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Research Article

Quantitative Risk Assessment of *Vibrio parahaemolyticus* Toxi Infection Associated with the Consumption of Roasted Shrimp (*Penaeus monodon*)

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In this study, a risk assessment on *Vibrio parahaemolyticus* infections was carried out in order to estimate the likelihood of gastroenteritis for Cameroonians after consumption of roasted shrimp (*Penaeus monodon*). The Codex Alimentarius Commission framework was used in this study. Based on the distribution of total *V. parahaemolyticus* in shrimp and literature information indicating that nonhaemolysing carrier strains could be pathogenic to humans, the cooking, and consumption patterns, the daily exposure level generated in this study, and the dose-response model from other studies, the infectious risk was evaluated and quantified by the Monte Carlo simulation. This simulation was realized based on 10,000 iterations using the Model Risk software, version 4.0, in combination with Microsoft Excel. To better quantify the exposure of consumers and the resulting risk of infection, several scenarios reflecting the minimal, average, and maximal exposures were undertaken. According to the results, the 90% confidence intervals for minimum and maximum exposures ranged from 15 to 24 colony-forming units per day (cells/day) and from 160 to 228 cells/day, respectively. Based on the modal scenario, 90% of the population consuming this shrimp is exposed to *V. parahaemolyticus* loads ranging from 74 to 110 cells/day, indicating a risk of infection ranging from 1.2 to 1.8 cases per million of consumption. The estimated number of annual disease cases based on annual production is between 1 and 10 cases. This reflects a relatively low risk of infection for roasted shrimp. Good hygiene practices during handling, cooking, and storage may help reduce the actual risk.

1. Introduction

For almost three decades, food-borne infections have been the center of concern worldwide. Europe is experiencing a decrease of infections while most underdeveloped African countries are still experiencing an increase. Raw or undercooked foods are generally the foods most implicated in food-borne illness. Among these foods, fish products (including shellfish), which are the most consumed, are the second most important source of food protein after meat [1]. Indeed, in 2016, its global production was estimated at about 171 million tonnes, valuing more than 362 billion dollars. In an estimate of 80 million tonnes of crustaceans, shrimp contributed to 65 million tonnes [2]. In 2017, in Cameroon, fishery products were estimated at about 140,100 tonnes, from which 1,150 tonnes of shrimp amounted to more than 1.3 billion CFA francs of commercial value [3].

Laws and standards are the main instruments used internationally for the adoption and prioritization of hazard criteria limits and risks to be managed. In this regard, risk assessment is the basic requirement for hazards' acceptable limits definition used in risk management [4]. Presently, in Cameroon, there is no risk management protocol regarding microbial hazards associated with shrimp, a product that is usually consumed along the sea touristic sites.

Among pathogens associated with shrimp, *Vibrio parahaemolyticus* seems to be the most implicated bacterium, especially when the product is raw or undercooked [5]. Tiger and pink shrimp (*Penaeus monodon* and *Penaeus notialis,* respectively), sold in national markets, are generally of nonexportable quality due to *V. parahaemolyticus* contamination [6, 7]. Since they are caught in marine waters, where this bacterium is naturally found because of its halophytic character, contamination becomes almost unavoidable [8].

In Cameroon, there is a paucity or almost no reported cases of *V. parahaemolyticus* food-borne infections. However, in Africa, at the level of some countries that have access to ocean waters (Mozambique, Nigeria, and Ivory Coast), cases of *V. parahaemolyticus* infections related to the consumption of seafood such as shrimp have been reported [9–11]. According to the literature reviewed, between the years 1990 and 2010, more than 800 outbreaks caused by *V. parahaemolyticus* and approximately 50,000 cases of disease and some cases of death were reported in the USA, Canada, France, Malaysia, and China, all related to seafood consumption [12–14].

The main production and consumption cities in Cameroon are Douala, Kribi, and Limbe, with roasting being the most commonly used form of preparation of the shrimp. It is therefore important to assess if different roasting protocols impact the risk associated with *V. parahaemolyticus* infection. The objective of this work was to assess the risk of acquiring gastroenteritis caused by *V. parahaemolyticus* as a result of the consumption of roasted *P. monodon* shrimp in Cameroon.

