. If any of the conditions of the inner circle are not met, the likelihood of cholera decreases (Jutla et al., 2015).

According to (Lugomela et al., 2014), there has been no analysis of data for the mainland coastal regions connected to the marine environment. Given the potential importance of such relationships for the prediction of cholera outbreaks in a country like Tanzania, they sought to analyse the relationship between cholera cases and climate in the coastal regions of mainland Tanzania, focusing on the years 2004 to 2010. They also aimed to establish whether any identified co-variations of cholera with seasonal or climatic cues would be useful in predicting outbreaks in coastal Tanzania regions ahead of time.

Cholera epidemics are likely to occur with high probability if hydroclimatological risk [defined as a combination of large scale geophysical processes and environmental conditions conducive to bacterial growth] and societal risk [defined as reduced or lack of availability of safe drinking water and sanitation] occur in a community or region (Jutla et al., 2015).

1.2.1 Theoretical framework for predicting cholera outbreaks.

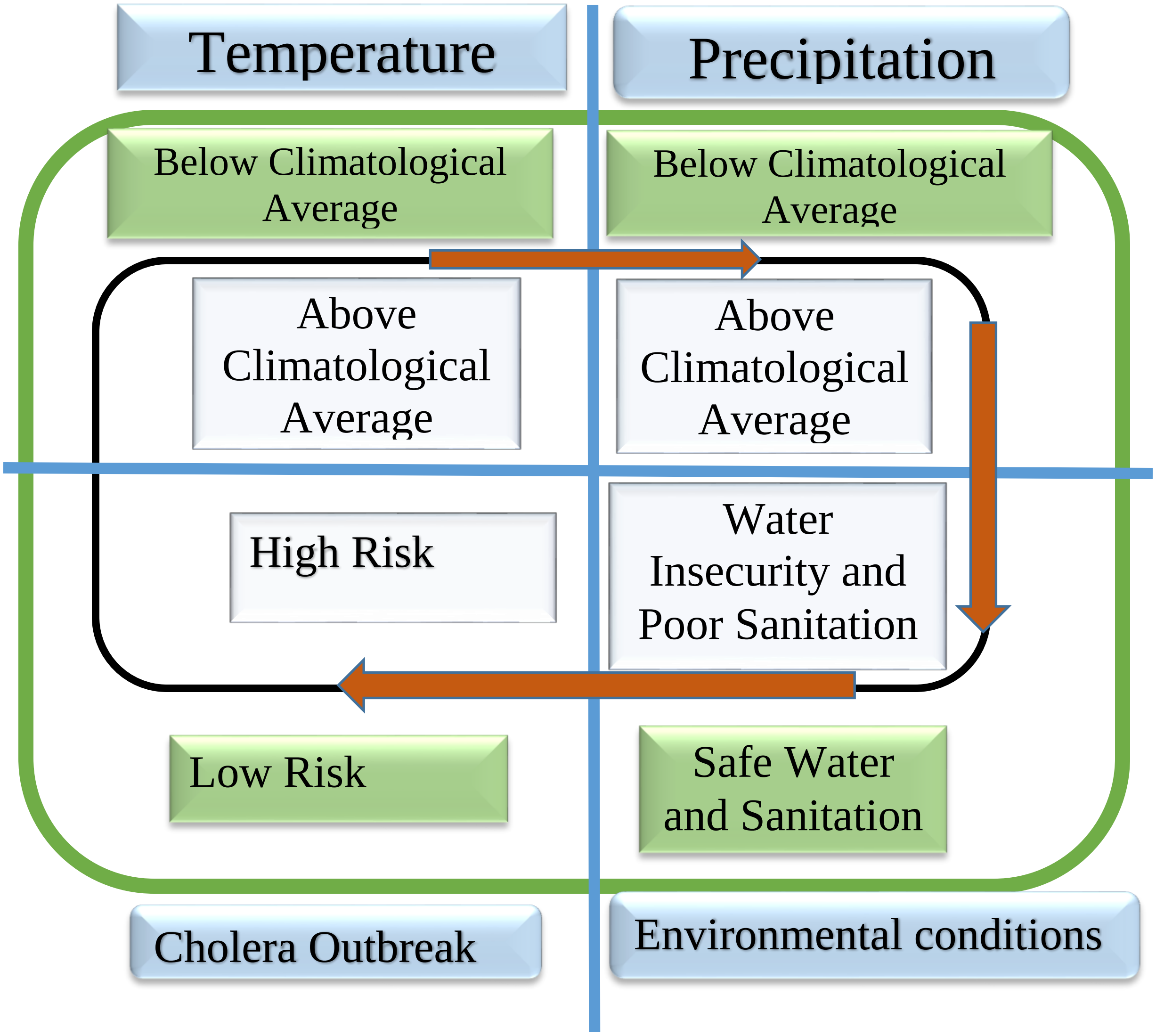

Figure 1: Adapted Theoretical Framework for predicting cholera outbreaks (Jutla et al., 2015).

A multifaceted approach is therefore, key to prevent and control cholera, and to reduce deaths. A combination of surveillance, water, sanitation and hygiene, social mobilization, treatment, and oral cholera vaccines are used. However, the variability of climatological conditions associated with endemic cholera should be adequately understood before implementing these interventions. Such understanding would enhance preparedness against cholera outbreaks and with proper executions of the preventive and control strategies and interventions, future outbreaks can be prevented and if they occurred could easily be controlled.

1.3 Research Question

What are the environmental factors associated with endemic Cholera in Lukanga swamps of Kapiri Mposhi district in Central Province, Zambia?

1.4 Objectives

1.4.1 General Objective:

To assess environmental factors associated with endemic Cholera in Lukanga Swamps of Kapiri Mposhi District in Central Province, Zambia.

1.4.2 Specific Objectives

i. To determine the cholera cases from Lukanga swamps in Kapiri Mposhi District from January, 2010 to December, 2016.

ii. To assess association between environmental conditions (temperature and precipitation) and cholera cases in Lukanga Swamps from January, 2010 to December, 2016.

iii. To describe the environmental conditions (water quality and sanitation) and microhabitat condition (water pH) in Lukanga swamps from September, 2017 to January, 2018.

iv. To determine the *Vibrio cholerae* contamination of 19 lagoons of Lukanga swamps that fall under Kapiri Mposhi catchment area from September, 2017 to January, 2018.

CHAPTER TWO

LITERATURE REVIEW

2.0 Cholera

Cholera is an acute diarrhoeal disease caused by *V. Cholerae* 01 (classical or El Tor) and 0139. Cholera is now commonly due to the El Tor biotype and 0139. Cases range from symptomless to severe infections. The majority of the infections are mild or asymptomatic. Typically cases are characterized by the sudden onset of profuse, effortless, watery diarrhoea followed by vomiting, rapid dehydration, muscle cramps and suppression of urine (Park, 2015). It also causes intense thirst, loss of skin turgor, wrinkled skin of hands and feet, sunken eyes, pinched facial expression, thread or absent peripheral pulses, and falling blood pressure and inaudible hypoactive bowel sounds. Unless there is rapid replacement of fluid and electrolytes, the case fatality may be as high as 30 to 40 percent (Park, 2015).

V. cholerae O139, which emerged in the Bay of Bengal in 1992, has so far been confined to South-East Asia. During 2013, only China reported cases due to O139 strains. Among the 49 laboratory-confirmed cases in China, 37 were O139 serogroup and 12 were O1. Countries are encouraged to test for both serogroups O1 and O139 when diagnosing *V. cholerae* infection (WHO, 2014).

Annually about 2.8 million cholera cases are reported globally and deaths from cholera are estimated to range from 28,000 to 142,000. Cholera has become endemic in Africa with large epidemics occurring in different parts of the continent. Over the course of 2007 to 2011, more than 100,000 cases of cholera were reported to WHO annually from at least 20 African countries including Zambia with case fatality rates reaching about 3 % (Ali, 2012).

The number of cholera cases reported to WHO continues to rise. For 2013 alone, a total of 129,060 cases were notified from the 47 countries, including 2,102 deaths. Many more cases were unaccounted for due to limitations in surveillance systems and fear of trade and travel sanctions. The true burden of the disease is estimated to be 1.4 - 4.3 million cases and 28,000-142,000 deaths annually (WHO, 2014).

Recent studies indicate that global warming creates a favourable environment for the bacteria. Cholera therefore, remains a global threat to public health and a key indicator of lack of social development. The dynamics of cholera occurrences since 2005, combined with the emergence of new strains that lead to a more severe clinical presentation; increased antimicrobial resistance and climate change, suggest that cholera may return to the forefront of the public health agenda (WHO, 2010b).

2.1 Epidemiological features

Cholera is both an epidemic and endemic disease. The epidemicity and endemicity of the disease will depend on the characteristics of the agent and those of the system (environment). Characteristics of the agent that influence its distribution include its ability to survive in a given environment, its virulence and, the average number of microorganisms required to cause infection. The characteristics of the system that affect the distribution of the agent include the number of susceptible hosts, and the opportunities it provides for transmission of the infection. Global experience has shown that the introduction of cholera into any country cannot be prevented, but cholera can create a problem only in areas where there is poor sanitation (Park, 2015).

Epidemics of cholera are characteristically abrupt and often create a public health problem. They have a high potential to spread fast and cause deaths. The epidemic reaches a peak and subsides gradually as the “force of infection” declines. Often-times, by the time control measures are instituted the epidemic would have already reached its peak and its waning. This cholera epidemic in a community is self-limiting. This is attributed to the acquisition of temporary immunity, as well as due to the occurrence of a large number of subclinical cases.

Global Map Showing Major Cholera Outbreaks from 2000 to 2010 to indicate Countries where cholera is endemic.

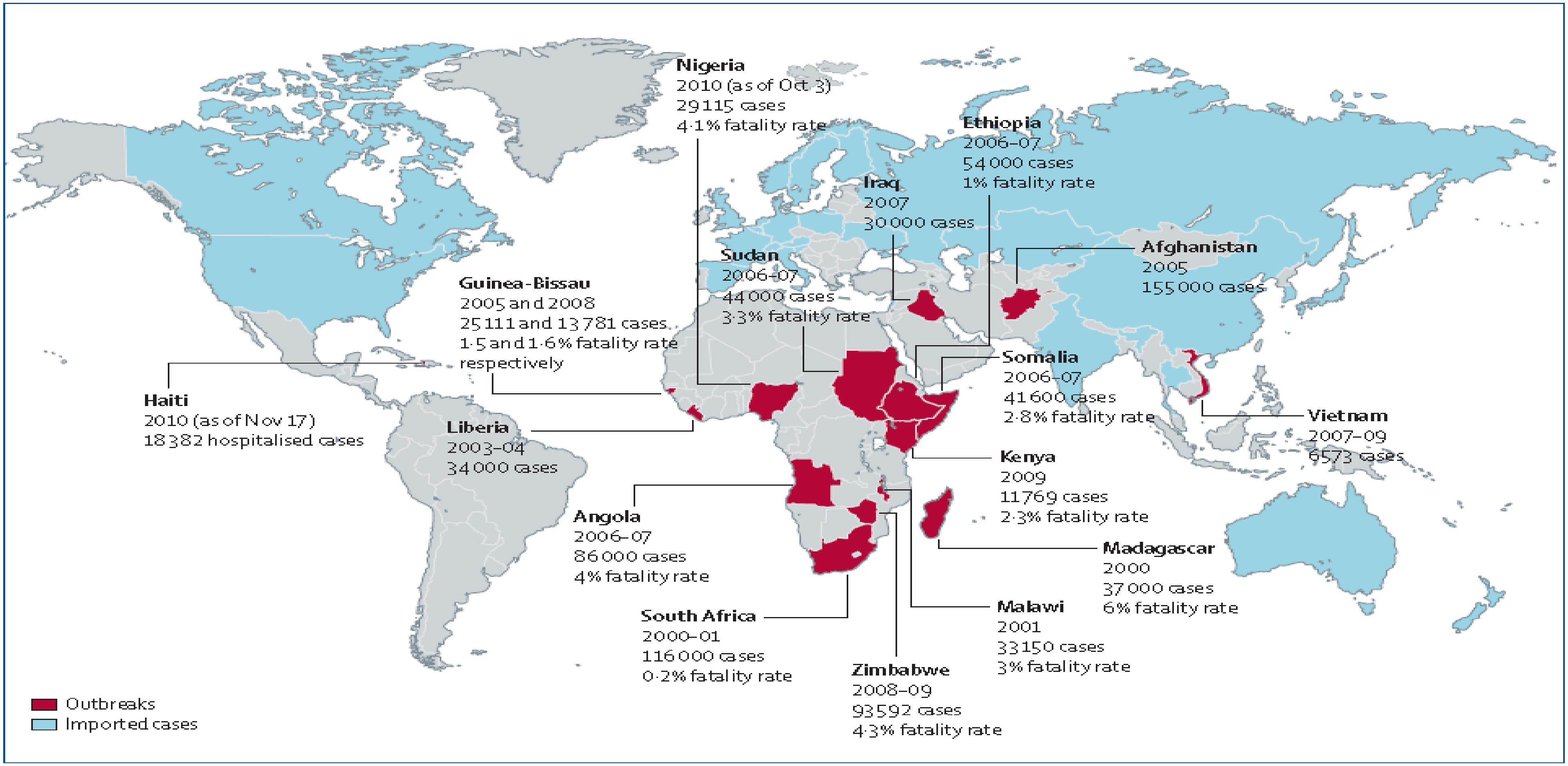

Figure 2: Major global cholera outbreaks from 2000 to 2010 (Taylor et al., 2015).

