Quantitative Exposure Assessment to *Vibrio cholerae* through Consumption of Fresh Fish in Lusaka Province, Zambia

By

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A research report submitted in fulfilment of the requirements for the degree of Master of Science in One Health Food Safety

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DECLARATION

I, **Mulenga Malata** do hereby declare that the contents of the research report being submitted herein are my own original work and they have not been previously submitted to this or any University for the award of a degree or any other qualification.

Signature …………………………………….. **Date……………………………**

CERTIFICATE OF APPROVAL

This research report submitted by, **Mulenga Malata** is approved as fulfilling the requirements for the award of the degree of Master in One Health Food Safety at the University of Zambia

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ABSTRACT

Fresh fish is one of the cheapest sources of proteins in the Zambian diet and widely consumed nationwide. Fresh fish harbours a number of spoilage bacteria and pathogens including *V. cholerae* which cause cholera in humans. Consumption of contaminated fresh fish has been linked to zoonotic transmission of diseases in humans. The aim of the study was to carry out a quantitative exposure assessment to *Vibrio cholerae* through consumption of fresh fish in Lusaka Province of Zambia. The data used to achieve this objective were obtained from reviews of scientific literature, government reports, questionnaire survey and expert opinions. The swift Quantitative Microbial Risk Assessment (sQMRA) model was used to model the risk of exposure. The study was driven by the lack of research-based information in this area despite the increase in the number of reported cases of cholera in Lusaka Province.

Results showed that the average serving portion for fresh fish in Lusaka District was 200g for fresh fish consumers in the restaurants and high socio-economic status households, while 100g were for the consumers in low socio-economic status households, respectively. The probability of exposure to *Vibrio cholerae* through consumption of fresh fish was estimated to be low. At serving portions of 100g and 200g in the low and high socio economic status households with a concentration of 50 cfu/g, infectious dose 50 (ID50) of 10^6 cells, one person would get ill, representing a probability of 0.000044%. At an average serving portion of 200g at restaurants level, 50cfu/g and ID50 of 10^6 cells, 47 people would get ill, translating into a probability of 0.0021% per year

The study concludes that the risk of exposure to *Vibrio cholerae* among residents in Lusaka Province, as a result of fresh fish consumption, would be generally low, attributed to preparation methods. The study also revealed that cross-contamination during preparation and consumption could be a source of exposure to the *Vibrio cholerae*. Improvement in food hygiene practices and processing are needed to minimize the occurrence of foodborne illnesses. Further, work is required to broaden the scope of the study and also undertake a microbiological evaluation of fresh fish from both household and restaurant risk exposure pathways.

DEDICATION

This research report is dedicated to my dearest friend and lovely wife Isabel N. Luambia, my beloved son Samuel Mulenga Malata, daughter Isabella Keziah Lukonde Malata, my mother Mary Samu, my nieces Natasha Mwamba and Natasha Chisanga who, for their sincere love, shared with me the challenges and the hardships during my studies away from home. I sincerely thank them all immeasurably for the endurance and understanding when I could not avail myself as much as I should have done during the period of my studies and stay in Lusaka. My best friend and wife Isabel, a woman of strong character, you missed me a lot and to you, this is the reward of your endurance and I am proud of you my wife.

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ABBREVIATIONS

%	:	Percentage
CAC	:	Codex Alimentarius Commission
CFR	:	Case Fatality Rate
cfu	:	Colon Forming Unit
DRGS	:	Directorate of Research and Graduate Studies
FAO	:	Food and Agriculture Organisation
FEHD	:	Food and Environmental Hygiene Department
g	:	Gram
ICMSF	:	International Commission on Microbiological Specifications for Foods
MoH	:	Ministry of Health
Spp	:	Species
SPS	:	Sanitary and Phytosanitary
sQMRA	:	Swift Quantitative Microbiological Risk Analysis
USFDA	:	United States Food and Drug Administration
UTH	:	University Teaching Hospital
WHO	:	World Health Organisation
WTO	:	World Trade Organisation

OPERATIONAL DEFINITIONS

Vibrio cholerae: A bacterial species of comma-shaped, motile bacillus that produces a soluble exotoxin and is the cause of Cholera in humans; it is the type species of the genus *Vibrio*.

Cholera: Is an acute infectious disease characterized by watery diarrhoea that is caused by the bacterium *Vibrio cholerae*, first identified by Robert Koch in 1883 during a Cholera outbreak in Egypt. The name of the disease comes from a Greek word meaning "flow of bile."

Fish: Any of various cold-blooded, aquatic vertebrates, having gills, commonly fins, and typically an elongated body.

Risk assessment: The process of identification, evaluation, and estimation of the levels of risks involved in a situation, their comparison against benchmarks or standards, and determination of an acceptable level of risk. In the food industry according to FAO/WHO it consists of hazard identification, hazard characterization, exposure assessment, and risk characterization.

Exposure assessment: A formal study of the impact of a hazardous substance on people, places, or things. It includes determination of the source(s) of the substance, its diffusion through the environment, its concentration, its duration and half-life, and the populations or media that are vulnerable to its effects

Food safety: Refers to handling, preparing and storing food in a way to best reduce the risk of individuals becoming sick from foodborne illnesses.

CHAPTER ONE

1.1 INTRODUCTION

Fish is a nutritious food that constitutes one of the desirable components of a healthy diet for humans' growth. Fish have been considered natural reservoirs of *Vibrio cholerae*, the deadly diarrheal pathogen (Hossain et al., 2018). However, little is known about the role of fresh fish in the transmission of *V. cholerae* in households and restaurants in Lusaka province. There are health risks associated with the consumption of fresh fish. One of the major risks involves the consumption of raw or undercooked fresh fish that may be naturally contaminated by foodborne pathogens present in the marine environment. Such a risk is further increased if fish is not handled hygienically during processing, where pathogens could gain access from external sources or intrinsically multiply exponentially under favourable conditions (FEHD, 2005).

In contrast to most other foodborne pathogens, *Vibrio* spp. have the aquatic habitat as their natural niche. As a result, *vibrios* are most commonly associated with fish and other seafood as natural contaminants. Foodborne infections with *Vibrio* spp. are common in Asia and other parts of the world (Sutherland and Varnam, 2000).

Cholera remains a public health threat globally, causing hundreds of thousands of cases every year. In former centuries, cholera was a continuous threat even to the developed countries in Europe, North America, and the northern part of Asia. Currently, cholera still remains endemic in some of the underdeveloped countries (Reidl and Klose, 2002). The global disease burden has been estimated to be 3-5 million cases and accounts for a total of 100,000-130,000 death per year (WHO, 2010). However, this threat is much reduced in places with safe water supply, good standards of hygiene, sanitation and good food safety management systems. The disease is an acute intestinal infection and its incubation period ranges from a few hours to five days, usually two to three days. Although asymptomatic infection is more common, clinical illness may be exhibited. Symptoms include a sudden onset of profuse painless watery diarrhoea that can quickly lead to rapid dehydration, acidosis, circulatory collapse, hypoglycaemia in children, renal failure and death if treatment is not promptly given. The disease is also characterized by nausea and vomiting which occurs early in the course of illness. Cholera is transmitted through ingestion of food or water contaminated with the bacterium, especially via faeces or vomitus of infected persons, directly or indirectly (WHO, 2010).

1.2 Problem statement

In recent years, Zambia experienced major cholera epidemics. During the outbreak in 2004 more than 12,000 cases were recorded resulting in mortalities accounting for 373.

Cholera has been an endemic condition in some parts of the country including Lusaka province which has been reporting the highest number of cases. The outbreak from 19th - 25th October 2009/2010 in Lusaka province alone recorded 4,464 cases and resulting in 73 deaths (CFR 1.63%). This was followed by another outbreak in 2010/2011, as of 16th January 2011, 173 cases were reported in Lusaka district (WHO, 2011a). Since the cholera outbreak was declared on 6th October 2017, it affected more than 3,534 people with more than seventy-seven (77) deaths (MoH, 2018). The outbreak disrupted all the economic activities in the country, especially in the affected areas. Additionally, the Government of Zambia spent a lot of funds in creating cholera treatment centres, providing medical supplies and controlling the disease. Cholera is responsible for significant mortality and economic damage in most parts of the world (Reidl and Klose, 2002).

