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

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ABSTRACT

Fresh fish is one of the widely consumed sources of proteins in Zambia. Consumption of contaminated fresh fish has been linked to zoonotic transmission of diseases in humans as fresh fish harbors several spoilage bacteria and pathogens including *V. cholerae* which cause cholera in humans. This study aimed at conducting a quantitative exposure assessment to *Vibrio cholerae* through consumption of fresh fish in Lusaka province of Zambia. Swift Quantitative Microbial Risk Assessment (sQMRA) model was used to estimate the risk of exposure. Data was obtained from reviews of scientific literature, government reports, questionnaire survey, and expert opinions. Fish consumers were categorised using three risk pathways; restaurants, households with low socioeconomic status and households with high socio-economic status.

Results revealed that at a serving portion of 100g in households with low socioeconomic status, a concentration of 50 cfu/g, and infectious dose 50 (ID50) of 10^6 cells, one person out of 2,251,898 population at risk would get ill, representing a probability of $4.4x10^{-7}$. At a serving portion of 200g in households with high socioeconomic status, a concentration of 330 cfu/g, and ID50 of 10^6 cells, 13 people out of 2,251,898 would get ill, representing a probability of $5.7x10^{-6}$. At an average serving portion of 200g in restaurants,

a concentration of 50 cfu/g, and ID50 of 10^6 cells, 47 people would get ill, translating to a probability density of 2.02×10^{-5}.

These results indicate that the risk of exposure to *Vibrio cholerae* through the consumption of fresh fish among the population at risk in Lusaka Province of Zambia is extremely low through all risk pathways.

Cross-contamination during preparation and consumption is the main source of exposure to the *Vibrio cholerae*. Therefore, improvement in good food safety handling and processing would further minimise the occurrence of foodborne illnesses.

Keywords: *cholera, cross-contamination, exposure assessment, fresh fish, vibrio cholera Zambia*

1.0 INTRODUCTION

Cholera remains a public health threat globally, causing hundreds of thousands of cases every year [1]. In previous centuries, cholera was a continuous threat even to the developed countries in Europe, North America, and the northern part of Asia [2]. Currently, cholera remains endemic in some developing countries [3]. The global disease burden is estimated at 3-5 million cases and accounts for a total of 100,000-130,000 death per year [4]. However, this threat is minimised in places with a safe water supply, good hygiene standards, sanitation and good food safety management systems.

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 [5].

Cholera has been endemic in some parts of the country including Lusaka province which has been reporting the highest number of cases. During the outbreak of 2009, Lusaka province recorded 4,464 cases and resulted in 73 deaths (CFR 1.63%) [6]. This was followed by another outbreak in 2010-2011, where 173 cases were reported in Lusaka district [7]. The Cholera outbreak was also declared in October 2017 and affected more than 3,534 people with more than 77 deaths [6]. The cholera outbreak disrupted many economic activities in the country, especially in the affected areas. Additionally, the Government of Zambia spent a lot of funds in creating cholera treatment centers, providing medical supplies, and controlling the disease [6].

Recently, Chiyangi *et al.* [8] reported the presence of *Vibrio cholerae*-O1 subtype and Ogawa serotype at a frequency of 40.8 per cent of the isolated bacteria from children between 12 and 23 months old at the Lusaka University Teaching Hospital (UTH). *Vibrio* species were also detected in Zambia's aquatic environment including fresh fish from both natural water bodies and ponds [9]. The Zambian government institutes a fishing ban during the rainy seasons; however, people still eat fish that could come from the illegal captures during the ban periods and cultured fish from local fish farmers or imported fish. Is there a risk of developing cholera through the consumption of infected fresh fish? Fresh fish is among the most highly nutritious foods as it is

a good source of proteins for humans [10]. However, there has been evidence of the presence of *Vibrio* species in fish from previous researches in other countries [11]. Equally, Mizanda [12] isolated *Vibrio cholerae* non-O1 in 27 per cent (n = 73) of fresh fish sampled from six markets in six sub-districts of Lusaka district of Zambia. Consumption of contaminated fresh fish has been incriminated to be the cause of cholera in some parts of the world [13, 14, 9]. In Zambia, cholera outbreaks have been recorded around 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 [15, 16], while 73 per cent of the cholera outbreaks in Zambia have been reported in Lusaka province [17]. In Zambia, Lusaka province is one of the leading provinces in the country in terms of fish consumption [17]. However, health risks, particularly cholera disease, associated with the consumption of fresh fish is little known by the community because in most cases, cholera outbreaks are attributed to the consumption of contaminated water and other foods, poor hygiene, and sanitation, among other factors. Therefore, this study used a swift Quantitative Microbiological Risk Assessment (sQMRA) model to estimate the likelihood of exposure to *V. cholerae* through consumption of contaminated fresh fish among the people of Lusaka province.