The “force of infection” is composed of 2 components, namely the force of infection through water and the force of infection through contacts. It is well known that the elimination of contaminated water does not immediately bring an outbreak to an end, but a so called “tail” of the epidemic is produced. This is due to the continuation of transmission through contacts. The El Tor biotype, where it has spread, has become endemic with periodic outbreaks. It appears to have greater “endemic tendency” than its classical counterpart in that it causes higher infection to case ratio (i.e; inapparent infections and mild cases) (Park, 2015). This is similar to the Lukanga swamps where there are periodic outbreaks denoting endemicity of the microorganism in that aquatic environment.

Cholera occurs at intervals even in endemic areas. A question that is frequently asked is about the fate of the *V. cholerae* in the inter-epidemic periods. Three explanations are offered: (a) the existence of long-term carriers; (b) the existence of diminished but continuous transmission involving asymptomatic cases; and (c) the persistence of the organism in the free-living, perhaps altered form in the environment. The existence of a free-living cycle may explain why cholera became endemic for varying periods in certain areas after introduction of the current pandemic strains. Atypical non-toxigenic V. *cholerae* 01 of the El Tor biotype have sometimes been found in surface waters in endemic and non-endemic areas without any related human infection or disease (Said et al., 2011).

Though the bacterium can live naturally in the environment, studies have shown that *V. cholerae* exist as natural inhabitants of aquatic ecosystems (Reidl and Klose, 2002). They usually occur as part of flora of streams, riverine, brackish water, estuarine and coastal waters. They attach to surfaces provided by plants, filamentous green algae, copepods (zooplankton), and insects (Juliana, 2011). *V. cholerae* has also been found associated with invertebrate members of the zooplankton such as crustaceans, dipterae, and shellfish; with vertebrates such as fish and waterfowl; and with other microorganisms such as *Acanthamoeba castellanii (Almagro-Moreno and Taylor, 2013a)*. Segments and slow flowing water can also lead to an increasing exposure of the organism. The Lukanga has large segments of slow or none flowing water which create an environment for the endemicity of the *V. cholerae*. According to Islam and others; the organism can survive in almost all kinds of aquatic environments including fresh water sources such as lakes, ponds, rivers and tanks. Cholera bacteria can also survive in non-aquatic environments such as refuse dumps sites, fruits, fresh vegetables, meat, cooked food, human and animal faecal waste, untreated or inadequately treated waste (Juliana, 2011). In its natural environment *V. cholerae* encounters two main predators: bacteriophages and protozoa. It has also been found that some antagonistic bacteria inhibit the growth of *V. cholerae* (Almagro-Moreno and Taylor, 2013a).

2.2 Cholera Transmission

The transmission of cholera occurs through different pathways. The Figure 3, first developed by Wagner and Lanoix in 1959, illustrates the major transmission pathways for excreta-related pathogens. The model depicts the five intermediary transmission environments from which an individual may become infected with a faecal-oral pathogen: fluids, fields, flies, fingers, and foods. Pathogens may travel via unwashed hands, in contaminated water, or by flies and other insects on to further human hosts (DFID, 2013).

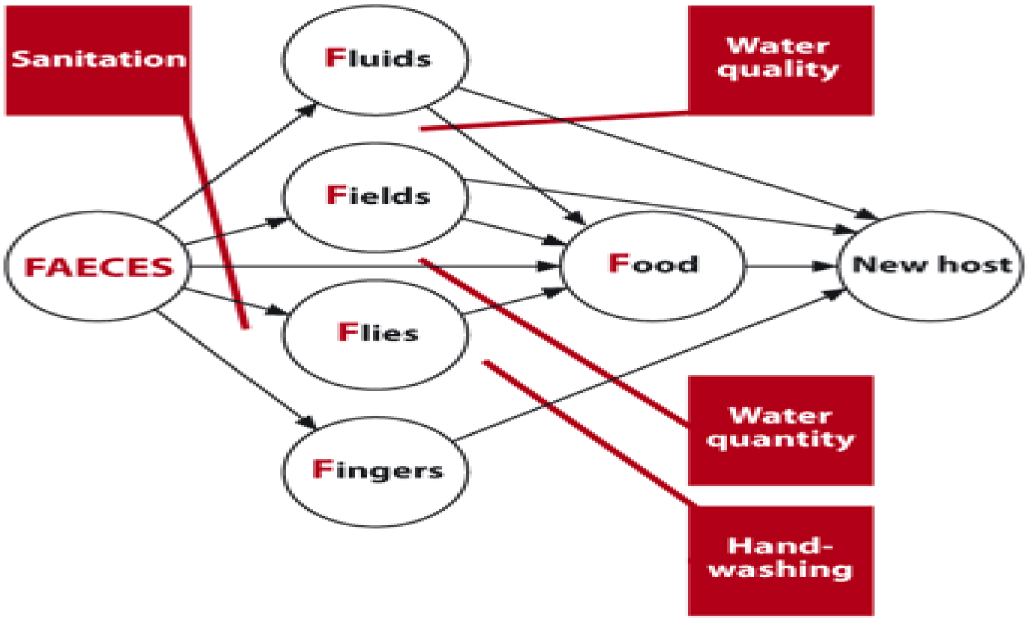

Figure 3: Major transmission pathways for excreta-related pathogens

2.3 Sources of Infection

Contaminated water with free living *V. cholerae* cell are the main source of cholera followed to a lesser extent by contaminated food particularly seafood like crabs, Oysters and shellfish. Bad sanitation practices in highly populated areas harboring the bacteria and the sources of intermittent outbreaks due to contamination of drinking water and or improper food preparation. The source of the infection is usually other cholera sufferers when their untreated diarrhoeal discharge is allowed to get into water ways or drinking water supplies. Drinking of any of the infected water and eating any foods washed in the water, as well as seafood living in affected water bodies, can cause a person to contract the infection (Nelson et al., 2009). The bacterium has been found in coastal environments around the world, both in areas where cholera is endemic and in cholera disease-free areas. It is now well known that cholera occurs in regions with natural aquatic reservoirs where the bacteria can persist in a free-living state, or in association with phytoplankton, zooplankton and detritus (Nelson et al., 2009). Using whole genome sequencing and subsequent genomic analysis, two distinct Vibrio populations, *V. cholerae* O1 and *V. cholerae* non-O1/O139, were identified and concluded to have contributed to the cholera epidemic in Haiti (Gaudart et al., 2013).

2.4 Transmission Mechanism of Cholera

There are two routes of cholera transmission namely, primary and secondary transmission. According to Osei; citing Hartley and other, primary transmission happen through an exposure to an environmental reservoir of *V. cholerae* or contaminated water sources regardless of previously infected persons or faecal contamination. Therefore, aquatic environments are essential for the spread of cholera. Secondary transmission route on the other hand occurs through exposure of faecally contaminated water sources, food or infected person. Osei argued that this route reflect a complicated transmission pattern since multiple factors may play a role in the spread of the disease (Collins et al., 2006).

2.5 Factors Influencing the spread of Cholera

Socio-economic, environmental including hydroclimatological and sociological factors and demographic conditions enhance the vulnerability of population to infection and contribute to the epidemic spread of cholera. The cholera germ is passed in the stool of infected persons. It is widely spread by consuming food or water which has been contaminated by the faecal waste/stools of an infected person. This happens more often in developing countries. This is so because developing countries lack adequate clean water for drinking and proper sewage disposal systems as well as also practices poor sanitation and poor hygiene (Igbinosa and Okoh 2008).

Once cholera is introduced to a population in a specific location, numerous complex factors decisively influence its propagation and may lead to prolonged transmission. In this study will focus on hydro-climatological factors and sociological conditions to assess how they influence and trigger the endemic cholera. The following may not be limited to the factors and conditions associated with endemic cholera in Lukanga swamps:

2.6 Environmental Factors

These environmental factors include not limited to all those conditions related to the natural aquatic environment such as air temperature, precipitation, humidity, salinity, water conductivity, Potential Hydrogen (pH) and water levels.

In areas where there is low temperature or low rainfall, transmission of vector-borne disease is restricted. However, climatic variations could also tip the ecological balance and trigger epidemics (McMichael, 2006). Cholera appears to wax and wane in endemic regions on time scales from 3 to 6 years, a pattern that has long been recognized. Early explanations for these cycles were already linked to climate (Pascual et al., 2002).

2.6.1 Air Temperature

It is well established that environmental factors, through seasonal variations or as a consequence of global climate change, play an important role in the resurgence and dynamics of infectious diseases. Weather conditions such as an increase in environmental or sea surface temperatures favor plankton bloom. This link with temperature could explain the surge of cholera in endemic zones in cycles of 3—6 years, its expansion and its re-emergence after an absence of several years In line with this observation, theoretical models were developed that included environmental variables as causal factors for cholera re-emergence in an attempt to describe its dynamics. In real-life conditions, positive correlations were shown to exist between an upsurge in the number of cholera cases during an outbreak and the increase in sea surface temperature 8 weeks earlier

(Fernández et al., 2009). Pathogens like salmonella and cholera bacteria proliferate more rapidly at higher temperatures (Pascal, 2005).

According to (Hashizume et al., 2008), in their study further indicate that high temperature and heavy rainfall are associated with increased number of diarrhoea cases suggesting that rainfall and temperature have adequate strength to project the epidemics of diarrhoea, implying that these weather factors provide valuable insights into the seasonality of diarrhoea.

The impact of temperature and rainfall, both associated with climate change, on cholera was studied in Tanzania and the conclusion was that temperature is significantly associated with cholera, i.e; a one degree Celsius increase in air temperature increases relative risk of cholera by 15–29% (Jutla et al., 2010). Water temperature, in particular, has been shown to be an important factor governing the seasonal and geographical variation in *V. cholerae* (Igbinosa and Okoh, 2008). The Figure 4 illustrates the relationship between temperature and cholera cases in Lusaka;

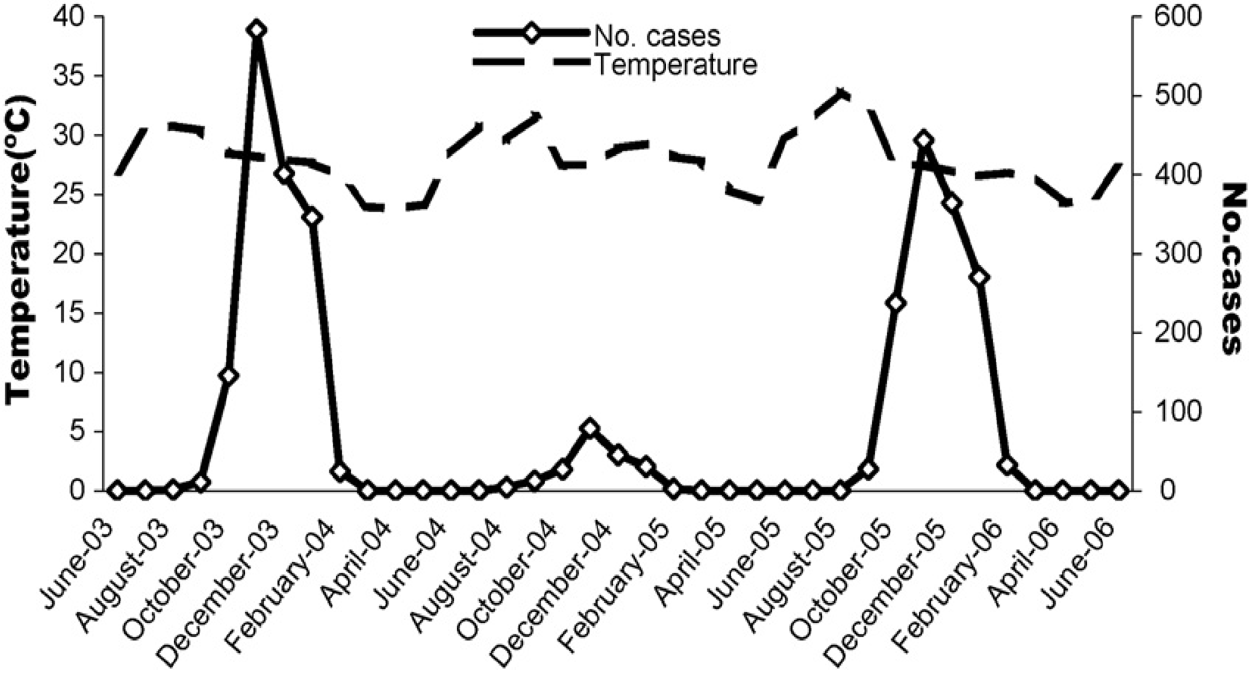

Figure 4: Time plots of number of cholera cases per month and monthly mean temperature (degrees Celsius) in Lusaka, Zambia, 2003-2006 (Fernández et al., 2009).