2. Material and Methods

This work was conducted in three phases: (1) a field survey on cooking, storage, and consumption patterns of shrimp, which are factors that impact *V. parahaemolyticus* infection levels; (2) mathematical modelling of the effect of roasting and postcooking storage parameters on the concentration variations of *V. parahaemolyticus*; and (3) a quantitative risk assessment of *V. parahaemolyticus* toxi infection during consumption.

2.1. Survey on Cooking, Storage, and Consumption Patterns of Shrimp Related to V. parahaemolyticus Infection. A survey was conducted in the main production and consumption town of Douala on a sample size of 96 people. It was done using a questionnaire with the aim of assessing factors such as precooking handling, roasting, and postcooking patterns that are factors that can impact the risk associated with *V. parahaemolyticus*.

2.2. Mathematical Modelling of the Effect of Roasting and Postcooking Storage Parameters on V Parahaemolyticus Load Variations. In the absence of standardized and validated *V. parahaemolyticus* enumeration methods in the literature, but inspired by the most probable number Polymerase Chain Reaction (MPN-PCR) analysis method [14], a protocol to evaluate the growth kinetics of this bacterium was set up in order to predict its initial concentration before enrichment in Alkaline Peptone Water (APW).

During this evaluation, three isolates of *V. parahaemolyticus* (VP_1, VP_2, and VP_3) from three shrimp samples, obtained following the protocol of [15] and identified using API 20 E, were cultured in APW for 6 hours. VP_1 and VP_2 cultures were previously incubated at 37°C overnight, while VP_3 was cultured at 37°C overnight and then stored at −20°C for 2 h, simulating bacteria present in shrimp during storage conditions. This was done in order to simulate the transportation done by some fishermen from harvest to retail. During incubation, the microbial concentration was evaluated every thirty (30) minutes using standard counting methods on Thiosulphate citrate bile salt sucrose (TCBS) agar. The enumeration data collected for the three isolates and representing the growth kinetics were fitted in the Baranyi and Roberts [16] model and growth kinetic parameters (μ; Lag) were estimated. The mean of the isolates' generation times (t_g) was used in the following equation to deduce the microbial concentration before enrichment (N_0).

$$\log_{10} N_0 = \log_{10} N_t - \left(\frac{t}{t_g}\right) \log 2. \quad (1)$$

The shrimp samples used for the experimental study were randomly selected from different markets, then aseptically packaged, and transported to the Food Safety Laboratory of the Biotechnology Center, Nkolbisson, University of Yaounde I. On arrival in the laboratory, shrimp samples were weighed, labelled, packaged, and stored at −20°C. For analysis purposes, the initial concentration of *V. parahaemolyticus* in the samples was determined.

From the survey data, the high-risk shrimp cooking protocol was identified as being the roasting because it was the most commonly used in shrimp consumption, with the lowest mean temperature in shrimp during transformation. The roasting temperature varied between 119°C and 175°C while in the shrimp this oscillated from 80°C to 99°C. To reproduce this roasting protocol in the laboratory, a $3^{(3-1)}$ fractional experimental plan was set up (Table 1). The factors selected for the experiment were the cooking time (min), storage time (hours), and initial concentration (cells/g) with three factors level.

From the experimental design, nine assays were performed (Table 2). The *V. parahaemolyticus* postcooking/storage concentration was evaluated in each assay and mathematically modelled using a multiple and nonlinear regression analysis with the STATISTICA version 10.2 software. There was no available simplified model for the present experimental setup in the literature; therefore, the polynomial type model was preferred and tested. A fractional factorial regression using a backward stepwise model building was used, starting from the global model. The variable selection performed with the model building takes into account the suggestions of Heinze et al. [17]. The best equation was selected based on the statistical significance of all parameters ($p < 0.05$) included in the model, the presence

Table 1: Coded values of the fractional experimental plan for the assessment of microbial loads after cooking and storage.

Factors	Level of factors		
	−1	0	+1
Cooking time (min)	10	20	30
Storage time (hours)	1	6	11
Initial concentration (cells/g)	2	50	100

Table 2: Real values used in the experimental plan for the assessment of microbial concentrations after cooking and storage.