A study conducted in Lusaka province by Chiyangi et al., (2017), found that *Vibrio cholerae* O1 subtype and Ogawa serotype (40.8%) were the most commonly isolated among other bacteria from children between 12 and 23 months old at the University Teaching Hospital (UTH). *Vibrios* were also detected in Zambia's aquatic environment including fresh fish from both natural water bodies and ponds (Kobayashi et al., 2010). Although fishing is prohibited during the rainy season, people in Zambia continue to eat fish which could come from those illegally captured during the ban periods, cultured fish from local fish farmers or imported fish from other countries.

Fresh fish is among the most highly nutritious foods as it is a good source of proteins for humans. However, fresh fish act as a reservoir for the pathogenic *Vibrio cholerae* bacteria (Senderovich et al., 2010). Consumption of contaminated fresh fish has been attributed to the cause of cholera in some parts of the world (Acosta et al., 2001 and Forssmain et al., 2007). Unhygienic handling of fresh fish that is contaminated can be a source of Cholera outbreaks (Kobayashi et al., 2010). Apart from health, this infectious disease may have a negative effect on the social and economic status of the community.

1.3 Study justification

Fish is an important source of protein in the Zambian communities and Lusaka province is one of the most leading provinces in the country in terms of fish consumption apart from the Copperbelt province (Genschick, 2018). However, while fish is a rich nutrient source, it can also be a potential vehicle of human foodborne illnesses including Cholera. Health risks associated with the consumption of fresh fish is little known by the community because in most cases cholera outbreaks are only attributed to contaminated water and other foods. Despite a number of risk factors for developing Cholera in Lusaka such as inappropriate fish handling processes, improper retail operations and consumption of fish, no study has been done to assess the risk of human exposure to this infectious disease through consumption of fresh fish in Zambia.

In some countries, *Vibrio cholerae* has been reported in both fresh fish and fresh waters, suggesting a possible source of human exposure through the fish value chain as well (Msyamboza et al., 2012). Some studies have indicated an association between cholera with the consumption of fresh fish, sardines, dried fish and other fish diets in different parts of the world (Acosta et al., 2001; Forssmain et al., 2007). In another study done by Pandit and Hora (1951), it was postulated that Cholera endemicity in India was due to consumption of hilsa fish. It was also reported in Berlin, in a case of Cholera caused by *V. cholerae* O1, the patient had most likely been infected while handling and preparing fish imported from Nigeria (Rmann et al., 2002). Other reports that connect *V. cholerae* to fish include the isolation of *V. cholerae* bacteria from Ayu fish in Japan (Kiiyukia et al., 1992).

There has also been evidence of the presence of *Vibrio* species in fish from previous researches in other countries (Senderovich et al., 2010). In a study done in Lusaka District by Mizanda, (2019 unpublished), *Vibrio cholerae* non-O1 was isolated. (26.9%) from fresh fish which were sampled from six markets in six sub-districts of Lusaka district. Most of the Cholera outbreaks in Zambia have been recorded from fishing camps and peri-urban areas of the Copperbelt, Luapula and Lusaka provinces. Cholera cases have been recorded every year in the Lukanga fishing camps in the last five years (Gama, 2017; Murebwa-Chirambo et al., 2017). Most (73%) of the Cholera outbreaks in Zambia have been reported in Lusaka Province followed by Luapula Province (7%) (Olu et al., 2013). However, very little is known about the exposure to *Vibrio cholerae* through consumption of fresh fish in Lusaka Province and Zambia in general. This study used a swift Quantitative Microbiological Risk Assessment (sQMRA) model to estimate the likelihood of exposure to *Vibrio cholerae* if they were to be transmitted through consumption of fresh fish, among the people of Lusaka Province of Zambia.

1.4 Objectives of the study

1.4.1 General objective

The main objective of this study was to quantitatively assess the risk of exposure to *Vibrio cholerae* species through consumption of fresh fish in Lusaka Province of Zambia.

1.4.2 Specific objectives

1. To assess the risk of human exposure to *Vibrio cholerae* through consumption of contaminated fresh fish.
2. To estimate the number of persons that are likely to be exposed to *Vibrio cholerae* as a result of consuming contaminated fish.

CHAPTER TWO

2.1 LITERATURE REVIEW

2.2 *Vibrio Cholerae*

The most important *Vibrio* species associated with disease problems in humans, due to ingestion or other routes of exposure, are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* (Tacket et al., 1984; Dalsgaard, 1998; FAO, 2001; Oliver and Kaper, 2001). Cholera is one of the most dangerous gastroenteric infections which are caused by enterotoxin-producing *V. cholerae* of serogroups O1 and O139. Cholera *vibrios* are commonly transmitted to humans through ingestion of contaminated food or water, and the disease occurs mainly in developing countries with inadequate sanitation conditions (Thompson et al., 2004).

Among the vibrios, *V. cholerae* is of most concern because of its ability to cause Cholera. *V. cholerae* can be divided into serogroups on the basis of the O antigen. Of the more than 200 *V. cholerae* serogroups that exist, only O1 and O139 are associated with the epidemiological features and clinical syndrome of Cholera. However, organisms of *V. cholerae* serogroups other than O1 and O139 (non-O1 non-O139 serogroups) have been associated with sporadic cases of foodborne outbreaks of gastroenteritis, but have not spread in epidemic form (Heymann, 2004). The most important virulence factor associated with *V. cholerae* O1 and O139 serogroups is the Cholera toxin. Non-O1 non-O139 serogroups are generally nontoxigenic (FEHD, 2005).

Vibrio species are Gram-negative, facultative anaerobic motile curved rods with a single polar flagellum. Among the members of the genus, 12 species have so far been reported to be pathogenic to humans, where eight of these may be associated with foodborne infections of the gastrointestinal tract (Oliver and Kaper, 2001). Most of these foodborne infections are caused by *V. parahaemolyticus* and *V. cholerae*, and to a lesser extent by *V. vulnificus* (FAO, 2001).

2.3 *Vibrio* in fish

Vibrio species are among other several pathogenic microorganisms that can affect the quality of fresh fish. Fish of various species are a reservoir or harbour *V.cholerae* and other *Vibrio* species in their digestive tract, gills and on their scales. In a study conducted in Israel by Senderovich et al., (2010), revealed that the intestines of fish harboured $5x10^3$ *V. cholerae* cfu per 1 gram intestine content high rates compared with known *V. cholerae* cfu numbers in the bacteria's natural reservoirs. Fish have been implicated in Cholera cases in the past (Senderovich et al., 2010).

2.4 Bacterial Contamination of fresh fish

The presence of human pathogenic bacteria in fish and fish products may also be attributed to contamination during processing (Håstein et al., 2006). Fish and water environments could be reservoirs of *Vibrio cholera*e (Senderovich et al., 2010). "*Vibrio cholerae* is a human pathogen and natural inhabitant of aquatic environments" (Traoré.Oumar et al., 2014). Fish has been implicated in the cause of some cholera outbreaks due to ingestion of undercooked contaminated fish (Scheelbeek et al., 2002). In some instances, fish get contaminated during the process of preparation due to unhygienic handling. In a study done in Monrovia, Liberia by Scheelbeek et al. (2002) they found fish samples positive of *V. cholerae* bacteria which was associated with limited hygienic measures during the gutting.

During a non-cholera outbreak period, *Vibrio cholerae* was isolated from some fresh fish samples in Tanzania in 2013, (Garrido-Maestu et al., 2014). This presented a food safety and public health concern. Fish has a favourable environment for the bacteria to thrive, and in case of vibrios have the ability to adhere to the host's intestinal epithelium (Sánchez, 2011). The bacteria are able to associate with aquatic life and prolong its survival (Faruque et al, 2002; Faruque, 2012). Scientists suggested that there was a commensal relationship between *V. cholerae* and fish because the isolated bacteria had the ability to degrade chitin" (Sujatha et al., 2011). Fresh fish is said to have a stable physio-chemical condition mainly pH and salinity that support the survival of *V. cholerae* O1 (Akoachere and Masalla, 2013). It is, therefore, suggested that the physical-chemical characteristics and conditions in fish gills may favour persistence of such toxigenic organisms in fish. "The isolation of *V. cholerae* O1 from different fish species samples is of food safety and health concern mainly due to risks of cross-contamination and potential for multiplication in different types of foods" (Senderovich et al., 2010). In Kenya, *V. cholerae* O1 was isolated from fresh fish samples during a non-outbreak period. It was concluded that, areas where Cholera was endemic, the bacteria existed in the biofilm-like aggregates and aquatic environments (Onyuka et al, 2011).