According to (Ali et al., 2013) citing Lobitz and others hypothesized that rise in the local sea-surface temperature influences the growth of phytoplankton concentrations, and an increase in sea-surface height increases human-Vibrio contact by transporting the bacteria into inland waters through tidal intrusion of plankton. They hypothesized that an increase of Surface Sea Temperature (SST) results in replication of phytoplankton populations, which are directly associated with the increase in V. *cholerae* bacteria and are linked spatially and temporally to zooplankton populations.

(Gaudart et al., 2013) in their study revealed that about 50% or more cholera outbreaks occurred when the air temperature is > 31°C, approximately one standard deviation from average air temperature over the previous 2 months (solid line). However, air temperature alone will not cause an epidemic, unless accompanied by appropriate transmission mechanisms such as poor water quality and lack of sanitation infrastructure, as well as rainfall. The triggering point of the epidemic may well have been the convergence of optimal environmental conditions of warm temperatures, heavy rainfall followed by flooding (as evidenced from time series analysis of historical cholera in North India), and destruction of an already inadequate water and sanitation infrastructure.

2.6.2 Precipitation

Disease rainfall relations are multifaceted, while heavy rainfall and flooding are associated with triggering outbreaks of diarrhoea; they may at the same time reduce vector or pathogen populations by flushing them from their environment in pooled water. There is also some evidence that heavy rainfall events may be followed by coliform re-growth in water distribution systems, presumably because of increased nutrients in water (Mudenda, 2014).

Previous research in Kolkata and Dhaka have shown that precipitation is associated with an increase in cholera cases most likely caused by the feeding of nutrient-rich runoff into water bodies and flooding of the water supply intended for human consumption with river water harboring *V. cholerae.* Rainfall provides the most appropriate mechanism for spread of cholera through cross-contamination of water, with the population having to rely on surface water for daily usage (Gaudart et al., 2013).

In 2009 in Lusaka, Zambia, Fernández and other found an association between increased rainfall and cholera cases as illustrated in Figure 5.

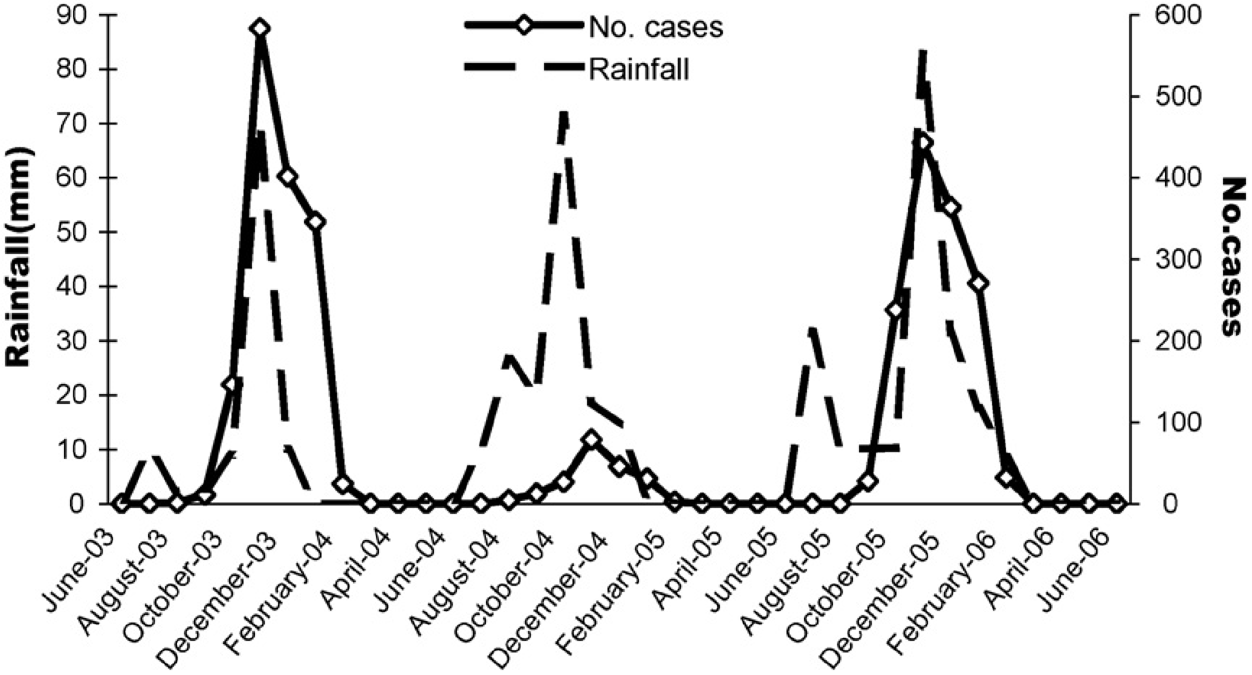

Figure 5: Time plots of number of cholera cases per month and monthly mean rainfall (mm) in Lusaka, Zambia, 2003-2006 (Fernández et al., 2009).

2.6.3 Humidity

Vibrio cholerae requires optimal temperature and physicochemical conditions (salinity, pH, humidity etc.) to survive. Nevertheless, it has also been shown to resist suboptimal conditions through specific associations of the bacterium with aquatic plants or animals such as oysters, crabs and copepods. As a result, the pathogen can persist for longer periods in aquatic habitats (Fernández et al., 2009). Other studies, although not predictive in design, linked climatic variables, e.g., precipitation, temperature, and humidity, to outbreaks of cholera, based on historical data (Jutla et al., 2015).

In Kolkata *V. cholerae* infection was associated with higher relative humidity (RH) (>80%) with 29°C temperature with intermittent average (10 cm) rainfall. Heavy rainfall indirectly influenced the *V. cholerae* infection, whereas no correlation was found with high temperature. Russel and Sundararajan established that high temperature, correspondingly high RH and intermittent rainfall acted as ideal climatic conditions for the incidence of cholera. In this study it was found that the temperature was neither low nor high but the humidity was high during rainfall. During this period, the zero difference day temperature highly favored survival of *V. cholerae* along with the gradual increase in RH throughout the day with controlled temperature. The RH remained almost constant during raining season but minimized considerably when there was no rainfall. The progressive nature of *V. cholerae* infection was correlated with high RH both during rain and non-raining periods.(Rajendran et al., 2011).

2.7 Microhabitat Conditions

2.7.1 Potential Hydrogen (pH) level

Rivers have long been recognized as an ecological corridor and habitat of cholera bacteria and its host, the copepod. Thus, warm air temperature, increased evaporation and water temperature, and decreased water levels in rivers, the primary source of water for household use in the region, are linked to cholera outbreaks. Overwhelming evidence in the literature shows that *V. cholerae* is autochthonous to the aquatic environment in both freshwater and estuarine systems. Previous studies have shown that the pH value of ∼8.0 and warm water temperatures between 19 and 28°c are related to an increased concentration of cholera bacteria (Gaudart et al., 2013)**.** This means that the *V. cholerae* thrive easily in an environment that has increased water conductivity and less acidic with pH level of ∼8.0 coupled with favorable temperatures.

2.8 Environmental Conditions

2.8.1 Sanitation

Cholera is hypothesized as a disease of deficient sanitation (Igbinosa and Okoh, 2008). Lack of adequate toiletry, cleaning, washing and drainage facilities results in sickness and increases the risk of transmission. Poor sanitation can be attributed to high population which leads to overcrowding putting strain on existing sanitation system, thereby putting the population at high risk (Anamzui-ya, 2012). Sub-Saharan Africa and South Asia have the lowest levels of access to improved sanitation at 30% and 41%. Notably, 1.1 billion people in the world still practice open defecation. Regionally, the highest levels of open defecation are found in South Asia (41%) and sub-Saharan Africa (25%). Over half of all people practicing open defecation globally live in India (DFID, 2013).

In the swamps there are no sanitary facilities where excreta can be disposed of safely. This is due to the fact that there is no available land where excreta disposal facilities can be constructed. In the exigencies of sanitary facilities in such areas, the common practice of excreta disposal is open defecation. High population exacerbates the sanitation problem and these people may increase in number in swamps due to the fact that the legal framework governing the fishing in swamps is weakened. There is inadequate monitoring and acknowledgement of how many people are in the swamps both fish traders and fishermen at any given point by the responsible authorities. The direct risk factors for cholera transmission are those facilitating the presence of faeces in the environment and its contamination of water, food, and hands, as documented by an extensive literature. Additionally, *V. cholerae* is known to be a regular resident of the aquatic environment, able to exist as a free-living aquatic organism (Collins et al., 2006). Inadequate and leaking sanitation provides opportunities for transmission of the virulent forms of the pathogen into environmental reservoirs (Namkinga et al., 2014) where it may survive and propagate depending on environmental and climatic conditions.

Hygiene behaviour is a key element in sanitation programmes. Sound sanitation practices such as hand washing with soap, safe disposal of children's faeces, and use of sanitary facilities for defecation are crucial to avoid the spread of disease (WHO, 2004).

The basic determinants of better health, such as access to water, and sanitation, are still in a critical state in Zambia. Limited access to water and sanitation facilities accompanied by poor hygiene is associated with skin diseases, acute respiratory infections (ARIs), and diarrhoeal diseases, the leading preventable diseases(Central Statistical Office (CSO) [Zambia], 2014).

2.8.2 Contaminated Water and Food

Drinking-water that has been contaminated at its source (e.g. by faecally contaminated surface water entering an incompletely sealed well), during transport and/or supply, or during storage (e.g. by contact with hands soiled by faeces). Unprotected water sources are very often contaminated. Arrangements should be made for the protection of water sources as an important measure for reducing the risk of contamination. Ice made from contaminated water and cooking utensils washed in contaminated water can also be sources of infection. Food contaminated during or after preparation. Moist foods (e.g. milk, cooked rice, lentils, potatoes, beans, eggs, and chicken), contaminated during or after cooking/ preparation and allowed to remain at room temperature for several hours, provide an excellent environment for the growth of Vibrio cholerae. Seafood, particularly crustaceans and other shellfish, taken from contaminated water and eaten raw or insufficiently cooked or contaminated during preparation. Fruit and vegetables grown at or near ground level and fertilized with night soil, irrigated with water containing human waste, or “freshened” with contaminated water, and then eaten raw, or contaminated during washing and preparation are also sources of infection (WHO, 2004). These poor hygienic practices can be exacerbated by low education level and poor quality of life.

2.9 Knowledge on Cholera

The major risk factors for diarrhoea and other water, hygiene and sanitation related-diseases are related to the consequences of poverty, such as under nutrition, micronutrient deficiency, lack of adequate sanitation, scarcity of sufficient quantities of safe water, poor hygiene, low level of education and poor health care access (DFID, 2013). Knowledge plays a great role in the prevention and control of cholera. Mostly the inadequate knowledge on the mode of disease transmission has contributed to the spread of the infection.