Test	Cooking time (min)	Initial concentration (cells/g)	Storage time (hours)
1	10	2	1
2	10	50	11
3	10	100	6
4	20	2	11
5	20	50	6
6	20	100	1
7	30	2	6
8	30	50	1
9	30	100	11

of all variables, the capacity of giving calculated data in the same order of magnitude as the observed data, the log-likelihood, and the Pearson chi-square (X^2) values.

2.3. Enumeration of V. parahaemolyticus in Raw Shrimp Samples for Distribution and during Experimentation. In order to determine the initial *V. parahaemolyticus* concentration distribution in raw shrimp, 87 shrimp samples were collected from fishermen at the sea coast, while for the experimentation aimed at reproducing roasting protocols, new batch samples constituted of twenty-seven raw fresh shrimp were bought from the local market. They were randomly divided into nine groups of three shrimp each. For the initial batch concentration, one group was cleaned and ground aseptically in an electric grinder. Then an equivalent volume of APW at 1% NaCl was added to the entire ground material in an Erlenmeyer flask. The mixture was incubated for 6 h at 37°C. After the enrichment incubation, the *Vibrio* concentration was evaluated using the standard counting method on TCBS agar [18, 19]. (1) was used to deduce the microbial concentration before enrichment (No).

2.4. Quantitative Risk Assessment of V. parahaemolyticus Infection during Consumption. The Model Risk software package, version 4.0 [20], in combination with Microsoft Excel, was used to carry out simulations. The risk estimate was calculated by using input data in the model, data generated in this study (a mathematical model describing the relationship between postcooking microbial concentration and the cooking, storage, and consumption patterns), and assumptions based on data from previous studies [13, 21]. A framework for microbiological risk assessment was applied according to the recommendation of the Codex Alimentarius Commission (CAC) [22]. All the calculations were performed by the Monte Carlo simulation method of sampling from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions. This simulation was realized based on 10,000 iterations. The likelihood of illness following exposure to *V. parahaemolyticus* from consumption of roasted shrimp (*P. monodon*) was calculated based on the following steps: hazard identification, exposure assessment, and risk characterisation.

2.4.1. Hazard Identification of V. parahaemolyticus. This was done by reviewing different papers on *V. parahaemolyticus* pathogenicity [1, 18, 19, 23, 24]. Based on the principle of precaution, the incapacity of the Cameroonian food safety system to selectively differentiate haemolytic and nonhemolytic carrier strains, and the fact that nonhemolytic carrier strains have been observed to cause severe illnesses, the hazard in this risk assessment was focused on the total number of *V. parahaemolyticus* in raw shrimp (*P. monodon*) and after roasting. Moreover, for the sake of comparison of the risk deviation caused by the assumption of total strains or only pathogenic strains, other calculations were performed using 8% [25] and 15% [1] pathogen species incidence. The adverse health effects include gastroenteritis. The adverse effect of daily consumption was assumed not to be cumulative.

2.4.2. Hazard Characterisation of V. parahaemolyticus. The dose-response relationship in this study was developed using the infectivity parameters (α, β, and γ) that were necessary for calculating the risk of infection generated by USFDA report [13, 14]. For more precision, values of α, and β were those of Anse [1] produced with the same data but not rounded up, while γ was the adjustment parameter applied on β in USFDA [14] in order to adapt the probability of infection obtained in FAO/WHO [21] to CDC data [26]. These parameters were extracted from the modified Beta-Poisson relationship using the Furumoto–Mickey approximation [27] under the conditions ($\beta >> 1$ and $\alpha < \beta$) [28]. Since there was no data on the dose-response relationship available for the

Cameroonian population, the same modified beta-Poisson parameters as those estimated during the modelling of the disease probability by the USFDA [14] were used.