In a study done by Sujatha et al. (2011) results indicated that five human bacterial pathogens were isolated from the two species of edible fishes, *Priacanthus hamrur* (demersal) and Megalaspis cordyla (pelagic) which were largely consumed by the coastal population of Chennai in India. The presence of these human pathogens in the marine waters indicated the pathogenic microbial contamination level of the habitat. Unhygienic fish handling practices of these infected fishes and inadequate cooking may further contribute to the spread of these pathogens (Sujatha et al., 2011).

2.5 *Vibrio cholerae* in humans

Vibrio cholerae is a human pathogen and natural inhabitant of aquatic environments (Traoré.Oumar et al., 2014). Human infections caused by microorganisms transmitted by fish or an aquatic environment are quite common, depending on the season, the patient's contact with fish or the environment, dietary habits, and the immune system status of the exposed individual (Novotny et al., 2004). Infection rates often peak after storms, floods, or sewage spills that transfer contaminated effluent to aquatic ecosystems.

One of the most dangerous gastroenteric infections is cholera. Epidemic cholera is caused by enterotoxin-producing *V. cholerae* of serogroups O1 and O139. Cholera *vibrios* are commonly transmitted to humans through ingestion of contaminated food or water, and the disease occurs mainly in developing countries with inadequate sanitation (Thompson et al., 2004; Mason, 2009; Gurbanov et al., 2011).

2.6 General overview of Cholera

Although Cholera is primarily known as a waterborne disease in the endemic regions including Bangladesh, contamination of foods can also be an imperative mode for Cholera transmission (Glass and Black, 1992). Many studies have reported that foods including seafood, fish and others can become contaminated with *Vibrio* spp. through improper handling, undercooking, washing with unhygienic water and by the use of untreated night soil (Feachem, 1981; Huq et al., 1983; Rabbani and Greenough, 1999). Fish has been found to serve as an important vehicle for Cholera transmission in the endemic areas (Rabbani and Greenough, 1999). Since the discovery of the bacteria *Vibrio cholerae* in the 19th century, it has spread rapidly across the globe affecting mainly the developing countries (Gaffga et al., 2007).

There has been a gradual shift in the epidemiology of the disease from high incidence in Asia and the Americas to Africa in the last 20 years (WHO, 2009). Since 2005, there has been a progressive increase in the yearly incidence rate of the disease globally (WHO, 2010). Increasing poverty, retarded economic growth and development and limited access to basic social services such as health, clean water and improved sanitation remain the underlying drivers of cholera transmission in Africa (Mintz and Guerrant, 2009).

According to WHO estimates, 115,106 cases and 3397 deaths of cholera were reported in Africa in 2010, representing 36.3% of the global incidence of the disease for that year (WHO, 2009). Since 2008, there has been a surge in cholera incidence in Southern Africa where huge and unprecedented outbreaks were reported in 2008/2009 (Mason, 2009).

In the year 2009, eight countries in Southern Africa, including Zambia, reported 109,008 cases of cholera which is about 50% of all the cases reported on the continent for that year (WHO, 2009). In recent years, Zambia experienced major cholera epidemics, the outbreak of 2004 recorded more than 12,000 cases and 373 deaths.

2.7 Global prevalence of Cholera

Cholera has been among other foodborne disease outbreaks in Zambia, Bangladesh and other parts of the world. The study conducted in Dhaka by Mrityunjoy et al., (2013) established that fish was among other food items from which *Vibrio cholerae* has been isolated, the presence of the bacteria observed indicated high risk to public health.

In October 2010, a Cholera outbreak was reported on the Island of Haiti, the *Vibrio cholerae* serotype Ogawa was isolated. This affected about 270,000 people resulting in 4,700 mortalities. The outbreak spread to other countries such as the Caribbean, Canada, United States and Dominic Republic (Farmer et al., 2011).

In the year 2010, in India, *V. cholerae* O1 Ogawa of the E1T biotype was implicated in the outbreak, mortality of Cholera-related increased by 24% within the five years. The approximated burden of Cholera in this region is about 3 to 5 million cases with estimated deaths of about 100,000 or more (Ali et al., 2015). Fresh fish was reported among other sources of the *V.cholarae* O1 Serotype Ogawa, biotype E1 Tor which affected 1,370 people resulting in thirty-two mortalities in the Ukraine cholera epidemic which occurred from 1994 and 1995 (Clark et al., 1998).

2.8 Regional prevalence of Cholera

Cholera is endemic in some parts of Central and Southern African countries. In 2011, the disease affected 2,787 people in the Democratic Republic of Congo and caused 153 deaths in a number of locations down along the Congo River (WHO, 2011b). In Morogoro (Tanzania), serotypes of *V. cholerae* O1 was isolated from fresh fish and vegetables during non-outbreak from October 2014 to February 2015 (Hounmanou et al., 2016). The other devastating outbreak occurred in Zimbabwe between 2008-2009, this had a fatality of 22-48%, Ogawa and Inaba were isolated as serotypes of *Vibrio cholerae*. The cases started spreading to the neighbouring countries such as South Africa, Zambia, Mozambique and Botswana (Mason, 2009). Another recent Cholera outbreak was recorded in Zimbabwe in September 2018, where 71 confirmed cases, and 32 deaths were reported out of 3,621 suspected cases (case fatality ratio: 0.8 %), of these, 98% (3564 cases) were reported from the densely populated suburbs in the capital Harare. The samples collected from patients tested positive for *V. cholerae* serotype O1 Ogawa (WHO, 2018a).

2.9 Local prevalence of Cholera

Zambia reported its first Cholera epidemic in 1977/78 and from then up until the early 1990s, the country experienced major outbreaks every three to five years. Starting in 2003, there has been a progressive increase in the yearly incidence of Cholera in the country. In 2010, the country reported 6,794 cases and 115 deaths (CFR 1.6%) of the disease with Lusaka Province accounting for 85% (5,798) of the total cases for that year. In 2010, Zambia accounted for 41.6% of all the 16,330 cases reported from four Southern African countries namely Malawi, Mozambique, Zambia and Zimbabwe. Outbreaks typically start between epidemiological weeks 40 to 45 of the year and end between weeks 20 to 25 of the following year (which corresponds to the Zambian rainy season) with the peak being reached between weeks one to thirteen (Olu et al., 2013).

Seventy-three per cent of all the 39,285 cases of Cholera reported from 2001 to 2010 were from Lusaka Province followed by 7%, 6%, 5% and 4% from Luapula, Southern, Central and Copperbelt provinces respectively. In Lusaka District, which is usually the epicentre of outbreaks, the majority of cases were reported from Kanyama, Chawama, Chipata, George, Kamwala and Mandevu wards, all of which are located in the western suburbs of the district. All the Cholera cases which tested positive (439) were identified with Eltor 01 as a predominant strain in Zambia since 2005 (Olu et al., 2013). Laboratory confirmation of *Vibrio cholerae* in the country on a yearly basis in the last ten years suggests that the country is now endemic for Cholera (WHO, 2010).

2.10 General Overview of the fish Sector in Zambia

The demand for fish in Zambia has grown which has led to most natural waters getting depleted of the fish stocks. It is because of this reason that the Government of Zambia has been encouraging its citizens to venture into fish farming so as to supplement the wild stocks (Musole et al., 2009). Fish makes up 55 % of animal protein in the diet of Zambians (FAO, 2006). Natural fisheries contribute 90% fish production of the average 70,000 to 85,000 metric tonnes in the recent past with the other 10% coming from fish farming (ACF/FSRP, 2009). There is a sharp increase in demand of fresh fish due to the increase in the human population. The per capita fresh fish supply was estimated to be at 11 kg per year compared to 6.3 kg per person per year in 2006 (Genschick et al., 2018)**.**

2.11 Risk assessment in fish

Studies on *Vibrio cholerae* risk assessment are scarce more especially on fish but some of the examples of vibrio species in seafood risk assessment were done which included a study in the USA on the risk of developing Cholera through consumption of warm-water shrimp in international trade containing choleragenic *Vibrio* O1 and O139. The process considered data gathering on the prevalence and concentration of choleragenic strains of *V.cholerae* at key points in the harvesting, processing, storage, preparation and consumption continuum. Two different approaches were used in order to develop a more quantitative estimation of the risk. Firstly, spread sheet-based and food-safety risk assessment tools were used (Ross and Sumner, 2002).