2.0 METHODS

Ethical approval and clearance was sought from Excellence in Research Ethics and Science (ERES) IRB Converge with Reference Number (2018-Oct-030).

2.1 Study Area

The study was conducted in Lusaka province of Zambia, an area with relatively high fresh fish consumption across all the socio-economic status groups [18]. The province has an area coverage of 21,896 km^2 with a population of 3,002,530 by 2018 (7). Lusaka is the commercial capital of Zambia and is inhabited by multiple ethnic groups. Fish sold in Lusaka province is sourced from locally grown, and outside Lusaka which includes imports from other countries [17].

2.2 Study Design

This was a simulation study using the Swift Quantitative Microbial Risk Assessment (sQMRA) model framework. The sQMRA-model was developed by Evers and Chardon [19]. It is implemented in a Microsoft Excel spreadsheet 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 15 parameters, always followed by intermediate model output broken down into categories of contamination, cross-contamination, and preparation. Model input and output are summarised and exposures, as well as cases, are attributed to the distinguished categories. As a relative risk measure, intermediate and final model outputs are 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 [19].

2.3 Data Sources

The study primarily used information generated from other studies. For additional secondary data, a cross-sectional questionnaire survey was undertaken to close the information gaps noticed in the literature. Therefore, the study used both secondary and primary data sources.

Secondary data was collected through a desktop study, which mainly involved reviewing scientific peer-reviewed papers and grey literature. The literature review was guided by research questions based on the sQMRA model. A 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. Furthermore, grey literature from reports from government institutions and Non-Governmental Organisations (NGOs) 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, etc., were used. Guided by questions in Table 1, literature that contained relevant data were included in the study and the rest were excluded

After an extensive literature review, we discovered that there were information gaps in serving portions and consumption patterns of fresh fish in Zambia. Therefore, a survey was conducted using a structured questionnaire to fill these information gaps. 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 households with high socio-economic status while the other ten (10) were from households with low socioeconomic status. Residential areas were used as a proxy for high socioeconomic status using the Central Statistical Office conditions of leaving survey [20]. High socioeconomic status was taken to be the households that were able to afford or meet basic needs such as education, clean water, shelter, food, health, and stable income according to the living conditions monitoring and survey report of 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 [21]. The other sixty (60) respondents were the restaurants owners or managers where clients consumed fresh fish in all the six (6) sub-districts. The sample size

Table 1: Literature review guide

Case definition • What is the pathogen of interest? • What is the food product of interest? • What is the population size? • What are the population characteristics? • What is the consumption period?	**Consumption data** 1. How many potions of fish are consumed in the population per consumption period? 2. What is the average size of one portion of fish? 3. What percentage of the portions of fish is contaminated at retail? 4. What is the concentration in log10 of colony-forming units (cfu) per gram in contaminated portions of fish?
Kitchen cross-contamination 5. Given contaminated portions of fish, what percentage of the portions of fish will contaminate the environment (Such as hands and kitchen equipment)? 6. Given contaminated portions, what percentage of the cfu's on a portion will contaminate the environment? e.g. hands and kitchen equipment? 7. Given cross-contamination, what percentage of cfu's in the environment ends up being ingested?	**Kitchen preparation** 8. What percentage of the portions of fish is prepared; cooked, half done (roasted), raw? 9. What percentage of cfu's on a portion will survive during preparation? Cooking, frying, and roasting?
Infection and illness 10. At which dose (number of cfu's) per portion will half of the exposed population get infected? 11. What percentage of infected people will get ill?	

was not based on statistics but was meant to give a general overview of fish 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.

3.0 RESULTS

The results were presented using the four steps of conducting food safety risk assessment under the Codex Alimentarius Framework. These steps include hazard identification, hazard characterisation, consumer exposure assessment, and risk characterisation.

3.1 Hazard identification

According to the WHO definition, choleragenic *V. cholerae* O1 and O139 are the only causative agents of cholera with epidemic and pandemic potential [18]. *Vibrio cholerae* was identified as hazardous bacteria [22]. The toxigenic *V. cholerae* species are zoonotic, with fresh fish and aquatic environments being their reservoir [11]. The pathogen can be transmitted from fresh fish to humans through the consumption of contaminated fresh fish if not handled hygienically during processing. *V. cholerae* can cause syndromes ranging from asymptomatic to cholera gravis. The pathogens cause cholera in humans which is characterised by 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 [22]. Zambia is endemic to cholera outbreaks where 75 per cent of cases are asymptomatic, 20 per cent are mild to moderate and 2-5 per cent are severe forms like gravis [23].