2.4.3. Exposure Assessment of V. parahaemolyticus. The exposure assessment component of a microbial risk assessment is an evaluation of the likelihood of ingesting a pathogenic microorganism via food and the likely level of exposure. In this assessment, the likelihood of exposure to *V. parahaemolyticus* after consumption of roasted shrimp (*P. monodon*) was evaluated. It was determined by integrating the initial concentration distribution function of shrimp into the predictive mathematical model for postcooking concentration and then multiplying by the shrimp weight randomly chosen from the distribution function, the average shrimp size consumed per dish, and the number of dishes per day. The general equation was as follows:

$$\begin{aligned} D_{ing/j} = {} & [f(\mathrm{Ci}(\mathrm{Weibull}(\alpha;\beta;\gamma)), \mathrm{ts}, \mathrm{tc},)] \\ & {}^{*}(\mathrm{Log}^{*}\mathrm{Logistic}(\alpha;\beta))^{*}A^{*}B, \end{aligned} \quad (2)$$

where $D_{ing/j}$: microbial load ingested per day (cells), f: predictive model of *V. parahaemolyticus* postcooking concentration per shrimp size (cells/g), ts: storage time (h), tc: cooking time (min), A: average shrimp size consumed per dish (g), B: number of dishes per day, Ci (Weibull3 (α; β; γ)): distribution function of the initial shrimps concentration of *V. parahaemolyticus*, and Log–Logistic (α; β): shrimp weight distribution function.

The estimated number of annual meals from locally harvested shrimp was obtained by dividing the annual quantities of shrimp provided by the Ministry of Fisheries produced annually by the daily meal size. We decided not to take into account the losses first because, in our opinion, estimating losses in our context introduces other bias factors since they are less directly estimated than total production. Secondly, it is better to overestimate than to underestimate. Thirdly, considering total annual shrimp as consumed is the worst-case option.

Its equation was as follows:

$$\frac{\mathrm{Nexp}}{\mathrm{year}} = \frac{\text{annual production}}{\text{daily meal size}}. \quad (3)$$

The average daily meal size was obtained by multiplying the shrimp's weight (distribution function) by the average number of shrimp consumed per dish and the average number of dishes consumed per day obtained during investigations. Its equation was as follows:

$$\begin{aligned} \mathrm{Meal}_{\mathrm{size/day}} = {} & [\text{shrimp weight}(\log - \text{logistic}\ (\alpha;\beta))] \\ & {}^{*}\left(\frac{\text{average shrimp}}{\text{dish}}\right) \\ & {}^{*}\left(\frac{\text{average number of dishes}}{\text{day}}\right). \end{aligned} \quad (4)$$

The different initial concentrations of *V. parahaemolyticus* in each shrimp (87 shrimp in total) and their respective weights were fitted to several distribution functions using EasyFit software version 5.6. It is a software that fits more than 150 distribution functions incorporated into its system to continuous data series. The choice of the best fitting distribution function was based on three tests, namely, the Anderson–Darling test, the Kolmogorov–Smirnov test, and the chi-square test.

2.4.4. Risk Characterisation of V. parahaemolyticus. In the risk characterisation, the estimated exposure is normally integrated with the dose-response model to provide a risk estimate and to determine the influence of different mitigation strategies on the risk estimate. The risk estimate was calculated by using the data generated from this study and assumptions based on data from other studies.

From the infectivity parameters (α, β, and γ), and the different doses ingested daily ($D_{ing/j}$), the infection likelihood ($P_{inf/j}$) was estimated using the following equation:

$$P_{inf/j} = 1 - \left[1 + \left(\frac{D_{ing/j}}{\gamma * \beta}\right)\right]^{-\alpha}. \quad (5)$$

The number of annual disease cases was estimated by multiplying the product of the likelihood infection by the number of annual exposures. Its equation was as follows:

$$\mathrm{Nber}_{\mathrm{Cases}} = P_{inf/j} * \text{Nber of annual exposure}. \quad (6)$$

The model simulations were implemented with Model Risk [20]. The Monte Carlo sampling method was used to perform all calculations from specified input distributions and appropriately combine the sample values to generate the corresponding output distributions. This simulation was realized based on 10,000 iterations.

3. Results

3.1. Survey on Cooking, Storage, and Consumption Pattern of Shrimp (Penaeus monodon) Related to V. parahaemolyticus Infection. The results of the survey of 96 households in the city of Douala are shown in Table 3. They indicate that shrimp are mostly eaten roasted; they are usually roasted using a charcoal oven for 20 min and stored at room temperature with a maximum storage time of 10 h. Most respondents visiting coastal areas usually consume two dishes per day, each containing two shrimp.