In the recent years, the world has seen the emergence of quantitative microbiological risk assessment as a tool for assisting the development of management strategies to improve food safety and safeguard public health, the risk assessments have since become a priority for Food and Agriculture Organization and World Health Organization (CAC, 1999). There has been scarce data on quantitative risk assessments conducted on *V. cholerae* in fish. However, FAO/WHO has conducted some quantitative risk assessment on *Vibrio parahaemolyticus* in seafood, including oysters (FAO, 2001). This was based on a framework developed by the U.S. Food and Drug Administration for *Vibrio parahaemolyticus* (U.S.FDA, 2005).

2.12 Research gaps

At the time of this review, no quantitative exposure assessments study had been conducted on developing Cholera through consumption of fresh fish.

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.2 Study area

The study was conducted in Lusaka Province of Zambia, an area with a relatively high fresh fish consumption across all the socio-economic status groups but with the low-income stratum spends a greater proportion of household expenditure on fish because it provides the cheapest source of animal protein (FAO, 2006). The province has an area coverage of 21,896km^2 with the population of 3,002,530 by 2018 (CSO, 2016). Lusaka is a commercial capital of Zambia and is inhabited by multiple ethnic groups. Fish sold in Lusaka Province is sourced from local farms, and outside Lusaka which includes imports from other countries (Genschick, 2018).

3.3 Swift Quantitative Microbiological Risk Assessment (sQMRA) model

The sQMRA-model was developed by Evers and Chardon (2010). It is implemented in a Microsoft Excel spread sheet. Deviating from a full-scale Quantitative Microbiological Risk Assessment (QMRA), where pathogen numbers are followed through the whole food chain. This model starts at retail and ends with the number of human cases of illness. The model is deterministic and includes cross-contamination and preparation (heating) in the kitchen as well as the dose-response relationship. The general setup of the sQMRA tool consists of consecutive questions for values of each of the 11 parameters, always followed by intermediate model output broken down into categories of contamination, cross-contamination and preparation. Model input and output were summarized and exposures, as well as cases, were attributed to the distinguished categories. As a relative risk measure, intermediate and final model outputs were compared with results from a full-scale. The model allows the results of the research to be quickly interpreted in terms of public health risk, given that pathogen concentration is determined from the model. It is also more accessible and understandable for scientists that are new to the QMRA research area or are not very mathematically inclined (Evers and Chardon, 2010).

3.4 Study design and data sources

This was a simulation study, employed sQMRA-model framework. The study primarily used information generated from other studies. Where information gaps were found, a cross-sectional questionnaire survey was undertaken to close the information gaps. Therefore, the study used both secondary and primary data sources.

Secondary data: This was a desktop study which mainly depended on a review of scientific peer-reviewed papers and grey literature (secondary data). The literature review was guided by research questions based on the sQMRA model. The literature search was conducted on major electronic databases including, Science Direct, Google Scholar, Research gate and PubMed using The University of Zambia (UNZA) library database.

Further, grey literature from reports from government institutions and Non-Governmental Organisations were obtained online using the "Google search engine". A search of key terms such as, "fish consumption, Quantitative risk assessment, cholera, fish value chain, Zambia", were used. Guided by questions in appendix 1, literature which contained relevant data were included in the study and the rest were excluded.

Primary data: After an extensive literature review, it was discovered that there were information gaps in serving portions and consumption patterns of fresh fish in Zambia. A survey was, therefore, undertaken to fill these information gaps. A structured questionnaire was used to address the information gaps in serving portions and consumption patterns. The study had a convenient sample size of one hundred eighty (180) respondents from all the six (6) sub-districts of Lusaka District. Twenty (20) respondents from each sub-district out of which ten (10) respondents were from the high socio-economic status households while the other ten (10) were from low socio-economic status. Residential areas were used as a proxy for socioeconomic status using the Central Statistical Office conditions of leaving survey (CSO, 2010). High socio-economic status was taken to be the households that were able to afford/meet basic needs such as education, clean water, shelter, food, health and stable income according to the living conditions monitoring and survey report 2015. This is because a poor household can be living in a high-cost housing area (an example of households staying in servants' quarters), or a rich person may live in a low-cost area (CSO, 2016). The other sixty (60) respondents were the restaurants' owners or managers were clients consumed fresh fish in all the six (6) sub-districts. The sample size was not based on statistics but was meant to give a general overview of fish average serving portions and consumption patterns in the province. Respondents were conveniently identified and interviewed from their residential areas and restaurants where they were found eating meals prepared from fresh fish.

3.5 Data Analysis

The data collected from the survey were coded and entered into SPSS Version 20. Descriptive statistics on average serving portions, consumption patterns, and kitchen preparation methods of fresh fish were calculated. Data from the literature review were entered in the Excel version of the sQMRA model developed by Evers and Chardon (2010). The model was then run for eight times to come up with results for the exposure assessment following the household and restaurant risk exposure pathways.

3.6 Ethical considerations

The study was approved and cleared by the School of Veterinary Medicine Board of Postgraduate and EREs Converge IRB an independent institution with a reference No. 2018-Oct-030. Permission to collect data from households and restaurants was also sought from Lusaka City Council. Written informed consents were obtained before the participants were enrolled in the study.

CHAPTER FOUR

4.1 RESULTS

4.2 Hazard identification

4.2.1 *Vibrio cholerae* species

According to the WHO definition, choleragenic *V. cholerae* O1 and O139 are the only causative agents of cholera, water and foodborne disease with epidemic and pandemic potential (FAO, 2006).

From the literature reviewed in this study, *Vibrio cholerae* was identified as hazardous bacteria. The toxigenic *V. cholerae* species are zoonotic, with fresh fish and aquatic environments being its reservoir. The pathogens cause Cholera in humans which is characterised with profuse rice watery stool, vomiting, fever, and severe dehydration leading to tachycardia, hypotension and vascular collapse due to dehydration and death if not managed early. Fresh fish act as a reservoir for the pathogens which habitats the intestines, scales and gills. This can be transmitted from fresh fish to human through consumption of contaminated fresh fish if not cooked adequately and handled hygienically during processing. *Vibrio cholerae* can cause syndromes ranging from asymptomatic to Cholera gravis. Zambia is endemic to Cholera outbreaks. In endemic areas, 75 % of cases are asymptomatic, 20% are mild to moderate, and 2-5% are severe forms like gravis (Bronze and Greenfield, 2005).

4.4 Hazard Characterization

This is the quantitative evaluation of the nature of the adverse effects associated with the consumption of fresh fish contaminated with *Vibrio cholerae*. According to WHO, (2004) about 20% of those who are infected develop acute, watery diarrhoea and 10 to 20 % of these individuals go on to develop severe watery diarrhoea with vomiting. A number of reports indicate that the numbers of choleragenic organisms required to cause illness is in the region 10^4 to 10^9 (Cash et al., 1974 and Novotny et al., 2004b). According to Butler et al. (2006) a remarkably high dose of *Vibrio cholerae* is required to produce or cause disease in humans. In a study done in United States of America by Hornick et al. (1971), it established the trend between dose and observed probability of response among the volunteers. The onset of symptoms as well as indication of asymptomatic infection were monitored. Additionally, both diarrhoea and cholera diarrhoea (very different in appearance) were also monitored as symptoms.

Table 1: Dose response for *Vibrio cholerae*.

Dose	Diarrhoea	No diarrhoea	Total
10	0	2	2
10^3	0	4	4
10^4	9	4	13
10^5	6	2	8
10^6	20	3	23
10^8	2	0	2

(Hornick et al., 1971)

4.5 Exposure Assessment

The retail-to-consumption risk pathway was considered. This assisted in the identification of the various points along the continuum that influence the prevalence and level of choleragenic *V. cholerae* in fresh fish. The study considered the retail-to-household pathway and retail-to-restaurant pathway.

4.5.1 Case definition

The pathogen of interest was *Vibrio cholerae* species and the targeted product was fresh fish. The population size of Lusaka province was taken to be 3,002,530 in this model as projected by Central Statistical Office (2018). A consumption period of one year was defined to assess the number of people who would get ill in this study (i.e., the number of people who would get ill per year). The population was in three categories, from high socio-economic status, lower socio-economic and restaurants' owners or managers. A case of Cholera was defined as a confirmed when *Vibrio cholerae* O1 or O139 was isolated from any patient with diarrhoea (WHO, 2019).