3.2 Hazard Characterisation

This is the quantitative evaluation of the nature of the adverse effects associated with the consumption of fresh fish contaminated with *V. cholerae*. According to WHO [24], about 20 per cent of infected individuals develop acute, watery diarrhea and 10 to 20 per cent of these individuals go on to develop severe watery diarrhea with vomiting. Several reports indicate that the number of choleragenic organisms required to cause illness is in the range of 10^4 to 10^9 [25, 26]. According to Butler *et al.* (27), a remarkably high dose of *V. cholerae* is required to cause disease in humans. A study done in the United States of America by Hornick *et al.* [28], established the trend between the dose and the observed probability of response among the volunteers (Table 2). The onset of symptoms as well as an indication of asymptomatic infection was monitored. Additionally, both diarrhea and cholera diarrhea (very different in appearance) was also monitored as symptoms.

Table 2: Dose-response for *Vibrio cholerae* (28)

Dose	Diarrhea	No Diarrhoea	Total
10	0	2	2
10^3	0	4	4
10^4	9	4	14
10^5	6	2	8
10^6	20	3	23
10^8	2	0	2

3.3 Consumer Exposure Assessment

The retail-to-consumption risk pathway was considered with three categories: (1) the retail-to-household with low socio-economic status risk pathway, (2) the retail-to-household with high socio-

economic status risk pathway, and (3) the retail-to-restaurant risk pathway. 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.

3.3.1 Case Definition

The pathogen of interest was *V. 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 the Central Statistical Office [29]. A consumption period of one year was considered 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 divided into three categories, the high socioeconomic status, the lower socio-economic and the restaurants' owners. A case of cholera was defined as a "confirmed case" when *V. cholerae* O1 or O139 was isolated from any patient with diarrhea [30].

3.3.2 Consumption Data

In this study, the portion size is defined as a whole fresh fish or a cutoff piece of fresh fish an individual consumes per meal. There was no available consumption data on fresh fish categorising the low and the high socio-economic status 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 the cheapest source of animal proteins. Everyone, irrespective of socio-economic status, enjoys fresh fish in Zambia [18]. Further, Genschick [17] found that the poorest households in Lusaka province relied on small fish products from capture fisheries while high socio-economic households consumed larger fresh fish such as tilapia.

The survey revealed that 100 per cent and 96 per cent of Lusaka province residents 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 socioeconomic status and restaurant fresh fish consumers were served with the whole piece of fish for one meal only.

According to the 2015 Labour Force Survey of Zambia, 75 per cent of the Lusaka population was in formal or informal employment [31]. Therefore, the study assumed that 75 per cent of 3,002,530 populations in Lusaka consumed fresh fish because of their purchasing power. The survey revealed that either the whole medium-size or half a piece of fresh fish was served (Lunch and dinner). Hence the number of portions consumed by the population was calculated to be 2,251,898 per consumption period multiplied by two servings for lunch and dinner (4,503,795 portions).

3.3.3 Contamination of Fresh Fish at Retail Outlets

There was no data found locally during the literature review hence the study used both shrimps and fresh fish contamination at retail outlets in other countries which were found from 3.5 per cent to 18.3 per cent in fresh fish retail centers and 0 per cent to 14.3 per cent in fresh fish in streets and restaurants [32]. A similar study in

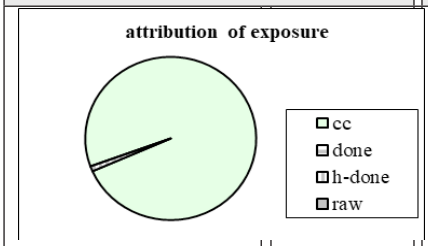
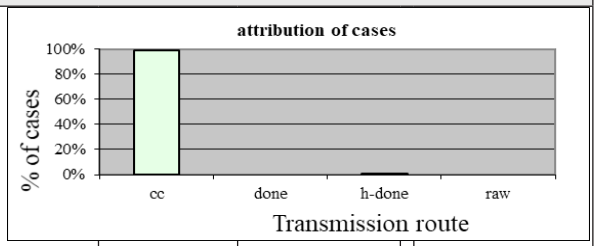
Bangladesh revealed that retail contamination of fresh fish stood at 20 per cent [33]. In another study conducted in Burkina Faso, the contamination was found at 6 per cent [34]. Some studies were also conducted in shrimps and shellfish in Ecuador, Berlin (Germany), and India, Cochin market contaminations were found at 11.3 per cent, 6.3 per cent, and 1 per cent, respectively [35, 36, 37].

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 [38]. As shown in Table 3, this study considered only minimum and high concentrations of *V. 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 [39].