With the knowledge of monitoring water and sanitation infrastructure, displaced populations and their convergence with environmental conditions and transmission pathways, a prediction capability of high probability can provide warning of potential cholera epidemics and allow mobilization of public health measures (Gaudart et al., 2013).

CHAPTER THREE

METHODOLOGY

3.0 Study Area

This study was conducted in Lukanga swamps in Central Province of Zambia. The catchment area of the wetland is 13,520 km^{2}. However, at times of high flow, the Kafue River causes water to backup into the Lukanga swamps, and during very high floods, the Kafue River itself overflows into them. It is estimated that about 60,000 people live in, or close to, the wetland (McCartney et al., 2011). Figure 6 locates the study site on the map of Kapiri Mposhi District.

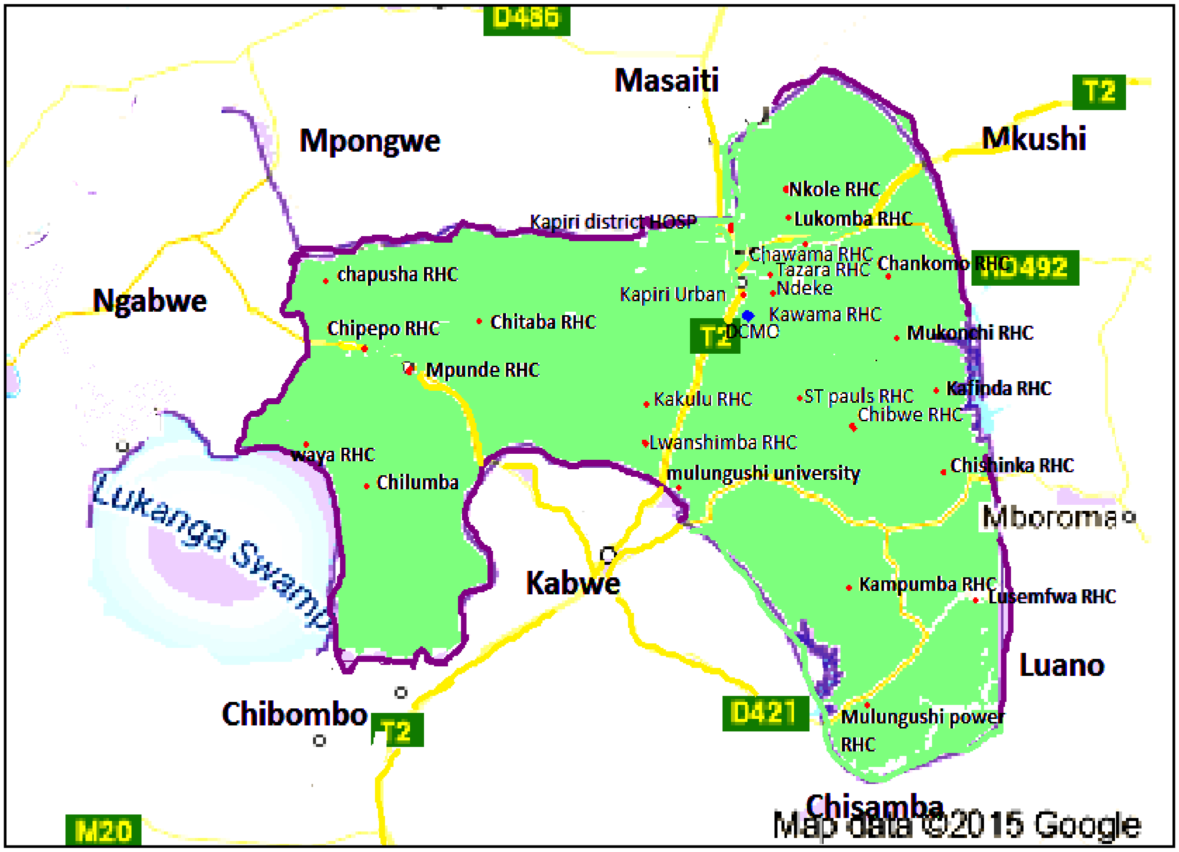

Figure 6: Map of Kapiri Mposhi District indicating the Lukanga Swamps; Source Kapiri DHO

The Lukanga Swamp is located approximately 50 km west of Kapiri Mposhi District, in the Central Province of Zambia, within the catchment of the Kafue River. Situated between latitudes 14^{o}08 S and 14^{o}40 S and longitudes 27^{o}10 E and 20^{o}05 E at an altitude of 1,090 masl, the swamps occupies a shallow depression with depths not exceeding 6.1

m, even at the height of the wet season. Although named a swamp, the wetland actually comprises a treeless lake and marsh ecosystem which is an intricate maze of reeds, pools, channels, and larger bodies of open water, covering an area of approximately 1,800 km^2. The wetland comprises two major types: palustrine (i.e.; marsh) and lacustrine (i.e. open water). Figure 7 provides actual locations of lagoons from where samples climatological data were collected.

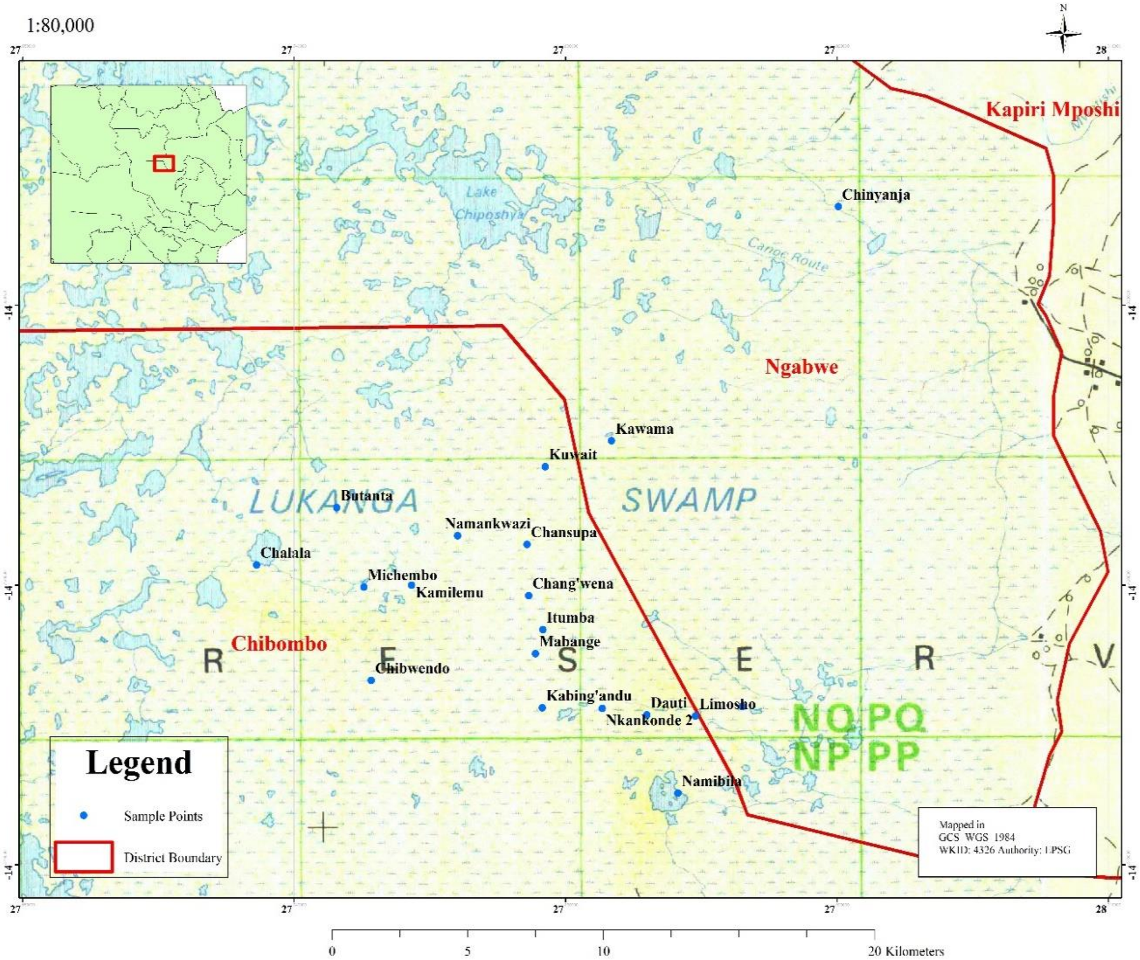

Figure 7: Study Site Map with Water Sample Collection Points

The palustrine wetland covers approximately 95 percent of the area and includes: stands dominated by reeds (Phragmites) mixed grass, cattail/reed-mace (Typha), and termitaria grasslands. The lacustrine area comprises about five percent of the total wetland. It provides habitat for a wide range of terrestrial and aquatic flora and fauna, including at least 316 species of birds, including cranes, storks, ducks, geese, pelicans, herons, egrets, and bitterns.

People mainly settle on floating Islands commonly known as Mitanda. The major economic activity in this area is fishing which is done by fishermen who reside on floating islands. The floating islands are artificial bases made of grass and reeds which are located on strategic points around the lagoon and are immovable by prevailing winds and water waves. Fish traders mainly men also come to settle on these floating islands as they await fishermen to bring fish from their catch. The indigenous people of the area are not mainly fishermen but their livelihood is farming and rearing of cattle and they settle around the swamps to utilize the natural resource for grazing of their animals. The people who do fishing mostly are migrants from other provinces including Northern, Luapula and Copper-belt. Women generally are not allowed to enter and do the fish trading in the swamps except using it for transport purposes from Waya harbor through to Chilwa Island.

Sanitation and hygienic conditions are greatly compromised in the swamps because people use the same body of water to dispose of their waste products and the same body of water provides as a source for drinking water. Due to low literacy levels, most of the fishermen have little time to domestically treat their drinking water which implies that little attention is attached to the importance of water treatment for drinking.

3.1 Inclusion and exclusion

All 19 lagoons namely Kamilemu, Chibwendo, Mabange, Michembo, Chalala, Kabinja'ndu, Chansupa, Namibila, Chang'wena, Butanta, Namankwazi, Mulilachembe, Kawama, Kankonde, Bunyanja, Dauti, Kuweti, Limosho and Itumba in Lukanga Swamps that are accessible through Kapiri Mposhi District were included in the study. No lagoon was excluded from the study.

3.2 Study type

A Times Series Correlational study design using a quantitative method was applied to assess environmental factors associated with epidemic cholera in Lukanga Swamps. This research design is useful for: Establishing a baseline measure; describing changes over time; keeping track of trends and forecasting future short term trends.

3.3 Variables

The dependent variable for the study was cholera cases and independent variables were Air temperature, Precipitation, Water quality, Sanitation system and Potential Hydrogen (pH) as detailed in Table 1.

Table 1: Shows the variable type, factor, variables, indicators, scale of measure and operational definitions.

TYPE OF VARIABLE	FACTOR	VARIABLE	INDICATOR	SCALE OF MEASURE	Type of Variable
Dependent	Prevalence of cholera	Cholera cases	Number of cholera cases in an outbreak per year	Number of Cholera cases per outbreak.	Continuous (Count)
Independent	Environmental Factors	Precipitation Pattern	Average monthly Rainfall in millimetres per lagoon	Millimetres (mm)	Continuous
		Air Temperature	Average monthly temperature in degrees Celsius per lagoon	Degrees Celsius	Continuous
		Humidity	Amount of moisture in the air	Percentage	Continuous
		Potential Hydrogen (pH)	The ability to attract hydrogen ions	pH readings	Continuous
		Water quality	Number of Colony forming Units (CFU) per 100mls of water	• Faecal coliform count per 100ml of water sample • Escherichia coli (E.Coli) Per 100ml of water samples • Vibrio Cholerae per 100ml of water	Nominal
		Sanitation	Excreta disposal method	Good Poor	Nominal

3.4 Sampling Procedure

Sampling in research is a process of obtaining information about an entire population by examining only a part of it (Mbuti, 2013). It serves the purpose of saving time and other resources and yet produces the required results. This happens by the researcher drawing inferences based on samples about the parameters of population from which the samples are taken. This study therefore, employed total sampling that included all the 19 lagoons in the Lukanga swamps that were accessible through Kapiri Mposhi district from which cholera has broken out before. Most of these lagoons fall within Chibombo district catchment area and partly Ngabwe district but can only be accessed through Kapiri Mposhi District.