4.5.2 Consumption data

In this study, portion size was defined as a whole fresh fish or a piece of fresh fish an individual consumes per meal. There was no available consumption data on fresh fish categorizing among the low and the high socio-economic status and restaurants fresh fish consumers in Lusaka province. The study assumed that residents in Lusaka province both in the high and low socio-economic status consumed fresh fish as it is a regular part of Zambian and cheapest source of animal proteins. Everyone, irrespective of socio-economic status, enjoys fresh fish in Zambia (FAO, 2006). According to the study done in Lusaka Province by

Genschick (2018), it was found that the poorest households relied on small fish products from capture fisheries while high socioeconomic households consumed larger fresh fish such as tilapia.

The survey carried out revealed that 100% and 96% consumed fresh fish at household and restaurant levels, respectively. People from low socio-economic households who were served with two portions of fresh fish (Lunch and dinner) accounted for 74.2 per cent, while 100 per cent of high socio-economic status and restaurant fresh fish consumers were served with the whole piece of fish for one meal only.

According to 2015 Labour Force Survey of Zambia , 75% of the Lusaka population were in formal employment (CSO, 2015). Therefore, the study assumed that 75% of 3,002,530 population in Lusaka who were in formal employment consumed fresh fish because of their purchasing power hence the number of portions consumed by the population in low economic status households was taken to be 2,251,898 per consumption period multiplied by 2 servings for lunch and dinner (4,503,795 portions).

4.5.3 Serving portions and consumption patterns

In 2014 per capita fresh fish supply in Zambia was estimated to be at 11kg capita/annum (Longley et al., 2014). A study done in Lusaka by Genschick (2018) revealed that Zambian aquaculture exclusively produces 99 per cent of tilapia fish weighing between 300 to 500 grams per piece and almost the commercially farmed fish was retailed in Lusaka in supermarkets, retail shops and open markets (Department of Fisheries (DOF) Zambia, 2015; and Kaminski et al., 2017). In terms of size preference, small fresh fish of about 50g to 100g were mostly on demand in places of high population density (low economic status) (Sililo et al., 2017). In addition, tilapia is a highly preferred fish for most Zambians though not always affordable (NFDS, 2016).

The results of the survey revealed that the average serving portion of fresh fish (without bones) per serving at a household level was 100 grams among low socio-economic status preferred small and medium tilapia species as the most consumed fresh fish which were mostly preferred cut into two pieces to cater for a household and 200g slight bigger fish were consumed as a whole fish per individual among high socio-economic status and restaurants fresh fish consumers (high fresh fish consumers). The households that prepared and consumed well done accounted for 98%, while 2 % fresh fish was consumed half done (by methods of roasting). The consumption patterns from the data showed that 41.7% of respondents consumed fresh fish once every week, 22.5% consumed once in every fortnight, 27.5% consumed fresh fish once a month and 2.5% consumed fresh fish every day either cooked or fried, while 5.8% of the respondents indicated that fresh fish is not part of their diet. At household level, fresh fish was cooked once but usually served in two different periods (2 serving portions lunch and dinner). A higher proportion of respondents 58.3% were served with two serving portions-lunch and supper, while 35.8% were only served with one portion for either lunch and or supper. At restaurant level, 100% of the respondents were served one portion for a once-off meal either lunch or mid-morning.

4.5.4 Contamination of fresh fish at retail outlets

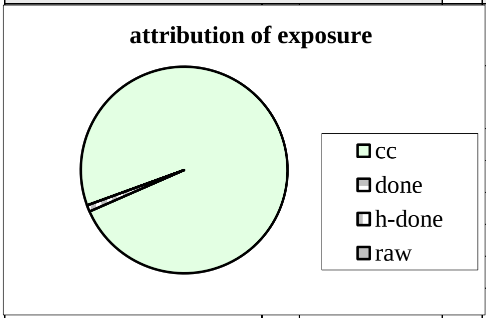
There was no data found locally during literature review hence the study used both shrimps and fresh fish contamination at retail outlets in other countries which were found from 3.5% to 18.3% in fresh fish retail centres and 0.0 % to 14.3% in fresh fish in streets and restaurants (Torres-vitela et al., 1997). A similar study in Bangladesh revealed that retail contamination of fresh fish stood at 20% (Hossain et al., 2018). In another study conducted in Burkina Faso, contamination was found at 6% (Traoré.Oumar et al., 2014). Some studies were also conducted in Shrimps, Shellfishes and Seafood in Ecuador, Berlin (Germany), and India, Cochin market contaminations were found at 11.3%, 6.3% and 1%, respectively (Sudha et al., 2014; Sperling et al., 2015; Vu and Huehn, 2018).

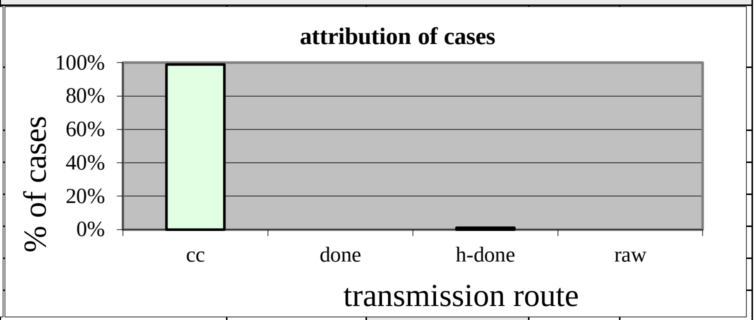
This study, therefore, used the data from Burkina Faso because it is within the same continent with similar weather conditions, experiences in the retail of fresh fish and handling practices like in many other low and middle-income countries in Africa (Traoré.Oumar et al., 2014). This study considered only minimum and high concentrations of *Vibrio cholerae* and hence the average concentration of colony forming units (cfu) per gram in a contaminated portion of fresh fish was taken to have a minimum value of 50 cfu/g, while a maximum value 3.3×10^{2}cfu/g (Chitov, 2009).

INPUT PARAMETERS			
Pathogen:			*Vibrio cholerae*
Food product			Fresh fish
Population size:			3002530
Population characteristics:			Population of Lusaka
Consumption period:			One year
Number	Para-meter	Question	Value
1	N	Portions consumed	4.5E+06
2	M	Portion size in grams	100
3	Sr/+	Prevalence in retail	20%
4	Cr/+	cfu per gram contaminated product	50.0
5	Scc/r	Portions causing cross. cont.	21%
6	Fcc	cfu's from portions to environment	23%
7	Fei	cfu's from environment to ingestion	2.5%
8	Sprd/cc	Portions prepared done	98%
8	Sprh/cc	Portions prepared half-done	2.0%
8	Sprr/cc	Portions prepared raw	0.000%
9	Fprd	cfu's surviving when prep. done	0%
9	Fprh	cfu's surv. when prep. half-done	2.0%
9	Fprr	cfu's surviving when prep. raw	100%
10	ID50	ID50 (number of cfu's)	1.0E+06
11	Pill/inf	% people infected who get ill	20%
time stamp:		23/01/2019 21:56	
sQMRA-tool			

Figure 1: Model input at 100g serving portion, 50 cfu/g and ID50 at 10^6 cfu (probability for low socioeconomic households fresh fish consumers risk exposure pathway)

EXPOSURE	EFFECT





transmission route	exposure	transmission route	calculation	attribution of cases
Cross contamination	76%	Cross contamination	Scc/r = 0%	75%
Prepared done	0%	Prepared done	Fprd = 0%	0%
Prepared half-done	24%	Prepared half-done	Fprh = 0%	24%
Prepared raw	0%	Prepared raw	Fprr = 0%	0%

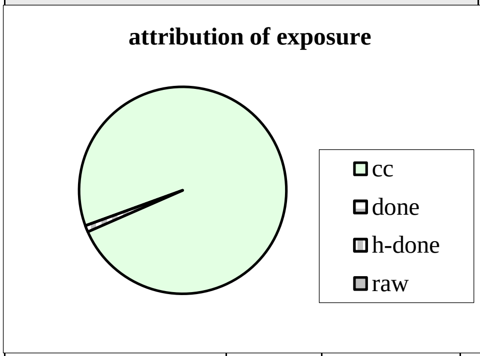
RELATIVE RISK	compared with QMRA *campylobacter* in chicken fillet		
point of comparison	model output	reference data	relative value
Portions consumed	4.5E+06	8.5E+07	5.30%
Contaminated portions (at retail) consumed	9.0E+05	3.3E+07	2.73%
Total number of cfu's before kitchen	4.5E+09	7.0E+10	6.43%
Total number of cfu's after kitchen	7.2E+06	6.1E+06	117%
Number of people ill	1.0	1.2E+04	0.01%