Table 3: Model input at 100g serving portion, 50 cfu/g, and ID50 at 10^6 cfu (probability for low socioeconomic household's fresh fish consumers risk exposure pathway)

INPUT PARAMETERS			
Pathogen:			*Vibrio cholerae*
Food product:			Fresh fish
Population size:			3002530
Population characteristics:			Population of Lusaka
Consumption period:			One year
No.	Parameter	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-contamination	21%
6	Fcc	cfu's from portions to environment	23%
7	Fei	cfu's from the 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 prepared done	0%
9	Fprh	cfu's surviving when prepared half-done	2.0%
9	Fprr	cfu's surviving when prepared raw	100%
10	ID50	ID50 (number of cfu's)	1.0E+06
11	Pill/inf	% people infected who get ill	20%
Timestamp:		23/01/2019 21:56	
sQMRA-tool			

Table 4: Model output at 100g serving portion, 50 cfu/g, and ID50 at 106 cfu (probability for the low socioeconomic households' 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%

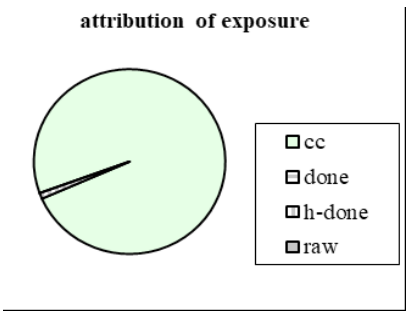
Table 5: Model input at 200g serving portion, 50 cfu/g, and ID50 at 106 cfu (probability for the restaurant fresh fish consumers risk exposure pathway)

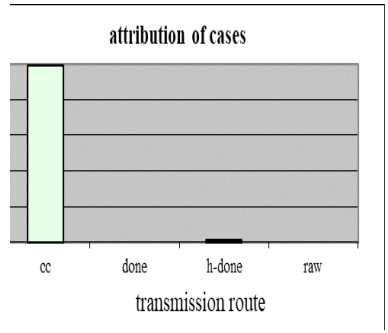
INPUT PARAMETERS			
Pathogen:			*Vibrio cholerae*
Food product:			Fresh fish
Population size:			3002530
Population characteristics:			Population of Lusaka
Consumption period:			One year
Number	Parameter	Question	Value
1	N	Portions consumed	4.5E+06
2	M	Portion size in grams	200
3	Sr/+	Prevalence in retail	20%
4	Cr/+	cfu per gram contaminated product	50.0
5	Scc/r	Portions causing cross-contamination.	21%

6	Fcc	cfu's from portions to environment	23%
7	Fei	cfu's from the 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 prepared done	0%
9	Fprh	cfu's surviving when prepared half-done	2.0%
9	Fprr	cfu's surviving when prepared raw	100%
10	ID50	ID50 (number of cfu's)	1.0E+06
11	Pill/inf	% people infected who get ill	20%
Timestamp:		23/01/2019 23:25	
sQMRA-tool			

Table 6: Model output at 200g serving portion, 50 cfu/g, and ID50 at 106 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

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%

Table 7: Summary of the outputs of 8 simulations under household and restaurant risk exposure pathways

Scenario	A portion (g)	cfu/g	ID50	Model output (No. People ill)	Qualitative Risk	Probability
Household with low socioeconomic status risk pathway						
1	100	50	10^6	1	Low	$4.4x10^{-7}$
2	100	330	10^7	0.7	Low	$3.1x10^{-7}$
Households with a high socioeconomic status risk pathway						
3	200	50	10^7	0.2	Low	$8.9x10^{-8}$
4	200	330	10^6	13.1	High	$5.8x10^{-6}$
Restaurant risk pathway						
1	200	50	10^6	46.9	Medium	$2.1x10^{-5}$
2	200	50	10^7	4.7	Low	$2.1x10^{-6}$
3	200	330	10^6	3,100	High	$1.4x10^{-3}$
4	200	330	10^7	31.0	Medium	$1.4x10^{-5}$

3.3.4 Kitchen Cross-contamination

In a study done by Hosain *et al.* [40] in Dhaka (Bangladesh), *Vibrio cholerae* cross-contamination was mostly originated in and around the kitchen area rather than the latrine area. Therefore, 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 per cent to 23 per cent (3.6% of cutting knives, 10.43% point of use pot surfaces, 2.90% latrine door handles, 22.69% food plates) [40]. The percentage of portions that would contaminate the environment such as the hands and kitchen was therefore set at 22.69 per cent for restaurants and the household risk exposure pathways.

In addition, Lee *et al.* [41] reported the presence of *V. cholerae* at 2 per cent on the hands of the food handlers working at the university in Kuala Lumpur, Malaysia. Further, Vibrio species were detected at 20.7 per cent in the food samples in the same country [42]. In this study, it was assumed that 2.5 per cent and 76.7 per cent of cfu on a portion in both household and restaurant risk pathways would end up being ingested as a result of daily consumption of fresh fish prepared half done.

3.3.5 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 per cent well done (well-cooked and or fried) while 2 per cent was half done (roasted). In this study, the percentage of surviving microorganisms in well-cooked fresh fish was assumed to be at zero because

of the over boiling or frying practices in Zambia; while it was assumed at 100 per cent in raw fresh fish due to poor hygiene practices along with the fish processing value in developing countries. Evers and Chardon [19] and Manyori *et al.* (44) also used 0 per cent in well-cooked and 100 per cent in raw chicken and beef in their sQMRA model.