3.5 Data Collection Techniques and Tools

Satellite remote sensing was used to obtain information for environmental variables including: air temperature and precipitation for the period of 2010 to 2016. Water quality monitoring and pH were also determined from September, 2017 to January, 2018. In addition sanitation condition of the Lukanga Swamps was also assessed.

3.5.1 Data for cholera cases

Information for cholera cases was obtained from the district health office Information System database and from Provincial Health Office line listing database from January, 2010 to December, 2016.

3.5.2 Climatological Data

Historic data for hydro-climatological factors such as air temperature and precipitation was collected for all 19 lagoons using satellite remote sensing. Due to lack of weather information in the study area, rainfall and temperature estimates were collected from Tropical Applications of Meteorology using Satellite and ground based observations (TAMSAT, 2017). TAMSAT was developed by the University of Reading in 1977 and has an archive of data from 1983 to the present (TAMSAT, 2017). TAMSAT is derived from a combination of geostationary Meteosat data and gauge observations using a calibration approach that exploits both data sets (Tarnavsky et al., 2014). The rainfall estimates are also compared with six other satellite based rainfall datasets. The primary objective has been to render historic and near real-time precipitation estimates for drought monitoring in sub-Saharan Africa where there is a lack or complete absence of in situ observations (Maidment et al., 2014). TAMSAT provides decadal (10 day) rainfall data at 4 kilometre spatial resolution across the whole African continent. The data is intended to enhance the capacity of African meteorological agencies and other organisations through the provision and support of the use of satellite derived rainfall estimates (TAMSAT, 2017). TAMSAT also provides temperature and evapotranspiration estimates.

Decadal rainfall and temperature data were collected and converted to monthly rainfall and temperature data. This was done in the ArcMap protocol of ArcGIS where decadal data within each month was summed to derive a monthly value. Within ArcMap, monthly rainfall and temperature values from January, 2010 to December, 2016 were extracted at each of the 19 sample points using the *Extract Multi Values to Points* tool in the Spatial Analyst toolbox resulting in a table. The monthly averages of rainfall and temperature for all 19 sample points were calculated and used to plot against the number of cholera cases reported at corresponding times in the study area. Furthermore, regression and correlation analysis were undertaken in STATA version 14 between rainfall and cholera cases and temperature and cholera cases respectively.

3.5.3 Water Sampling

Water samples were collected from all lagoons under study and it was aimed that 2 samples would be collected per lagoon per month for five months from September, 2017 to January, 2018 to assess the presence of total coliforms, Escherichia coli and *V. cholerae*. It was designed that a total of 10 water samples would be collected per lagoon and delivered to two different laboratories; Food and Drugs Control Laboratory and Center for Infectious Diseases and Research in Zambia (CIDRZ) Laboratory respectively for analysis in Lusaka for quality control purposes. The total number of water samples planned to be collected were 190 from the 19 lagoons under study. However, we managed to collect 164 water samples from the study sites due to logistical challenges. Sampling techniques were upheld to maintain the efficacy of the samples. Water samples were collected using sterilized 500ml bottles for bacteriological analysis to Food and Drugs Control Laboratory while Whatman 903 filter papers were used to collect and store water samples for CIDRZ Laboratory for isolation of *V. cholerae*. A cooler box was used to carry water samples for analysis to Lusaka at the Food and Drugs Control Laboratory. The samples were delivered to the laboratories within 48 hours of their collection.

Procedure taken at Food and Drugs Lab: Water samples were enriched in alkaline peptone broth (1% peptone, 1% Sodium Chloride (NaCl, pH 8.4), using appropriate concentration of broth relative to sample volume. Enriched cultures were incubated for 6 to 8 hrs at 35degrees celsius, then streak a loopful of the enrichment broth onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubate these plates at 35 degrees for 18 to 24hours. Suspected *V. cholerae* colonies appear yellow, a result of sucrose fermentation.

There were 74 water Samples that were delivered to Centre for infectious Diseases and Research in Zambia (CIDRZ) from October, 2017 to January, 2018 from the 19 Lagoons of the Lukanga Swamps monthly for isolation of *Vibrio Cholerae*. Water samples were collected and stored on Whatman 903 filter papers. The filter papers were delivered to CIDRZ on a monthly basis for the detection of *V. cholerae* 01/0139 by Polymerase chain reaction (PCR).

Procedure: In order to perform PCR, deoxyribonucleic acid (DNA) had to be extracted from the bacterial cells. The thermal shock was used to lyse the bacterial cells that were present on the filter papers and free the DNA. The Chelex matrix was used to absorb cell lysis products that interfere with the PCR amplification process. Then a multiplex PCR assay for rapid detection of toxigenic *Vibro cholerae* 01 and 0139 was comprehensively done.

3.5.4 Potential Hydrogen Levels

The pH level which is important in creating a conducive environment for the growth of microorganisms including *V. cholerae* was measured on monthly from each lagoon. Water samples from each study lagoon were collected for five months in one litre container and were submitted to Mahatma Gandhi Memorial Clinic in Kabwe for pH analysis in a Porter Lab. The readings were recorded on the check list formulated for that purpose. However, the instrument used to measure pH was calibrated from 0 to 8.5 and above 8.5 it was giving a greater than Sign making it difficult to know the exact pH readings for all those that indicated the greater than sign.

3.6 Assessment of Sanitation Status in 19 Lagoons of Lukanga Swamps

The evaluation of sanitation status in all the 19 sampled lagoons was done through observations as initially designed and also by requesting where to answer the call of nature from within the swamps.

3.7 Data Processing and Analysis

Data on temperature and Precipitation was collected for 84 months from January, 2010 to December, 2016 and average monthly temperatures and precipitation were reported. A total of 164 water samples for water quality monitoring were collected from 19 lagoons for five months from September, 2017 to January, 2018. Data was thoroughly cleaned by the Principal investigator for completeness, consistence and correctness before, entering in Microsoft Excel and then exported to Stata version 14 (Stata Corp., College Station, TX, USA) for analysis. To assess association between cholera cases and air temperature, precipitation and humidity Poison Regression Model and Zero-inflated negative binomial regression were used and compared to choose the best fit model. Zero-inflated negative binomial regression was the best fit model with (AIC 92.7, BIC 107.3) compared to Poisson regression model with (AIC 296.5, BIC 303.8). The level of significance was set at less than 0.05 with 95% confidence interval. Results were presented as frequencies in tables and graphs. Data was restricted and only the research team had access.

3.8 Ethical considerations

The conduct of the study had no risk either to the individual or the community, the study had minimal direct contact with humans. The benefit of the study may not be immediate but could help to understand environmental factors associated with endemic Cholera in Lukanga swamps and prevent future disease outbreaks.

The ethical approval of this research was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC). The rights of institutions were respected. The researcher sought permission from the Local Authority, District health Office and from the Health center with the catchment area covering partly the Lukanga swamps to conduct the study. The Fishing Committees in Lukanga swamps were also informed of the study. Special permission was also sought from the District

Meteorological department for the extraction of hydro-climatological conditions data. Confidentiality of the data was upheld.

3.9 Limitations of the study

The study was not without constraints and the notable ones were: failure to extract humidity through remote sensing for it required more time to be processed. Humidity was supposed to be assessed together with temperature and precipitation in order to understand the best predictor associated with endemic cholera.

Water quality monitoring to isolate faecal coliforms was only done for two months December, 2017 and January, 2018 due to inadequate funds for analysis. The implication of inadequate sampling period could not give a good trend of the faecal coliform concentration.

CHAPTER FOUR

PRESENTATION AND INTERPRETATION OF RESULTS

4.0 Cholera Cases from 2010 to 2016 in Lukanga Swamps

The study revealed that there were 133 cholera cases in the period under study. It was observed that 2014 had the highest number of Cholera cases whilst the lowest was in 2015 where 6 cases were recorded as indicated in Figure 8.

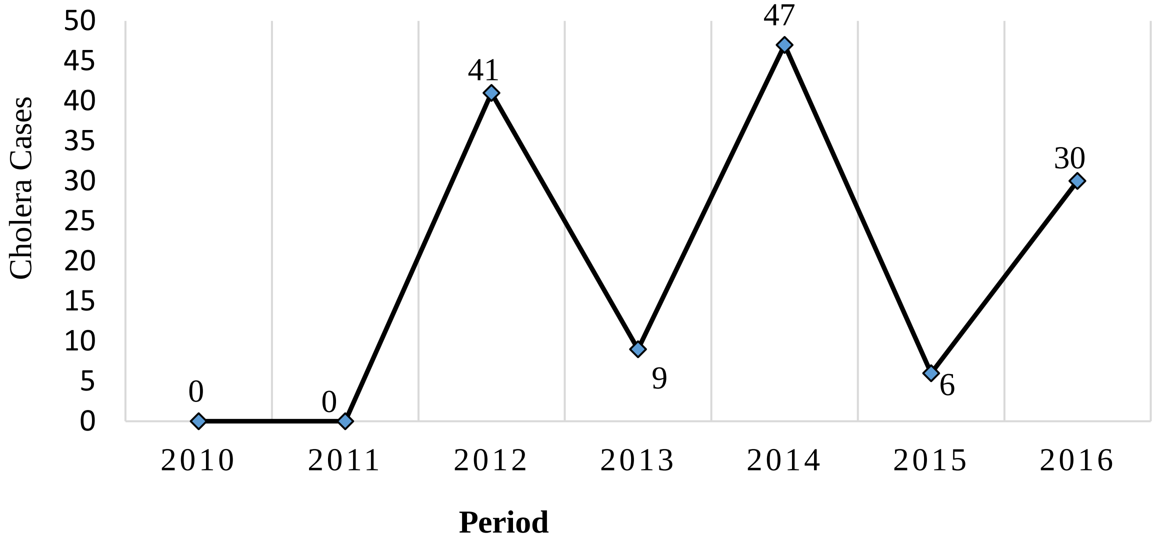

Figure 8: Cholera Cases in Lukanga Swamps from January, 2010 to December, 2016

4.1 Precipitation and Temperature in Lukanga Swamps from 2010 to 2016

The study revealed that air temperature from January, 2010 to December, 2016 in the Lukanga Swamps ranged between 16 degrees celsius and 28 degrees celsius and the lowest was in the month of June, 2010 while the highest was in October, 2016. Precipitation had a wider range in the seven years period from as low as 0.05mm in April, 2016 to as high as 248mm in December, 2013 as illustrated in figure 9.