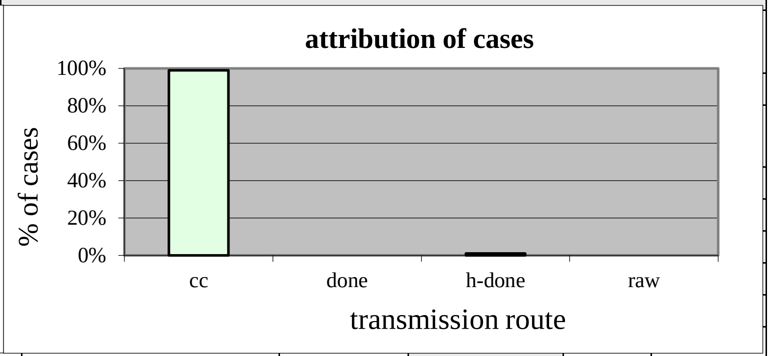
Figure 2: Model output at 100g serving portion, 50 cfu/g and ID50 at 10^6 cfu (probability for the low socioeconomic households risk exposure pathway)

INPUT PARAMETERS			
Pathogen:			*Vibrio cholerae*
Food product:			Fresh fish
Population size:			3002530
Population characteristics:			Population of Lusaka
Consumption period:			One year
Number	Para-meter	Question	Value
1	N	Portions consumed	4.5E+06
2	M	Portion size in grams	200
3	Sr/+	Prevalence in retail	20%
4	Cr/+	cfu per gram contaminated product	50.0
5	Scc/r	Portions causing cross. cont.	21%
6	Fcc	cfu's from portions to environment	23%
7	Fei	cfu's from environment to ingestion	77%
8	Sprd/cc	Portions prepared done	98%
8	Sprh/cc	Portions prepared half-done	2.0%
8	Sprr/cc	Portions prepared raw	0.000%
9	Fprd	cfu's surviving when prep. done	0%
9	Fprh	cfu's surv. when prep. half-done	2.0%
9	Fprr	cfu's surviving when prep. raw	100%
10	ID50	ID50 (number of cfu's)	1.0E+06
11	Pill/inf	% people infected who get ill	20%
time stamp:		23/01/2019 23:25	
sQMRA-tool			

Figure 3: Model input at 200g serving portion, 50 cfu/g and ID50 at 10^6 cfu (probability for the restaurant fresh fish consumers risk exposure pathway)

EXPOSURE	EFFECT





transmission route	Exposure	transmission route	calculation	attribution of cases
Cross contamination	99%	Cross contamination	Scc/r = 0%	99%
Prepared done	0%	Prepared done	Fprd = 0%	0%
Prepared half-done	1.01%	Prepared half-done	Fprh = 0%	1.01%
Prepared raw	0%	Prepared raw	Fprr = 0%	0%

RELATIVE RISK	compared with QMRA *campylobacter* in chicken fillet		
point of comparison	model output	reference data	relative value
Portions consumed	4.5E+06	8.5E+07	5.30%
Contaminated portions (at retail) consumed	9.0E+05	3.3E+07	2.73%
Total number of cfu's before kitchen	9.0E+09	7.0E+10	13%
Total number of cfu's after kitchen	3.4E+08	6.1E+06	5548%
Number of people ill	46.9	1.2E+04	0.38%

Figure 4: Model output at 200g serving portion, 50cfu/g and ID50 at 10^6 cfu (probability for the restaurant fresh fish consumers risk exposure pathway).

4.5.5 Kitchen cross-contamination

In a study done by Hossain et al. (2016) in Dhaka (Bangladesh), results indicated that *Vibrio cholerae* cross-contamination is mostly originated in and around kitchen area rather than latrine area. The percentage of portions that would cross-contaminate the environment such as hands and household kitchen used in the model was assumed to range from 2.0% to 23 % (3.6% of cutting knives, 10.43% point of use pot surfaces, 2.90% latrine door handles, 22.69 % food plates) (Hossain et al., 2016). The percentage of portions that would contaminate the environment such as the hands and kitchen was therefore set at 22.69% for restaurants and the household risk exposure pathways.

In a study conducted by Lee et al., (2017) in Kuala Lumpur, Malaysia detected 2% of *Vibrio cholerae* on the hands of the food handlers working at the University. In another study done by (Tunung et al., 2010) in Malaysia, enumerated the density of *Vibrio* species in the food samples. The occurrence of Vibrio species detected was 20.65% (Tunung et al., 2010). In this study, household and restaurant risk pathways were assumed that 2.5% and 76.7% of cfu on a portion would end up being ingested as a result of daily consumption of fresh fish prepared half done.

4.5.6 Kitchen preparation

From the questionnaire survey on fresh fish preparation, the percentage of doneness on the portion of fresh fish at both household and restaurant kitchen levels was; 98% well done (well-cooked and or fried) while 2% was half done (roasted).

The percentage of micro-organisms surviving was assumed to be zero when well-done. This was attributed to over boiling and, or frying of fresh fish which is normally practised in Zambia; 100% when raw due to poor hygiene practices along the fish processing value in developing countries. Evers and Chardon (2010) also used 0% in well done and 100% when prepared raw, in their sQMRA model.

4.5.7 Infection and illness

The infectious dose of *V. cholerae* in humans varies greatly depending on the bacterial strain and the host. Doses of 10^8–10^{11} cells were required to produce consistent colonization in healthy North American volunteers (Cash, 1974; Wachsmuth, Blake and Olsvik, 1994; Oliver and Kaper, 2001). Most of the research work shows that the infectious dose is between 10^8 and 10^{11} colony forming units (cfu). It is also widely believed that the infectious dose of *V.cholerae* during Cholera epidemics is likely to be considerably less than 10^6 cfu (Butler et al., 2006).

In this study, the infectious dose (number of cfu’s) per gram of portion that would cause half of the exposed population to get Cholera infection (ID50) was taken to be a minimum of 10^6 cfu/g and the maximum of 10^9 organisms needed to be ingested (Novotny et al., 2004b). The study assumed that 20% of the exposed population would get ill when they ingested such doses of Cholera species. About 10% - 20% individuals develop severe watery diarrhoea with vomiting (WHO, 2004). The average concentration of cfu’s per gram in a contaminated portion of raw fresh fish/ seafood was a minimum of 50 cfu/g and maximum $3.3x10^2$ cfu/g of *Vibrio* species (Chitov, 2009).

4.6 Risk characterization

A total of eight (8) simulations which included four from the households risk pathway (2 for the low socio-economic status fresh fish consumers, while the other 2 simulations were for high socio-economic status fresh fish consumers) and four for the restaurant (high fresh fish consumers) risk pathway, were run. Each run produced a summary of input parameters (Fig.1) and the output (Fig.2) model results for the low risk of exposure to *Vibrio cholerae* species among the high socio-economic status, (Fig.3) and (Fig.4) input and out parameters in a restaurant risk pathway. Table 2 (risk characterization) summarises the results of all the outputs of the 8 simulations. Of the 4 case scenarios for the low socio-economic status and high socio-economic status of fresh fish consumers (through the household risk pathway), scenario one, two and three recorded low risk with one person out of a population of 3,002,530 people would develop cholera through the consumption of fresh fish contaminated with *Vibrio cholerae*, representing a probability of 0.000044%. Among the high consumers of fresh fish (through the restaurant) risk pathway, 47 people out of a population of 3,002,530 people would risk developing cholera through consumption of *Vibrio cholerae* contaminated fresh fish, representing a probability of 0.0021%.

Table 2: Summary of the outputs of 8 simulations under household and restaurant risk exposure pathways.

Household risk pathway					
Scenario	**A portion (g)**	**cfu/g**	**ID50**	**Model output (No. People ill)**	**Qualitative Risk**
1	100g	50	10^6	1	Low
2	100g	330	10^7	0.7	Low
3	200g	50	10^7	0.2	Low
4	200g	330	10^6	13.1	High
Restaurant risk pathway					
1	200	50	10^6	46.9	Medium
2	200	50	10^7	4.7	Low
3	200	330	10^6	3,100	High
4	200	330	10^7	31.0	Medium

Quantitative Risk Characterization Using SQMRA at Household level (Low socio-economic and high socioeconomic status households- fresh fish consumers)

Scenario 1: 1 person per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish in low-income households

Scenario 2: 1 people per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish in low-income households

Scenario 3: 1 person per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish in high-income households

Scenario 4: 13 persons per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish in high-income households

Quantitative Risk characterization using sQMRA for restaurant/high-risk pathway (Table 1)

Scenario 1: 47 persons per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish

Scenario 2: 5 persons per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish

Scenario 3: 3100 persons per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish

Scenario 4: 31 persons per year in a general population of 3,002,530 people of Lusaka Province would develop cholera through consumption of *Vibrio cholerae* contaminated fresh fish. In general, a combination of higher fresh fish contaminations levels and a lower infectious dose and (ID50) would result in more people becoming infected.