3.3.6 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 to 10^{11} cells were required to produce consistent colonisation in healthy North American volunteers [25, 43, 45]. 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 [27].

In this study, the infectious dose (number of cfu's) per gram of portion ingested that would cause half of the exposed population to get cholera infection (ID50) was considered to be 10^6 cfu/g and 10^9 cfu/g as the minimum and the maximum, respectively [26]. The study assumed that 20 per cent of the exposed population would get ill when they ingest such doses of cholera species. About 10 to 20 per cent of individuals develop severe watery diarrhoea with vomiting [24]. 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 a maximum of 3.3×10^2 cfu/g of *Vibrio* species [39].

3.4 Risk Characterisation

A total of 8 simulations were conducted. These simulations included four from the household's risk pathway (2 for the low socio-economic status fresh fish consumers, and the other two simulations for high socioeconomic status fresh fish consumers), and four from the restaurant risk pathway. Each stimulation produced a summary of input parameters (Table 3) and the output (Table 4) model results for the low risk of exposure to *V. cholerae* species among the high socio-economic status, (Table 5) and (Table 6) input and out parameters in a restaurant risk pathway. Summarises the results and risk characterisation of all the outputs of the 8 simulations are shown in tables 7 and 8. Of the 4 case scenarios for the low and high socioeconomic status of fresh fish consumers (through the household risk pathway), scenario one, two, and three recorded the lowest risk with 1 person out of a population of 2,251,898 people being at risk of developing cholera through the consumption of *V. cholerae* contaminated fresh fish, representing a negligible probability of 0.00000044. Among the high consumers of fresh fish (through the restaurant risk pathway), 47 out of a population of 2,251,898 people would develop cholera through consumption of *V. cholerae* contaminated fresh fish, representing a probability of 0.000021.

Table 8: Quantitative Risk Characterisation Using sQMRA

Scenario	Household-level (Low socio-economic and high socio-economic status households- fresh fish consumers)	Restaurant/high-risk pathway
1	1 person per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish in high-income households.	47 people per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*–contaminated fresh fish.
2	1 person per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish in high-income households.	5 people per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish.
3	1 person per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish in high-income households.	3100 people per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish.
4	13 people per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish in high-income households	31 people per year in a general population of 2,251,898 people of Lusaka province would develop cholera through consumption of *V. cholerae*-contaminated fresh fish. In general, a combination of higher fresh fish contaminations levels and a lower infectious dose (ID50) would result in more people becoming infected.

4.0 DISCUSSION

This study assessed the risk of exposure to *V. cholerae* species through consumption of fresh fish in Lusaka province of Zambia. The results revealed that the risk of developing cholera through consumption of contaminated fresh fish is generally low for both exposures from restaurants and households risk pathways. The low risk in this study could be attributed to preparation methods of fresh fish and meat in Zambia, which involves boiling and frying for long hours as highlighted by Manyori *et al.* (44). 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 was scaled, gutted, gills removed, and washed before cooking. The fresh fish that was prepared and consumed well done through boiling or frying accounted for 98 per cent, while 2 per cent half done (roasted). This is similar to a study done by Traoré *et al.* [34] in Coastal towns of Côte d'Ivoire where food preparation practices were found to prevent human infections, as most households (96.7 per cent) boiled crustaceans before consumption. Although consumption of well-done fresh fish does not pose a significant risk of exposure to cholera species, other ways of getting an infection with *V. cholerae* are through 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 per cent 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 boiled food. This is consistent with an experimental study conducted in Houston, where the growth of *V. cholerae* 01 was low in foods that were held between 55 or 60^{o}C after 40 or 60 minutes from the time the food was kept hot [46]. Furthermore, WHO [47] states that Vibrio spp. do not survive at the cooking temperature of at least 65^{o}C (149^{o}F).

According to the study, it was observed that fresh fish was scaling, gutting, and slicing was done on preparation surfaces (wooden or stainless) with a water source nearby, for low-income households, while in restaurants water was usually in buckets for cleaning. Lack of proper handwashing and re-using of water for cleaning kitchen equipment was observed in low-income households and restaurants visited. This has the potential to cause cross-contamination during the preparation of food. This is consistent with the findings in a study done in Dhaka (Bangladesh), where poor sanitation was cited in low-income households and restaurants [48]. In this study, 53.3 per cent of the restaurants did not have cooling facilities for foodstuff. This compelled some of the restaurant owners to store their fresh fish at their homes with the potential of cross-contamination during the transportation.

It was revealed that infection due to cross-contamination at both household and restaurant levels accounted for 76 per cent and 99 per cent respectively, while 24 per cent and 1 per cent were attributed to consumption of half-done contaminated fresh fish when the concentration of *Vibrio* spp. in retail fresh fish was 50cfu/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.* [9] who observed that the 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 was identified as becoming infected with *V. cholerae* while handling and preparing imported fresh fish from Nigeria [48]. In a study done in the USA by WHO [49], it was established that cross-contamination from raw food-stuffs to cooked fish and seafood could commonly occur [50].