3.2. Modelling of Postcooking and Poststorage V. parahaemolyticus Concentration in Roasted Shrimp

3.2.1. Growth Kinetics of Three Isolates of V. parahaemolyticus. The growth kinetics revealed the different growth phases: latency, exponential, stationary, and decay. Figures 1(a)–1(c) show the growth kinetics of the three isolates of *V. parahaemolyticus* (VP) adapted to the Baranyi and Robert [16] model. The VP_3 isolate (Figure 1(c)), which previously underwent thermal stress at −20°C for 2 hours, presented a latency phase of 0.89 h while no latency phase was observed with VP_1 and VP_2 isolates

Table 3: Data from the survey of 96 households in the city of Douala with the highest consumer density.

Questions	Parameters	Values
Treatment modalities		
Cooking time (min)	Minimum	10.0
	Maximum	45.0
	Average	17.5
	Standard deviation	5.8
	Mode	20.8
Storage time (hours)	Minimum	1.0
	Maximum	10.0
	Average	4.8
	Standard deviation	2.0
	Mode	5.7
Consumption modalities		
Number of shrimp per dish	Minimum	1.0
	Maximum	7.0
	Average	3.2
	Standard deviation	1.3
	Mode	2.0
Number of dishes per day	Minimum	1.0
	Maximum	3.0
	Average	1.7
	Standard deviation	0.6
	Mode	2.0
Average number of meals per month	Minimum	2.0
	Maximum	18.0
	Average	8.1
	Standard deviation	3.4

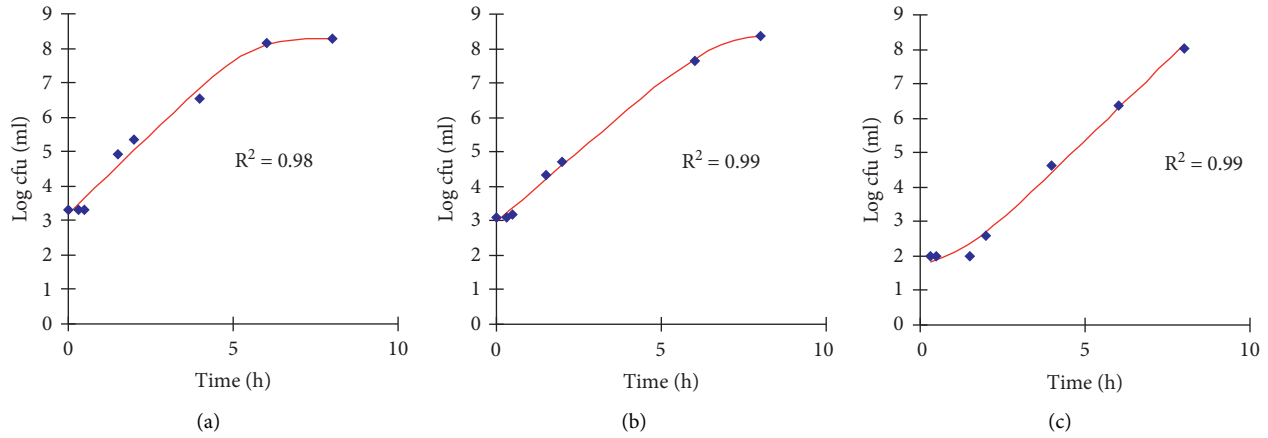

Figure 1: Growth kinetics of three isolates of *V. parahaemolyticus* adapted to the model of Baranyi and Robert [16] (curves a, b, and c). Unstressed isolates of VP1 (a), VP2 (b), and the isolate stressed at −20°C for 2 h VP3 (c).

that were not stressed (Figures 1(a) and 1(b)). The growth rates of the three isolates were 0.91 (VP_1), 0.82 (VP_2), and 0.93 (VP_3) log CFU/ml/h. The generation times for the three strains VP1, VP_2, and VP_3 were 0.33 h, 0.37 h, and 0.32 h, respectively.