4.7 Uncertainty

The study was based on simulation model and much of the data was from the reviewed literature. Data was difficult to find on exposure to *vibrio* species as no studies have been done on quantitative exposure assessment to *Vibrio cholerae* through consumption of fresh fish in Zambia, hence, there were some missing data as input parameters in the model. To cover up for these information gaps, the study used some data from other countries and a simple survey on the consumption patterns and serving portions of fresh fish in the population was done to get the average serving portions. The study was also conducted in a post cholera outbreak period which proved difficult to get information from residents and restaurants owners, who were suspicion of having their restaurants closed by the local authorities for non-compliance to legislative requirements. However, the reference point in the model was for *Campylobacter* in chicken fillet in the Netherlands where the model was developed. This, however, does not have much effect on the model because the epidemiology of these pathogens are less the same (Evers & Chardon, 2010) and only serving portions would differ with regard to African countries.

CHAPTER FIVE

5.1 DISCUSSION

Fresh Fish plays an important role in the diets of people in Lusaka Province (Genschick, and Kaminski, 2017). It provides 55% of animal protein consumed by Zambians (Longley et al., 2014). However, when not handled hygienically it can cause foodborne infections such as Cholera. Fresh fish acts as a reservoir for *Vibrio cholerae* (Senderovich et al., 2010).

This study was conducted with the aim of assessing the risk of exposure to *Vibrio cholerae* species through consumption of fresh fish in Lusaka Province of Zambia. The key question was to find out whether with the current consumption patterns of fresh fish sold in Lusaka Province would pose a risk of Cholera infection through it's consumption as prepared at home and those consumed in restaurants. In this study, it was observed that the risk of developing Cholera as a result of fresh fish consumption would be generally low for both exposures from restaurants and in households risk pathways. The low risk in the study would be attributed to low serving portions per meal, preparation methods of fresh fish. However, the serving portion of fresh fish has the potential to contribute to the risk of Cholera infection in humans. In this study, the average serving portion of fresh fish (without bones) was 100g and 200g per meal for low and high-income households and 200g per meal in restaurants. The study revealed that fresh fish were scaled, gutted, gills were removed and washed before cooking. The fresh fish were prepared and consumed well done through boiling or frying which accounted for 98%, while 2% was half done (roasted). This is similar to a study done by Traoré et al. (2016) in Coastal towns of Côte d'Ivoire where food preparation practices were found to prevent human infections, as most households (96.7%) boiled crustaceans before consumption. Although consumption of well-done fresh fish does not pose a risk of exposure to Cholera species other way of getting an infection with *Vibrio cholerae* would be cross-contamination in the kitchen which could occur when handling contaminated fresh fish. In addition, eating with dirty hands could be other ways in which fish can be contaminated.

The study revealed that both households and restaurants served 98% well done fresh fish, prepared at an average temperature of $120^{o}C$ for a minimum period of three hours. This practice does not allow microorganisms to survive in adequately boiled food. This is consistent with an experimental study conducted in Houston (USA), the growth of *V. cholerae* 01 was low in foods which were held between 55 or 60 ^{o} C after 40 or 60 minutes from the time the food was kept hot (Makukutu and Guthrie, 1986). Furthermore, WHO

(2018) states that *Vibrio* spp. do not survive at the cooking temperature of at least 65^0c (149^0 F).

In this study, it was observed that fresh fish were scaled, gutted and slicing was done on preparation surfaces (wooden or stainless) with a water source nearby; for low-income households and restaurants water was usually in buckets for cleaning. Lack of proper hand washing and re-using of water for cleaning kitchen equipment was observed in low-income households' and restaurants visited. This has potential to cause cross-contamination during preparation of food. This was consistent with the findings in a study done in Dhaka (Bangladesh), where poor sanitation was cited in low-income households and restaurants (Wahed, 2013). In this study, 53.3% of the restaurants did not have cooling facilities for foodstuff. Lack of cold storage facilities (fridges and freezers) in the restaurants compelled some of the restaurant owners to store their fresh fish at their homes, where there was potential to cause cross-contamination during moving of food from homes to restaurants.

It was revealed that infection due to cross-contamination at both household and restaurant levels accounted for 76% and 99%, respectively, while 24% and 1% were attributed to consumption of half-done contaminated fresh fish when concentration of *Vibrio* spp. in retail fresh fish was 50 cfu/g of fresh fish and infectious dose fifty (ID50) of 10^6 cfu/g. Cross-contamination to fish from different foodstuffs could take place in the cold storage facilities during storage. An infected or contaminated food handler could contaminate fish during preparation or serving. This is consistent with a study done in Zambia by Kobayashi et al., (2010) who observed that unhygienic handling of fresh fish that is contaminated can be a source of a Cholera outbreak. This was also consistent with a study done in Berlin (Germany), where a patient became infected with *Vibrio cholerae* while handling and preparing imported fresh fish from Nigeria (Schurmann et al., 2002). In another study done in the USA by WHO (1976), it was established that cross-contamination from raw food stuffs to cooked fish and seafood could commonly occur (WHO, 1976).

In the current study, there were low numbers of predicted cases of cholera at low contamination (50cfu/g) and high ID50 (10^7cfu/g). In Zambia, fish is usually eaten cooked; therefore, fish may be a low-risk food when it is adequately cooked. This is in agreement with a study done in the USA by Sumner, (2011) where seafood was adequately cooked before consumption and estimated risk was zero. However, there is always the possibility of cross-contamination during food preparation steps and storage.

Poor hygiene and improper food preparation practices in consumers' homes and restaurants have previously been demonstrated as contributing to foodborne diseases although many have been unreported. This is consistent with the results from the swab samples that indicated 34% presence of *Vibrio cholerae* on food preparation surfaces in some food outlets in Lusaka District during 2017/18 Cholera outbreak (MOH/ZNPH/WHO, 2018).

In general, the low risk of developing Cholera in the current study is in contrast to the study done in Washington, DC by Pan American Health Organization, where seafood is usually consumed raw; it was observed that consumption of fresh raw fishery products poses the greatest risk (PAOH, 1991). This simulation study model could be a starting platform for further studies on the contamination of fresh fish with *Vibrio* spp. from harvesting to the consumption level in Zambia.

CHAPTER SIX

6.1 Conclusions

The risk of exposure to *Vibrio cholerae* from consumption of contaminated fresh fish would be generally very low among the fresh fish consumers in Lusaka province. This is attributed to preparation and adequate cooking methods. Cooking alone is not enough; retailers and consumers must avoid cross-contamination through improved food handling practices. However, consumption of half-cooked fish poses risk to consumers, especially those eating from the restaurants. Another risk of exposure could come as result of cross-contamination with other ready-to-eat products handled in the same kitchen with raw fish.

6.2 Recommendations

More robust studies must be carried out at a national level to come up with data on consumption patterns throughout the year, general risk and the Disability-adjusted life years (DALYs) due to Cholera after consumption of contaminated fresh fish.

Policy makers and stakeholders such as the Food and Nutrition Council of Zambia, Jesuits for Theoretical Reflection (JSTR) and fresh fish processors should also come up with the mechanism of data collection on consumption patterns of various food items, including fresh fish.

Awareness campaigns should be carried out to encourage consumers to adequately cook fresh fish to reduce contamination. This is because it has been demonstrated in this study that consuming fresh fish which is well cooked reduces the risk of developing Cholera.

Generally, consumers and food handlers have to be educated on the dangers of half-cooked fresh fish and the retail contamination levels need to be taken care of so that there is a reduction in contamination levels of fresh fish at retail markets.

6.2 Study Limitations

This was a research report done in partial fulfilment of the course requirement in One Health Food Safety research project within a short period of time and inadequate funding hence covered a small sample size. The study was also done during a post Cholera outbreak period which made it difficult for the research team to get responses from some households and restaurant owners who were suspicion of premises closure from the regulatory authorities for non-compliances to legislative requirements.