In this study, there were low numbers of predicted cases of cholera at low contamination (50 cfu/g) and high ID50 (10^7cfu/g). In Zambia, fish is usually eaten cooked; therefore, fish may be a low-risk food in transmitting cholera when it is adequately cooked. This is in agreement with a study done in the USA by Sumner [51] where seafood was adequately cooked before consumption and the 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 to be contributing factors to foodborne diseases. This is consistent with the results from the swab samples that indicated 34 per cent presence of *V. cholerae* on food preparation surfaces in some food outlets in Lusaka district during the 2017/18 cholera outbreak [6].

In general, the low risk of developing cholera in the current study is in contrast to the study done in Washington D.C. by Pan American Health Organisation, where the seafood is usually consumed raw. It was observed that the consumption of fresh raw fishery products poses the greatest risk [52]. 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 developing countries.

The limitation of this study was that there was substantial missing data as input parameters in the model. To address this limitation, a survey on the consumption patterns and serving portions of fresh fish in the population was conducted to get the average serving portions, so as avoid too much reliance on logical assumptions and use of data from other countries. The pathogen numbers were followed through the food chain, which in this case starts at retail and ends with the number of human cases of illness.

5.0 Conclusion

The risk of exposure to *V. cholerae* from consumption of contaminated fresh fish is generally very low among the fresh fish consumers in Lusaka province. This is attributed to the preparation and adequate cooking methods. However, the consumption of fresh fish, which is not well done, poses risk to consumers, especially those eating from restaurants. Equally, cross-contamination with other ready-to-eat products handled in the same kitchen with raw fish may increase the risk of exposure to *V. cholerae*.

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6.0 References

1. Ali M, Nelson AR, Lopez AL, Sack D. Updated Global Burden of Cholera in Endemic Countries. PLoS Negl Trop Dis. 2015; 9(6): e0003832. doi:10.1371/journal.pntd.0003832.
2. Banerjee R, Bhabatosh D, Balakrish N, Surajit B. Dynamics in genome evolution of Vibrio cholera, volume23; https://doi.org/10.1016/j.meegid.2014.01.006
3. Reidl J, Klose KE. Vibrio cholerae and cholera: out of the water and into the host. Rontgenring 11, 97070 Wurzburg, Germany. FEMS Microbiol. Rev. 2002; 26: 125-139.
4. WHO. World Health Organisation. Cholera, Weekly Epidemol Rec. 2010; 86:325-340.
5. Olu O, Babaniyi O, Songolo P, Matapo B, Chizema E, Kapin'a-kanyanga M, Musenga E, Walker O. 2013. Cholera epidemiology in Zambia from 2000 to 2010: Implications for improving cholera prevention and control strategies in the country, Lusaka, Zambia. East African Medical J. 2013; 10: 324.
6. MoH. Ministry of Health. Situational Report No. 122 Outbreak Name: Cholera Investigation start date: 4th October 2017; Lusaka, Zambia. 2018; 1–8.
7. WHO. World Health Organisation. Zambia Global Task Force on Cholera Control, Cholera Country Profile: Lusaka, Zambia. 2011; 1-2
8. Chiyangi H, Muma JB, Malama S, Manyahi J, Abade A, Kwenda G, Matee MI. Identification and antimicrobial resistance patterns of bacterial enteropathogens from children aged 0–59 months at the University Teaching Hospital, Lusaka, Zambia: A prospective cross-sectional study 1–9.2017; https://doi.org/10.1186/s12879-017-2232-0
9. Kobayashi T, Mudenda HB, Yamamoto H. The Survival of Vibrio cholerae in the Natural Environment of Zambia: Fish, the suspicious Gateway of Cholera Outbreak? Lusaka, Zambia. J. Int. Heal. 2010; 25: 33–39.
10. Pal J, Shukla BN, Kumar AM, Verma OH, Pandey G, Amitha. A review on the role of fish in human nutrition with special emphasis on essential fatty acid, College of Fisheries NDUA & T Kumarganj Faizabad Uttar Pradesh,