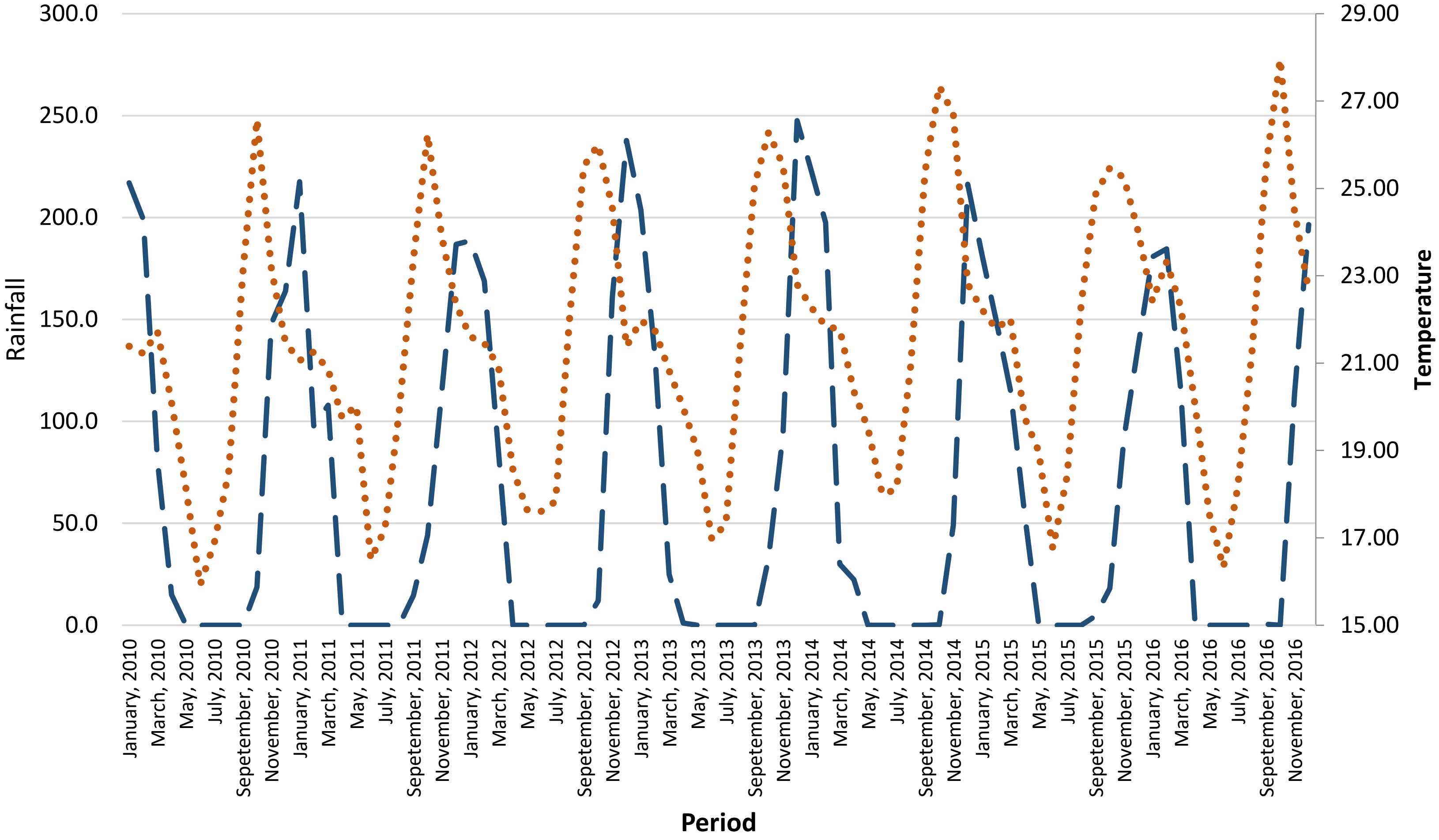

Figure 9: Rainfall (dashed) and Temperature (doted) from 2010 to 2016 in 19 Lagoons of Lukanga Swamps

4.2 Association between Environmental conditions and Cholera Cases in Lukanga Swamps from 2010 to 2016

Figure 10 provides a relationship between climatological conditions (rainfall and temperature) and Cholera Cases in Lukanga Swamps from January, 2010 to December, 2016. The study revealed a significant association between increased temperature (p=0.001) and cholera cases while increased amount of rainfall was associated with decreased risk of cholera cases (IRR = 0.992, 95% CI: 0.988 – 0.997, p=0.002). The study also revealed that cholera in Lukanga Swamps occurs mainly in the dry season.

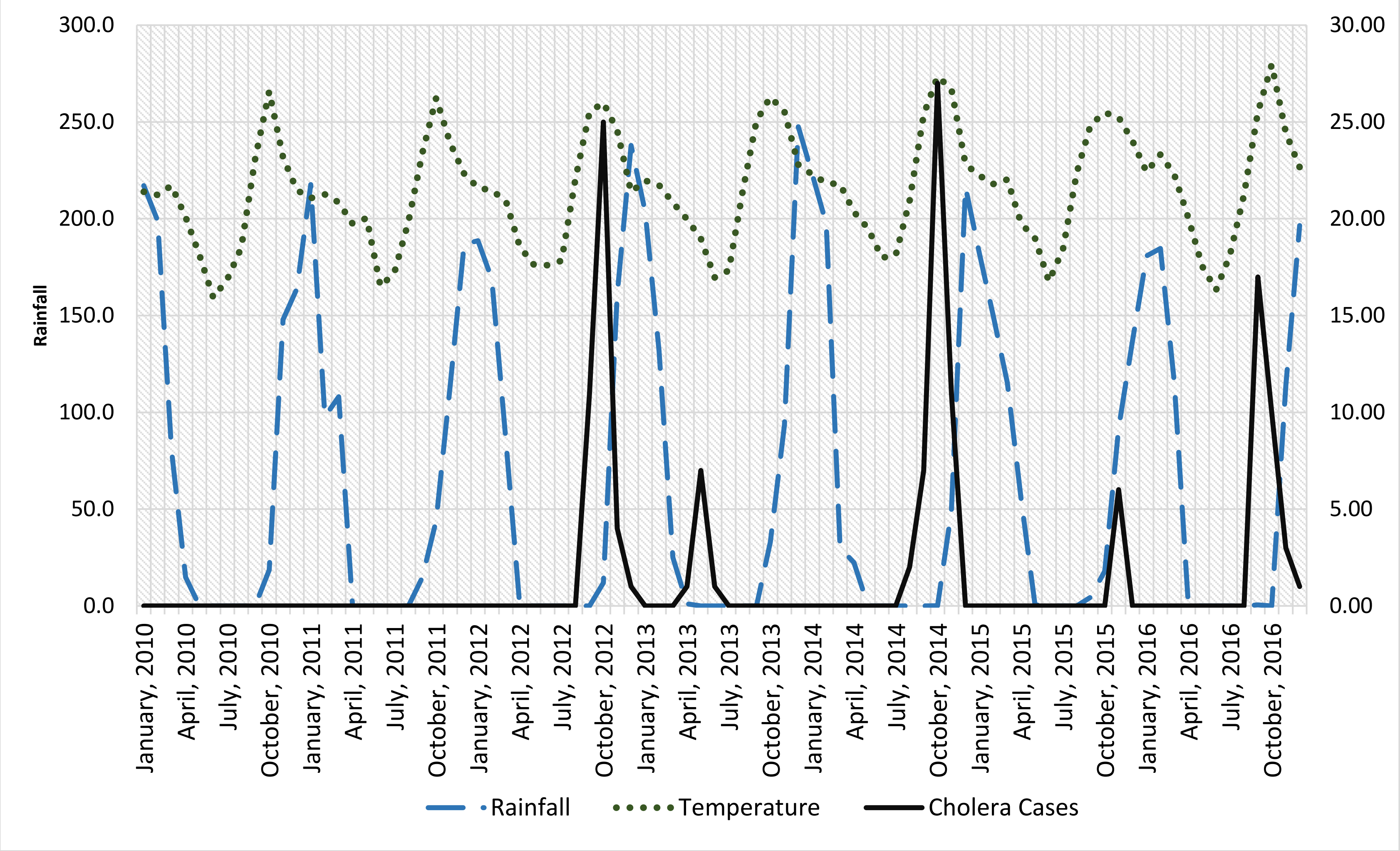

Figure 10: Association between Environmental Conditions (Rainfall and Temperature) and Cholera Cases in Lukanga Swamps from 2010 to 2016.

4.2.1 Zero-Inflated Negative Binomial Regression Analysis of the Association between Temperature, Precipitation and Cholera Cases in Lukanga Swamps

The air temperature and rainfall data was collected for 84 months and the study revealed that increased amount of rainfall was associated with decreased risk of cholera cases (Incidence RR = 0.992, 95% CI: 0.988 – 0.997, p=0.002). The results also revealed that one degree increase in temperature increases the risk of cholera cases by 27% and was statistically significant with (IRR = 1.265, 95% CI: 1.142 – 1.402, P=Value 0.0001).

Table 2: Association between rainfall, temperature and cholera cases using Zero Inflated Negative Binomial Regression Analysis.

Predictor Variable	**Incidence Rate Ratio (IRR)**	**P-Value**	**[95% CI]**
Temperature	1.265	0.0001	1.142 - 1.402
Rainfall	0.992	0.002	0.988 - 0.997

IRR = Incidence Rate Ratio CI = Confidence Interval (N=84 months)

4.3 Depiction of Sanitation, Water Quality and Water pH in Lukanga swamps from September, 2017 to January, 2018.

4.3.1 Sanitation Situation in 19 Lagoons of Lukanga Swamps

Through observations, the study revealed that in Lukanga Swamps there is no land that provides for digging and construction of sanitary facilities. That illustrates an impossibility of having a sanitary facility in that aquatic environment. Figure 11 provides some selected sites of the floating Islands within Lukanga Swamps.

Figure 11: Photos of some selected Floating Islands within Lukanga Swamps

The study further revealed that people within the Swamps camp on strong floating surfaces that have been naturally established though reinforced with grass and rids to strengthen the base. The study has also shown that both Fishermen and Fish Traders have specific areas designated for sanitation and also for drawing water for both drinking, washing cooking utensils and cooking within the same body of water.

4.3.2 Water Quality Monitoring from the 19 lagoons in Lukanga swamps

The water quality monitoring was done for two months December, 2017 and January, 2018 to isolate faecal coliforms as shown in figure 12: The results of the monitoring showed that 19/36 (53%) of water samples were contaminated with faecal coliforms. The study also indicated that in December, 2017 there were 12/19 (63%) of the lagoons with contamination and the faecal coliform contamination level per 100ml of water sample ranged from 1-200 Colony Forming Units (cfu). The results also show that in January, 2018 there were 7/19 (37%) contaminated lagoons and the faecal contamination level ranged from 2-200 Colony Forming Units as indicated in Figure 12.

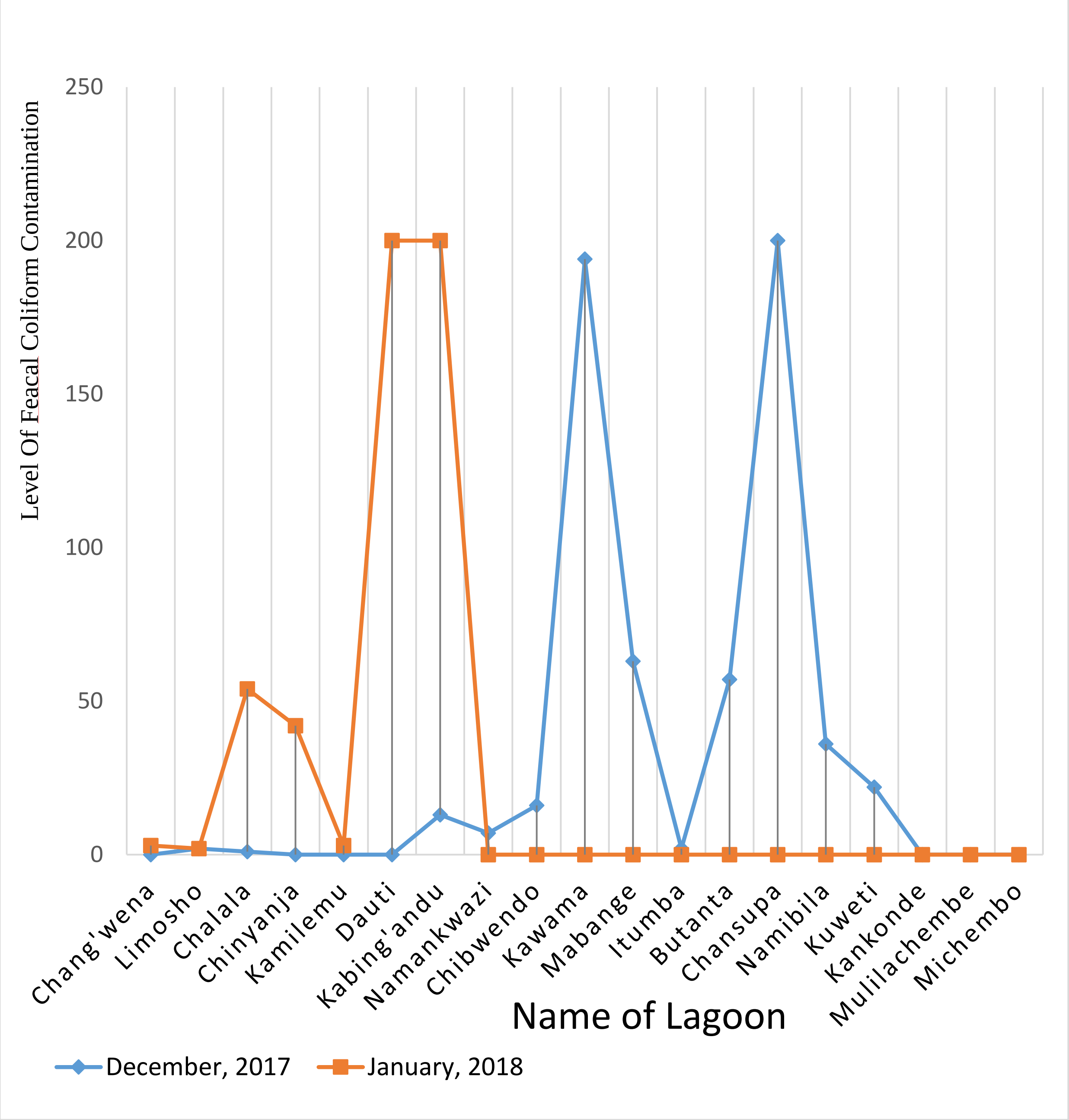

Figure 12: Faecal coliform (CFU/100ml water) for December, 2017 and January, 2018 per Lagoon.