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APPENDICES

Appendix 1: Literature review guide

Case definition 1. What is the pathogen of interest? 2. What is the food product of interest? 3. What is the population size? 4. What are the population characteristics? 5. What is the consumption period?	**Consumption data** 1. How many pieces of fish are consumed in the population per consumption period? 2. What is the average of the fish contaminated at retail? 3. What percentage of the fish contaminated at retail? 4. What is the average concentration of colony forming units (cfu) per gram in contaminated fish?
Kitchens cross contamination 1. Given contaminated pieces of fish, what percentage of the pieces of fish will contaminate the environment? Such as hands and kitchen equipment? 2. Given cross contamination, what percentage of cfu's in the environment ends up being ingested	**Kitchen preparation** 1. What percentage of the portions/whole fish is prepared; cooked, half done (roasted), Raw 2What percentage of cfu's on a portion/piece will survive during preparation? Cooking, frying and roasting
	Infection and illness 1. At which dose (number of cfu's) per portion will half of the exposed population get infected? 2. What percentage of infected people will get ill?

Appendix 2: Information sheet

Introduction:

My names are Mulenga Malata, a second year MSc student in One Health Food Safety under the school of Veterinary Medicine at the University of Zambia conducting research on the Quantitative risk exposure assessment to *Vibrio cholerae* through consumption of fresh fish in Lusaka Province, Zambia.

Be assured that the information collected from you in this research will be kept strictly confidential. No name will be written on the interview schedule only numbers.

There are no risks involved in this study, except it will take some of your time as you will need to answer the questions on the questionnaire. There are benefits to you by participating in this study. If you are involved you will be able to be aware of issues concerning the risk of developing Cholera through consumption of fresh fish. In addition, there are other benefits arising from this study because the results will be used by Lusaka City Council, District Health Office, policymakers and other Stakeholders to strategize prevention of Cholera outbreaks.

Your participation in this research is entirely voluntary and you do not have to participate if you do not wish to do so. Be assured that your refusal to take part will not in any way result in penalty or loss of services to which you are otherwise entitled. If you decide to take part, you are still free to withdraw at any time without giving a reason for your withdrawal. You also have the right to end the interview at any time, and to choose not to answer particular questions that are asked in the study.

If you choose to participate in this research study, please sign the informed consent form below

Appendix 3: Consent form

I **……………………………………………………………**have agreed to take part in this research with a title "Quantitative risk exposure assessment to *Vibrio cholerae* through consumption of fresh fish in Lusaka province, Zambia. I confirm that the study has been adequately explained to me and I understand it to the best of my knowledge. I attest to participate voluntarily and that I can withdraw at any time without repercussions.

I understand that disguised extracts from my responses may be quoted in the thesis and any subsequent publications.

I agree to provide the necessary information needed for this study

Participant…………………..........................sign……………………..Date…………………

Name of investigator: ………………………sign………………………Date………………...

Appendix 4: Questionnaire for households

Quantitative Risk Exposure Assessment to *Vibrio cholerae* Through Consumption of Fresh Fish in Lusaka Province, Zambia.

Section A: Demographic Characteristics

Name of interviewer………………………………………… Questionnaire No. ☐

Date of interview dd/mm/yy ☐

Respondent

1.0 Gender

(a) Male ☐

(b) Female ☐

2.0 Age of the respondent

a) 15-20 ☐

b) 21-30 ☐

c) 31-40 ☐

d) 41-50 ☐

e) 51-60 ☐

3.0 Marital status of the participants

a) Married ☐

b) Single ☐

c) Divorced ☐

d) Cohabiting ☐

e) Widowed ☐

4.0 Number of people living in the household:

a) 0-2 year ☐

b) 3-12year ☐

c) 13-18 year

d) Above 18 years

(e) Others. Specify……………………………….

5.0 Highest level of education

a) Never attended school

b) Primary (grade 1-7)

c) Junior secondary (grade 8-9)

d) Senior Secondary (grade 10-12)

6.0 Employment status

a) Government/salary worker

b) Self-employed

e) Unemployed

d) Others specify…………………………………..

7.0 Location of Household

a) Urban

b) Peri-urban

c) Rural

8.0 If urban, is it

a) High density

b) Medium density

c) Low density

9.0 Does the household have electricity?

a) Yes

b) No

10.0 Household's main source of fuel cooking?

a) Electricity

b) Firewood

c) Charcoal

d) Gas

e) Other specify…………………………………………….

11.0 Household's cooling facilities

a) Refrigerator

b) Freezer

c) Both refrigerator and freezer

d) Others specify……………………………………..

Section B: Household fish Consumption Patterns

12.0 Do you prepare fish in your household?

a) Yes

b) No

If no give reasons…………………………….

13.0 If your answer in 12 was yes, how often do you eat fish in your household?

a) Every day

b) Once every two weeks

c) Once every week

d) Once a month

e) Others specify……………………………

14.0 How many kilograms of fish have you bought?

15.0 How many meals with fish are normally prepared from the amount of fish bought?
16.0 How is the fish bought consumed?

a) Raw

b) Half cooked

c) Well cooked

d) Others specify

17.0 Where do you normally buy the fish consumed in your household?

a) Supermarket ☐

b) Local fish stalls ☐

c) Local market ☐

d) Others specify ☐

18.0 How much portion per client for a meal? ……………………………………………

THANK YOU VERY MUCH FOR YOUR TIME

END OF QUESTIONNAIRE

Appendix 5: Questionnaire for restaurants' managers

Quantitative Risk Exposure Assessment to *Vibrio cholerae* species Through Consumption of Fresh Fish in Lusaka Province, Zambia.

Section A: Demographic Characteristics

Name of interviewer………………………………………… Questionnaire No. ☐

Date of interview dd/mm/yy ☐

Respondent

1.0 Gender

(a) Male ☐

(b) Female ☐

2.0 Age of the respondent

a) 15-20 ☐

b) 21-30 ☐

c) 31-40 ☐

d) 41-50 ☐

e) 51-60 ☐

3.0 Marital status of the participants

a) Married ☐

b) Single ☐

c) Divorced ☐

d) Cohabiting ☐

e) Widowed ☐

4.0 Highest level of education

a) Never attended school ☐

b) Primary (grade 1-7) ☐

c) Junior secondary (grade 8-9)

d) Senior Secondary (grade 10-12)

e) Tertiary level

5.0 Restaurant/Household's main source of fuel cooking

a) Electricity

b) Firewood

c) Charcoal

d) Gas

e) Other specify…………………………………………….

6.0 Restaurant's cooling facilities

a) Refrigerator

b) Freezer

c) Both refrigerator and freezer

d) Others specify……………………………………..

Section B: Restaurant fish Consumption Patterns

7.0 Do you usually prepare fresh fish for your clients in this restaurant?

a) Yes

b) No

8.0 If the answer is yes in 9, how much portion per client for a meal?..............................

9.0 If your answer in 9 was yes, how often do your clients eat fish in your restaurant?
a) Every day

b) Once every two weeks

c) Once every week

d) Once a month

e) Others specify……………………………

10.0 How many kilograms of fresh fish do you buy?

11.0 How many meals with fresh fish are normally prepared from the amount of fish bought?

12.0 How is fresh fish bought consumed by clients?

a) Raw ☐

b) Half cooked ☐

c) Well cooked ☐

d) Others specify ☐

13.0 Where do you normally buy the fresh fish consumed in your restaurant?

a) Supermarket ☐

b) Local fish stalls ☐

c) Local market ☐

d) Others specify ☐

THANK YOU VERY MUCH FOR YOUR TIME

END OF QUESTIONNAIRE

Appendix 6: Letter of permission from Lusaka City Council

LUSAKA CITY COUNCIL
OFFICE OF THE TOWN CLERK

P O Box 30077
CIVIC CENTRE
LUSAKA, ZAMBIA 10101

TELEPHONE : 260 01 252997
TELEFAX : 260 01 252141

Our Reference: HK/gc
TCD/7/58/10

02nd January, 2019.

TO WHOM IT MAY CONCERN

RESEARCH STUDY – MR. MULENGA MALATA

The above mentioned is a student at University of Zambia pursuing a Master's Degree in One Health Food Safety, he is currently undertaking a research study on **"Quantitative Risk Exposure Assessment to Cholera species through Consumption of Fresh Fish in Lusaka District, Zambia".**

This study is purely for academic purpose, kindly therefore assist him with the needed information for the successful completion of her research project.

Yours faithfully,
LUSAKA CITY COUNCIL

Alex Mwansa
TOWN CLERK

Cc: The Director - Human Resource & Administration