India. Int. J. Fisheries and Aquatic Stud. 2018; 6(2): 427-430

11. Senderovich Y, Izhaki I, Halpern M. Fish as Reservoirs and Vectors of Vibrio cholerae; Columbia University, United States of America. PLoS ONE. 2010; 5(1) 8607 5. https://doi.org/10.1371/journal.pone.0008607
12. Mizanda C. Investigation and Antimicrobial Susceptibility of Vibrio species isolated from the Tilapia Fish Marketed in Lusaka District, Zambia. 2019. The University of Zambia Thesis.
13. Acosta C, Galindo C, Kimario J, Senkoro K, Urassa H. Cholera outbreak in southern Tanzania: Risk factors and patterns of transmission. Emerg. Infect. Dis. 2001; 75: 83-587.
14. Forssmain B, Mannes T, Musto J, Baumann B, Frei U, Gottlieb T, Robertson G, Natoli JD, Shadbolt C, Biffin B, Gupta L. *Vibrio cholera* O1 E1 Tor cluster in Sydney linked to imported whitebait, New South Wales. Med. J. Aust. 2007; 187 (6): 345-347.doi:10.5694/j.1326-5377.2007.tb01278.x
15. Gama A, Kabwe P, Nanzaluka F, Langa N, Mutale LS, Mwanamoonga L, Moonga G, Chongwe G, Sinyange N. Cholera Outbreak in Chienge and Nchelenge Fishing Camps, Zambia, 2017. Health Press Zambia Bull. 2017; 2 (1): 12-19.
16. Murebwa-Chirambo R, Mwanza R, Mwinuna C, Mazaba ML, Mweene-Ndumba IMJ. Occurrence of cholera in Lukanga fishing camp, Kapiri-Mposhi District, Zambia. Health Press Zambia Bull. 2017;1(1).
17. Genschick S. Fish consumption in urban Lusaka: The need for aquaculture to improve targeting of the poor, Lusaka, Zambia. Aquac. 2018; 492: 280–289. https://doi.org/https://doi.org/10.1016/j.aquaculture.2018.03.052
18. FAO. Food and Agriculture Organisation. Fishery country profile report: The republic of Zambia, general economic data, Lusaka, Zambia, FID/CP/ZAM. 2006; 1-10. Evers EG, Chardon JE. A swift Quantitative Microbiological Risk Assessment (sQMRA) tool; BA Bilthoven, The Netherlands. J. Food Control. 2010; 21(3): 319-330.
19. CSO. Central Statistical Office living conditions monitoring survey. Retrieved from http://www.zamstats.gov.zm/report/Lcms/2006-2010.LCMS Report Final
20. CSO. Central Statistical Office. The 2015 Living conditions monitoring survey report, Lusaka, Zambia: CSO. 2016.
21. Dalusi L, Thomas J, Lugomela C, Ken MM, Sjoling. Toxigenic *Vibrio cholerae* identified in estuaries of Tanzania using PCR techniques, Dar es Salaam, Tanzania. 2015. doi: 10.1093/femsle/fnv009
23. Bronze MS, Greenfield RA. Biodefense Principles and Pathogens horizon bioscience. Norfolk England; FEMS Immunology & Medical Microbiology. 2005; 45(2): 351–352, DOI: 10.1016/j.femsim.2005.06.002.
24. WHO World Health Organisation. Global task force on cholera control; assessing the outbreak response and improving preparedness. Geneva: World Health Organisation. 2004
25. Cash RA, Music SI, Libonati JP, Craig JP, Pierce NF, Hornick RB. Response of man to infection with *Vibrio cholerae*. II. Protection from illness afforded by previous disease and vaccine. J. Infect. Dis. 1974; 130: 325–333.

26. Novotny L, Dvorska L, Lorencova A, Beran V, Pavlik I. Fish: a potential source of bacterial pathogens for human beings, Brno, Czech Republic; Vet Med Czech. 2004; 49: 343-358.
27. Butler SM, Nelson EJ, Chowdhury N, Faruque SM, Stephen B, Calderwood AC. Cholera stool bacteria repress chemotaxis to increase infectivity; Boston, MA 02111, USA. Mol. Microbiol. 2006. https://doi.org/10.1111/j.1365-2958.2006.05096
28. Hornick RB, Music SI, Wenzel R, Cash RA, Libonati JP, Snyder MJ, Woodward T. The Broad Street Pump Revisited: Response of Volunteers to Ingested Cholera *Vibrios*. Bull. New York Acad. Med. 1971; 47(10): 1181-1191.
29. CSO. Central Statistical Office. Zambia in Figures-2018, Lusaka. 2018
30. WHO. World Health Organisation. Prevention and control of cholera outbreaks: WHO Policy and Recommendations. 2019
31. CSO. Central Statistical Office Labour force Survey –Republic of Zambia. 2015.
32. Torres-vitela MAR, Castillo A, Finne G, Rodriguez-Garcia MAO, Martinez-Gonzales N E, Navarro-Hidalgol V. Incidence of Vibrio cholerae in Fresh Fish and Ceviche in Guadalajara, Mexico. 1997; 60: 237–241.
33. Hossain ZZ, Farhana I, Tulsiani SM, Begum A. Transmission and Toxigenic Potential of *Vibrio cholerae* in Hilsha Fish (Tenualosa ilisha) for Human Consumption in Bangladesh. Front. Microbiol. 2018; 9: 222,1–13. https://doi.org/10.3389/fmicb.2018.00222
34. Traoré SG, Bonfoh B, Krabi R, Odermatt P, Utzinger J. Europe PMC Funders Group Risk of Vibrio Transmission Linked to the Consumption of Crustacean in Coastal Towns of Côte d' Ivoire. 2016; 75: 1004–1011. https://doi.org/10.4315/0362-028X.JFP-11-472.
35. Sperling L, Alter T, Huehn S. Prevalence and Antimicrobial Resistance of Vibrio spp. in Retail and Farm Shrimps in Ecuador. J. Food. Prot. 2015. https://doi.org/10.4315/0362-028X.JFP-15-160.
36. Sudha S, Mridula C, Silvester R, Mohamed HA. Prevalence and antibiotic resistance of pathogenic Vibrios in shellfishes from Cochin market, Cochin 682 016, Kerala, India. Indian J. Geo-Marine Scie. 2014; 43(5): 815-824.
37. Vu TTT, Alter A, Huehn S. Prevalence of Vibrio spp. in Retail Seafood in Berlin, Germany. J. Food Prot. 2018; 81 (7): 1079-1086
38. Oumar T, Martikainen O, Anja S, Nicolas B, Kaisa H. Occurrence of Vibrio cholerae in fish and water from a reservoir and a neighboring channel in Ouagadougou, Burkina Faso. J. Infect. Dev. Countries. 2014; 8(10): 1334- 1338. https://doi.org/10.3855/jidc.3946
39. Chitov T. Occurrence of potentially pathogenic *Vibrio* species in raw, processed, and ready-to-eat seafood and seafood products. 2009. https://doi.org/oai:doaj.org/e:d22a41b861646c3939b155dc337c7ab
40. Hossain ZZ, Farhana I, Tulsiani SM, Sultana R, Jensen PM, Begum A. Investigation of Household Transmission of *Vibrio cholerae* in Bangladesh. Poster session presented at American Society for Microbiology, Bostom, United States. 2016.