4.3.3 Microhabitat condition (water pH) of the 19 Lagoons in Lukanga Swamps

A total of 89 water samples were collected and tested for pH from 19 Lagoons monthly from September, 2017 to January, 2018. Figure 13 shows the results per lagoon: The pH in the lagoons under study ranged from 7.1 – 8.5. The study indicates that 2/19 lagoons (Chansupa and Chang'wena) gave the pH readings more than 8.5 in all the months beyond the pH testing machine calibration range of 6.5 – 8.5 while other lagoons had some months with readings beyond the capacity of the testing machine. The study shows variants in pH readings from all the 19 lagoons for the period of five months as provided in Figure 13.

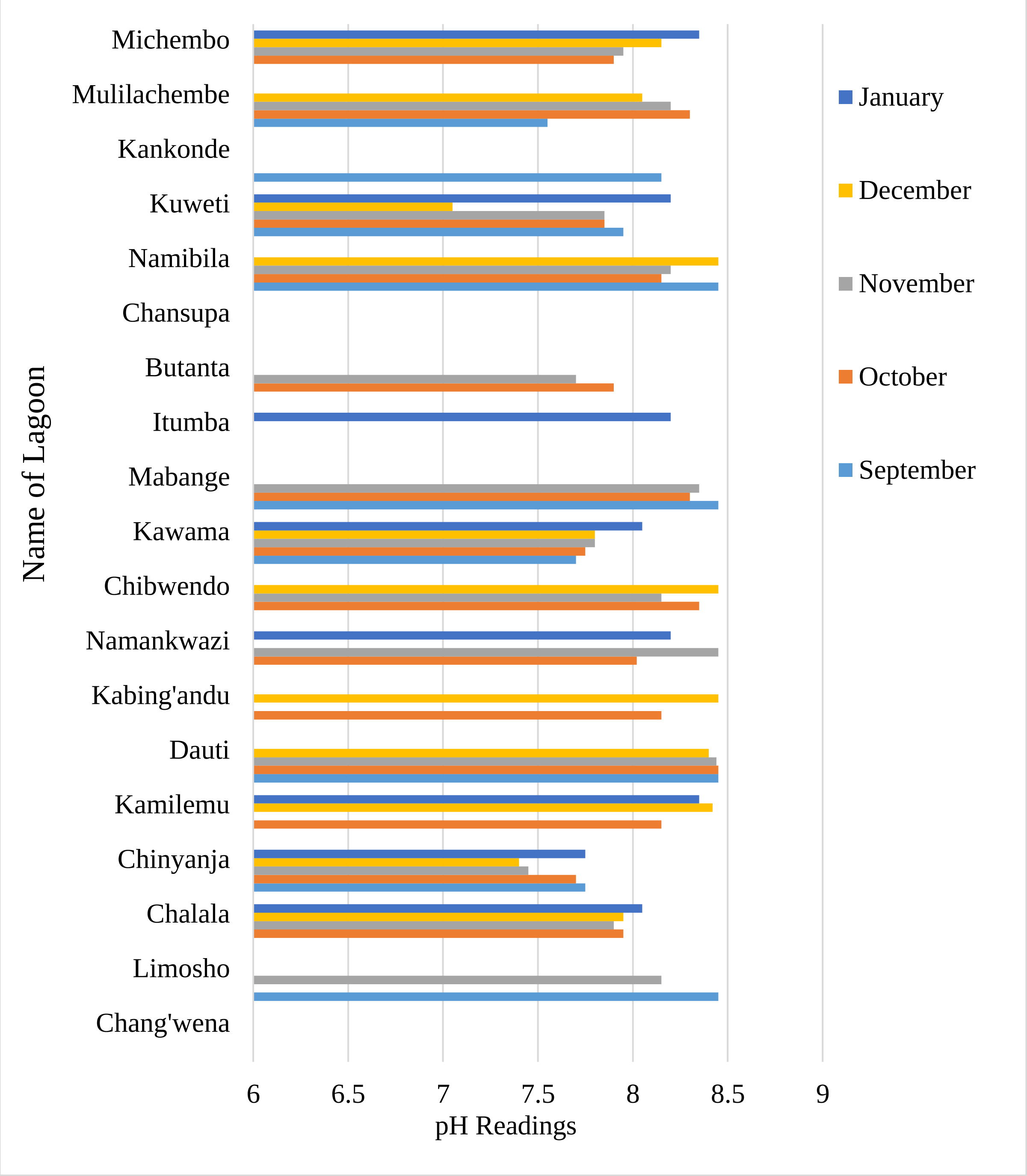

Figure 13: Monthly pH levels in water per Lagoon from September, 2017 to January, 2018

4.4 Water Sample Analysis

Table 3 shows water samples analysed by two different Laboratories to Isolate *V. cholerae.* The study revealed that of 90/164 (55%) water samples submitted to Food and Drugs Control Laboratory to isolate Vibrio were all non-reactive. It shows further that of the 74/164 (45%) water samples submitted to Centre for Infectious Diseases and Research in Zambia (CIDRZ) similarly to isolate Vibrio were also non-reactive.

Table 3: Water samples analysed by two different Laboratories to isolate V. Cholerae from 19 Lagoons of Lukanga Swamps

Period	Food and Drugs Laboratory			CIDRZ Laboratory		
	Samples	Method used	Results	Samples	Method used	Results
September, 2017	14	Culture	Negative	Nil	Nil	Nil
October, 2017	19	Culture	Negative	19	PCR	Negative
November, 2017	19	Culture	Negative	19	PCR	Negative
December, 2017	19	Culture	Negative	19	PCR	Negative
January, 2018	19	Culture	Negative	17	PCR	Negative
Total= 164	90			74		

CHAPTER FIVE

DISCUSSION

5.0 Discussion

A total of 133 cholera cases were recorded during the study period. The study indicates precipitation and temperature to be significantly associated with recurring cholera outbreaks in Lukanga Swamps. Environmental and microhabitat conditions provided supportive environment of Vibrio thriving and triggering the Cholera outbreaks within Lukanga Swamps. Water quality monitoring showed heavy contamination of faecal coliforms ranging from 1 – 200 cfu/100ml of water samples. The study also showed that all the 164 water samples submitted to Food and drugs Control Laboratory and Center for Infectious Disease and Research in Zambia (CIDRZ) respectively from September, 2017 to January, 2018 to isolate Vibrio were non-reactive.

This study revealed that cholera outbreaks mainly occurred during the dry season. This is in agreement with the study done in Ghana on assessment of response to Cholera outbreaks in two district in 2016 which found out that most of the cases occurred before the rain season (Ohene et al., 2016b). This was contrary to the study conducted in Tanzania on co-variation of Cholera with climatic and environmental parameters in coastal regions which revealed that Cholera cases occurred every month between 2004 and 2010 (Namkinga et al., 2014).

The study also found that one degree increase in temperature increases the risk of cholera cases by 27% and that was statistically significant. This agrees with the study done in Tanzania which concluded that temperature was significantly associated with cholera, i.e; a one degree Celsius increase in air temperature increased relative risk of cholera by 15–29% (Jutla et al., 2010). Water temperature, in particular, has been shown to be an important factor governing the seasonal and geographical variation in *V. cholerae* (Igbinosa and Okoh, 2008). Another study done in Tanzania shows a significant relationship between cholera cases and temperature and predicts an increase in the initial risk ratio for cholera in Tanzania in the range of 23-51% for a 1 degree Celsius increase in annual mean temperature (Trærup, 2010). The significant relationship between temperature and cholera cases could be due to the fact that increased temperature is supportive of growth and multiplication of the *V. cholerae.* A study conducted in China in 2012 shows that *Vibrio* is able to grow within the temperature of 20^{o}c and 45^{o}c and that it cannot survive at 4^{o}c for extended periods (S. Fu, 2012). This finding is similar to the study of Gaudart and others which revealed that about 50% or more cholera outbreaks occurred when the air temperature was > 31°C, approximately one standard deviation from average air temperature over the previous 2 months (solid line) (Gaudart et al., 2013).

On the other hand according to the study done in India using a time series statistical model, incidence of cholera has not been correlated with air temperature (Rajendran et al., 2011). This finding could be due to an accession that air temperature alone will not cause an epidemic, unless accompanied by appropriate transmission mechanisms such as poor water quality and lack of sanitation infrastructure, as well as rainfall. However, according to time series analytical study done in Bangladesh, Ali and others, hypothesized that an increase of Surface Sea Temperature (SST) results in replication of phytoplankton populations, which are directly associated with the increase in *V. cholerae* bacteria and are linked spatially and temporally to zooplankton populations (Ali et al., 2013).

This study has shown that increased amount of rainfall was associated with decreased risk of cholera cases and was statistically significant. This may be linked to the occurrence of cholera outbreaks mostly during the dry seasons with high peaks between August and October in Lukanga Swamps and reduced cholera outbreaks during rain seasons. This finding was consistent with a time series model study done in India for 11 years from 2000 to 2010 which showed initially that from 2000 to 2004, there was a significant association between rainfall and cholera cases but from 2005 to 2010 the study indicated a negative significant association between rainfall and cholera cases (Sebastian et al., 2015). They related the initial positive association that it could be due to the power of the study, insufficient time period, due to changes in the drainage system and non-availability of good quality drinking water by the government. On the other hand a study done in Bangladesh showed an association between cholera and rainfall (Wu et al., 2018) and this finding was similar to a study done in Zambia by Mudenda in 2014 that heavy rainfall and flooding were associated with triggering outbreaks of diarrhoea.

In this study an assessment of sanitation status through observations in all the 19 sampled lagoons revealed that there was no land where to dig and construct sanitary facilities. A further assessment indicated that there was open defecation within the aquatic environment. The inhabitants separated two areas where to defecate and from where to draw drinking water with different directions. The Lukanga Swamps has large segments of slow or none flowing water which create an environment for the endemicity of the *V. cholerae*. According to Juliana; the organism can survive in almost all kinds of aquatic environments including fresh water sources such as lakes, ponds, rivers and tanks. Cholera bacteria can also survive in non-aquatic environments such as refuse dump sites, fruits, fresh vegetables, meat, cooked food, human and animal faecal waste, untreated or inadequately treated waste (Juliana, 2011). The implication of open defecation in an aquatic environment would be a great contribution to the reservoir of *V. cholerae*. The challenge is that due to low initial concentrations of choleragenic *V. cholerae*, the carriers can show no symptoms of the disease. Nonetheless, these asymptomatic carriers will shed pathogenic clones in their stools, further enriching the water sources with virulent bacteria and facilitating the initiation of an epidemic (Almagro-Moreno and Taylor., 2013b). This implies that *V. cholerae* is usually shed from humans in the aquatic environment in smaller numbers and as they get attached to phytoplanktons and copepods do multiply and when the environment is conducive, they cause virulent epidemics. A study undertaken in Ghana in two districts, Akatsi District in Volta Region and Komenda-Edina-Eguafo-Abirem (KEEA) Municipal in Central Region during the 2012 cholera epidemic provided a warning that Cholera remains a threat to areas where access to safe drinking water and adequate sanitation cannot be assured (Ohene et al., 2016a). Poor sanitation coupled with unhygienic practices in highly populated areas harboring the bacteria as a source of intermittent outbreaks due to contamination of drinking water and or improper food preparation is a serious concern in Lukanga Swamps.