41. Lee HK, Abdul Halim H, Thong Kwai L, Chai LC. Assessment of Food Safety Knowledge, Attitude, Self-Reported Practices, and Microbiological Hand Hygiene of Food Handlers. Kuala Lumpur, Malaysia Int. J. Env. Res. Pub. Health. 2017; 14 (1): 55. https://doi.org/10.3390/ijerph14010055
42. Tunung R, Margaret SP, Jeyaletchumi P, Chai LC, Zainazor TCT, Ghazali, FM. Prevalence and quantification of Vibrio in raw salad vegetables at retail level Prevalence and Quantification of Vibrio parahaemolyticus in Raw Salad Vegetables at Retail Level. J. Microbiol. Biotechnol. 2010; 20(2): 391-396. https://doi.org/10.4014/jmb.0908.08009
43. Oliver JD, Kaper JB. Vibrio species. In Food Microbiology: fundamentals and frontiers (M.P. Doyle, ed.), 2nd Ed. ASM Press, Washington, DC, 263-300 2nd ED.ASM. 2001.
44. Manyori CI, Mumba C, Muma JB, Mwale MM, Munyeme M, Bwanga EK, Häsler B, Rich KM, Skjerve E. Quantitative risk assessment of developing salmonellosis through consumption of beef in Lusaka Province, Zambia. Food Control. 2017. doi:10.1016/j.foodcont.2016.10.027
45. Wachsmuth IK, Blake PA, Olsvik Ø. Vibrio cholerae and Cholera: Molecular to Global Perspectives. Washington D.C: ASM Press. 1994; 345–356.
46. Makukutu CA, Guthrie RK. The behavior of *Vibrio cholerae* in Hot Foods; Houston, Texas, USA. Am. Soc. Microbiol. 1996; 52: 824–831.
47. WHO. World Health Organisation. Cholera –Zimbabwe: Emergencies preparedness response, Disease outbreak news: Update, 5 October 2018.
48. Wahed T. Knowledge of, attitudes toward, and preventive practices relating to cholera and oral cholera vaccine among urban high-risk groups: findings of a cross-sectional study in Dhaka, Bangladesh. BMC Public Heal. 2013; 13:242. https://doi.org/doi: 10.1186/1471-2458-13-242
49. Schürmann D, Ebert N, Kampf D, Baumann B, Frei U, Suttorp N. Domestic Cholera in Germany Associated with Fresh Fish Imported from Nigeria. Eur. J. Clin. Microbiol. Infect. Dis. 2002; 21: 827–828. https://doi.org/10.1007/s10096-002-0832-z
50. WHO. World Health Organisation. Microbiological aspects of the food hygiene; Report of a WHO Expert Committee with the participation of FAO, Geneva, Technical report series No. 598. 1976.
51. Sumner J. Food Safety Risks Associated with Prawns Consumed in Australia, Seafood CRC Project: 2009/787. 2011
52. PAOH. Pan American Health Organisation. Veterinary Public Health Program, Risk of transmission of Cholera by food: Washington, DC, USA. 1991