This study revealed that water sources in the Lukanga swamps were heavily contaminated. A water quality monitoring done in December, 2017 and January, 2018 indicated heavy water contamination with faecal coliforms ranging from 1 – 200 Colony Forming Units (cfu) /100ml of water samples and that could be due to a cracked safe excreta disposal system and hygiene practices in the aquatic environment of Lukanga

Swamps. These results have been substantiated by other studies and a study conducted in the USA in 2011 indicated that substantial populations of fecal coliforms and *E. coli* are harbored in freshwater bottom sediments, bank soils, and beach sands (Pachepsky, 2011). Another study in USA revealed similar findings on assessment of the available wastewater treatment plant. Treated effluent data and in-stream sampling data indicated that water quality criteria for fecal coliform in recreational waters exceeded 800 cfu/100ml water samples at all locations of the Santa Cruz River (Emily C. Sanders, 2013). Faecal Coliform contamination seems to be very common in water bodies that are not protected and this could be due to bacteria concentrations discharged as treated effluent and from nonpoint sources into these open water bodies.

The assessment of microhabitat condition ie potential Hydrogen (pH) showed variations in all 19 sampled lagoons for five months. The pH in 89 water samples collected and analysed to assess whether it was within the range that supported the thriving of *V. cholerae* and it ranged from 7.1 to above 8.5. These results agree with previous studies which have shown that the pH value of ∼8.0 and warm water temperatures between 19 and 28°c were related to an increased concentration of cholera bacteria (Gaudart et al., 2013)**.** Another study done in Haiti to monitor water sources for environmental reservoirs of Toxigenic *Vibrio cholerae* O1 **s**howed that because the sites varied from mountains to floodplain to estuaries where their study was conducted, there was relatively wide variability in salinity (0–21.6 g/L), pH (6.4–8.6), and temperature (24.3–33.7°C) (Alam et al., 2014).This means that the *V. cholerae* thrive easily in an environment that has increased water conductivity and less acidic with pH level of ∼8.0 coupled with favorable temperatures. Another study carried out in the USA found that the timing of *V. cholerae* prevalence was coupled to the water characteristics they measured. For example, temperature and pH decreased in most sites through July/August, as did the prevalence of V. cholerae (Ryan et al., 2017). This provides a clear picture that lower temperature and pH do not support survival and proliferation of V. *cholerae.*

In this study the analysis of water samples to isolate V. *cholerae* in 19 sampled lagoons of the Lukanga Swamps from September, 2017 to January, 2018 did not yield positive results. A similar study revealed that in most cases *V. cholerae* has been found to persist in its natural environment mainly in two forms: viable but not culturable (VBNC) and conditionally viable environmental cells (CVEC). VBNC is a dormant state that *V. cholerae* enters in response to nutrient deprivation and other environmental conditions (Almagro-Moreno and Taylor, 2013a). This could probably be one of the reasons isolation of V. *cholerae* during the study period. On the other hand in a study done in Haiti, they managed to isolate *V. cholerae* O1 (toxigenic and nontoxigenic) and all isolations occurred at water temperatures of >31°C. The study further indicated that, there was evidence that *V. cholerae* O1 isolation was more common in the environment preceding epidemic peaks of disease among humans (Alam et al., 2014). Water samples were collected for analysis to isolate *V. cholerae* during a non-epidemic period making it relatively difficult to isolate the organism as elucidated by Alam and others in their study. Their study noted the importance of transporting water samples at room temperature which promotes the thriving of the microorganism. We however, maintained low temperatures when transporting water samples to Food and Drugs Laboratory to hinder further multiplication of microorganisms which would lead to depletion of air and food contained in the sampling receptacle.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study revealed that there were 133 cholera cases in the period under study with the highest number of cases recorded in 2014 with 47 cases and no cases in 2010 and 2011 respectively. Air temperature was found to be the main predictor associated with cholera outbreaks in the Lukanga Swamps and it shows ability in predicting these outbreaks with a lag time of two months prior to temperature rise. This implies that if temperature is monitored regularly and when it rises to levels that are supportive of cholera outbreak, then disease preventive measures could be implemented to prevent the outbreak. The study also revealed that increased amount of rainfall was associated with decreased risk of cholera cases. In Lukanga Swamps therefore, increased amount of rainfall plays a preventive role to cholera outbreaks and the risk of cholera outbreaks increases when there is reduced amount of rainfall. This is very important for policy makers and implementers to note when it comes to application of intervention to prevent the disease outbreak in Lukanga swamps. Microhabitat condition (pH) showed supportive environment for growth of *V. cholerae* within the Lukanga Swamps and coupled with poor sanitation, the risk of cholera outbreak increases. Environmental factors therefore, provide early warning systems of cholera outbreaks for impactful implementation of identified interventions in that aquatic environment. Health promotion based interventions coupled with disease surveillance and vaccination of individuals in order to prevent and control cholera outbreaks in Lukanga Swamps should be used as fundamental elements.

6.2 Recommendations

The Environmental factors within Lukanga Swamps require a multifaceted approach to tackle them in order to prevent and control cholera outbreaks. Proper implementation of the successive recommendations at different levels would help to prevent such outbreaks.

6.2.1 Ministry of Health and its Partners

i. To develop an Environmental Management policy that will address environmental factors associated with cholera outbreaks in Fishing Camps of Lukanga Swamps
ii. Establishment of a robust surveillance system to detect trend variations on environmental factors and conditions that trigger cholera outbreaks in Lukanga Swamps
iii. Development of individual cholera vaccination programme in Fishing Camps for cholera prone areas

6.2.2 Provincial and District Health Offices

i. Promote safe water and hygiene within the Lukanga Swamps
ii. Advocate for individual vaccination programme to be implemented prior to the peak temperature upsurge.
iii. Strengthen and conduct an integrated disease surveillance system within the Lukanga Swamps
iv. Strengthen and conduct regular monitoring of environmental factors associated with cholera outbreaks in that aquatic environment.
v. Develop and implement an epidemic preparedness plan for mounting a timely and appropriate response

6.2.3 The Community within Lukanga Swamps

i. Ensure domestic treatment of water, food safety and enhance hygiene practices
ii. Early referral of individuals who develop diarrhoea from the Swamps to the nearest health facility
iii. Increase awareness on preventive and control measures of cholera within the Swamps

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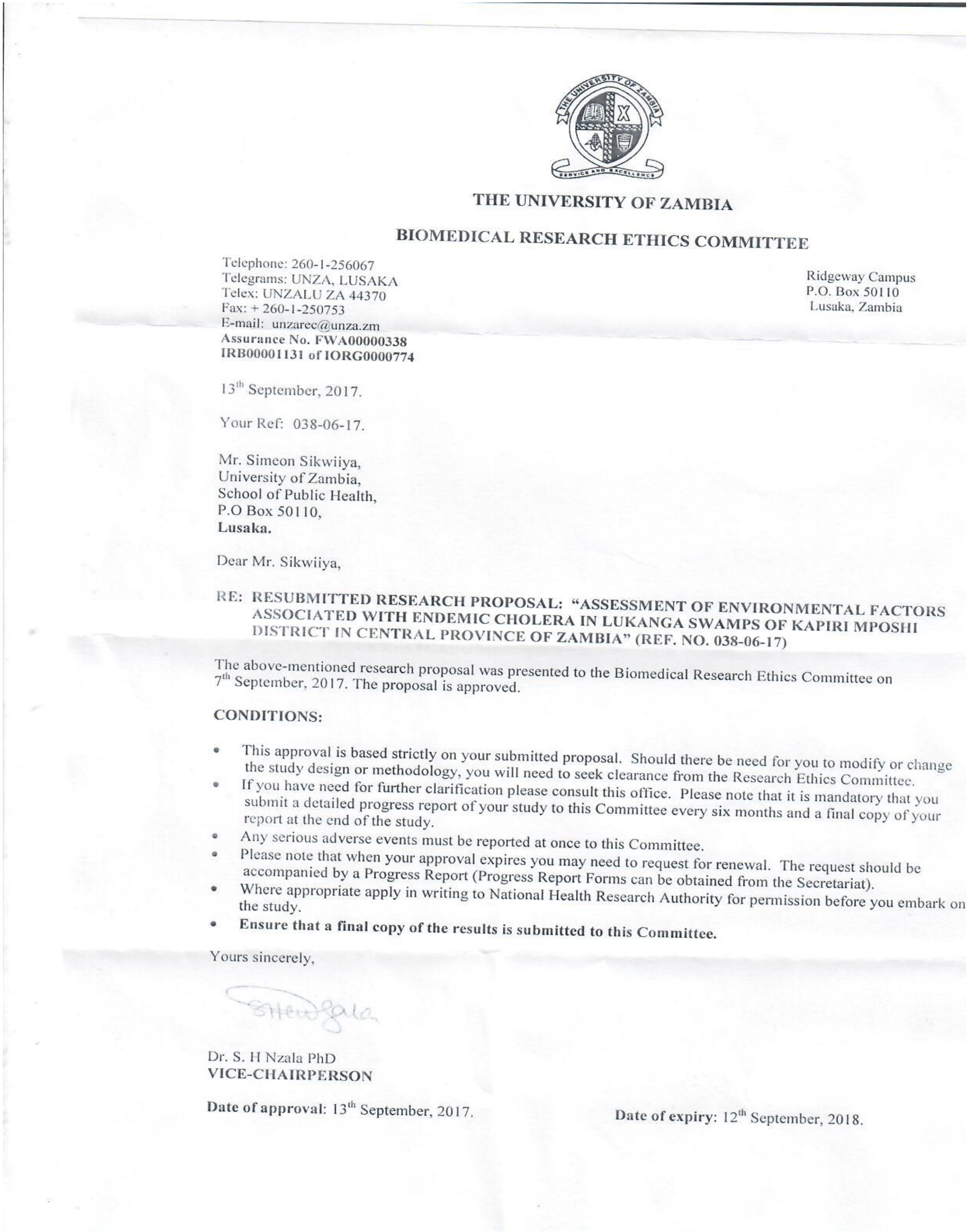
APPENDICES

Appendix 1: Approval Letter from Research Ethics Committee



THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

13th September, 2017.

Your Ref: 038-06-17.

Mr. Simeon Sikwiiya,
University of Zambia,
School of Public Health,
P.O Box 50110,
Lusaka.

Dear Mr. Sikwiiya,

RE: RESUBMITTED RESEARCH PROPOSAL: "ASSESSMENT OF ENVIRONMENTAL FACTORS ASSOCIATED WITH ENDEMIC CHOLERA IN LUKANGA SWAMPS OF KAPIRI MPOSHI DISTRICT IN CENTRAL PROVINCE OF ZAMBIA" (REF. NO. 038-06-17)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 7th September, 2017. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- Where appropriate apply in writing to National Health Research Authority for permission before you embark on the study.
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr. S. H Nzala PhD
VICE-CHAIRPERSON

Date of approval: 13th September, 2017.

Date of expiry: 12th September, 2018.

Appendix 2: Observational Checklist for Sanitation, water and pH levels in Lukanga swamps

Name of Lagoon ……………………………………..Month…………………………

S N	Observed and checked parameter for sanitation	Yes	No	Date	Time
1	Is there any form of safe excreta disposal facility at the lagoon?				
2	Is the excreta disposal facility kept clean and dry?				
	Is there sufficient number of toilets provided at the lagoon?				
3	Is there open defecation within the swamps?				
4	Is there separate drinking water source?				
5	Is domestic waste disposed of safely elsewhere apart from water body?				
6	Is domestic waste kept in refuse pits with cover before disposal				
7					
8					
	Potential Hydrogen 2017 monthly measures	**Readings**		**Date**	**Time**
	September				
	October				
	November				
	December				
	January				
	Water Conductivity monthly measures	**Readings**		**Date**	**Time**
	September				
	October				
	November				
	December				
	January				

Appendix 3: Water and Food Sampling Form

MF NO 206   FOOD AND DRUGS ACT CAP 303, 1995.   SAMPLING FORM

1.Sample No.		2.Date Collected:
3.(a) Product Name and Description:		
(b) Collector’s identification on packages and seal:		
4. Reason for collection:		
Manufacturer:		Dealer:
7.Size of lot sampled:		8.Date dispatched:
9.Delivered to:	10.Date	11.Lusaka Food and Drugs Laboratory, LUSAKA
12.Records obtained:	(a)Invoice No. and date:	Shipping records and date:
	(c)Other Documents:	
13.Remarks:		
14.Sample cost:	15.Collector (Print Name and Signature):	