3.2.2. Modelling the Concentration of V. parahaemolyticus Affected by Cooking and Storage Conditions. Based on the information collected during the survey and the initial *V. parahaemolyticus* concentration of the sampled batch (2.02 cells/g) estimated using the protocol described previously and equation (1), an experimental design was set up to evaluate the postcooking concentration of *V. parahaemolyticus*. The results obtained are shown in Table 4. For the same cooking scale, the residual microbial concentration increases proportionally to the initial concentration. After applying multiple regression analysis, a mathematical equation predicting the residual concentration after roasting as a function of tested parameters was obtained with a log-likelihood of 0.902 and a Pearson chi-square (X^2) of 0.431. In this equation, all the estimated parameters were statistically significant at $p < 0.05$.

Table 4: *V. parahaemolyticus* concentration at consumption time as a function of cooking time, initial concentration, and storage time at ambient temperature.

Assay	tc (min)	Ci (cells/g)	ts (hours)	Post-cooking concentration (cells/g)
1	10	2	1	0
2	10	50	11	10.76
3	10	100	6	1693.74
4	20	2	11	0
5	20	50	6	6.03
6	20	100	1	129.52
7	30	2	6	0
8	30	50	1	0.41
9	30	100	11	169.37

$$Z = \mathrm{EXP}\left(-2,92042 + 0,12893 * \mathrm{Ci} - 0,11593^{*}\mathrm{ts} + 0,01308^{*}\mathrm{ts}^{*}\mathrm{tc} - 0,00263^{*}\mathrm{tc}^{*}\mathrm{Ci}\right), \quad (7)$$

where Z is the postcooking/storage concentration of *V. parahaemolyticus* in cells/g, Ci is the initial concentration of *V. parahaemolyticus* in cells/g, ts is the storage time in an hour, and tc is the cooking time in minutes.

A representation of equation (7) when the initial concentration is among the highest is given in Figure 2. The model predicts that the increase in the postcooking concentration of *V. parahaemolyticus* is inversely proportional to the cooking time but proportional to the storage time.

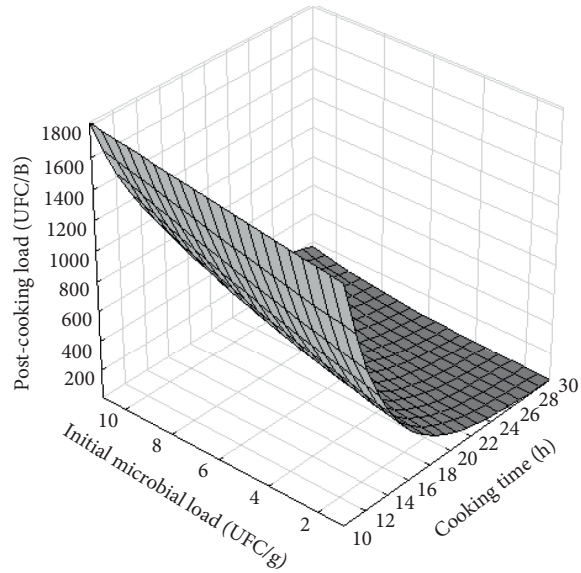

Figure 2: Response surfaces expressing the variation of the postcooking concentrations of *Vibrio parahaemolyticus* as a function of cooking time and storage time for an initial concentration (Ci) of 100 (cells/g).

3.3. Quantitative Risk Assessment of *V. parahaemolyticus* Infection during Consumption

3.3.1. Hazard Identification of V. parahaemolyticus. *Vibrio parahaemolyticus* is a halophilic bacterium that naturally occurs in the sea and brackish waters. This bacterium is often transmitted to humans through the consumption of raw, inadequate, or recontaminated cooked seafood [29].

Vibrio parahaemolyticus is rarely incriminated in a health issue in Africa and Europe, but the incidence of infections of this pathogen has increased sharply since 1996 in Asia and North America. The assumptions for this increase are global warming, eutrophication of coastal water, increased consumption of raw seafood, changes in strain virulence, and an increased proportion of subpopulations at risk [1]. However, the incidence has dropped recently in Japan. This is attributed in part to the decline in seawater contamination and especially to the hygienic measures taken downstream during the harvest [30]. Exposed for 2-3 h at room temperature, a growth of *V. parahaemolyticus* of 10^2-10^3 CFU/g up to or more than 10^5 CFU/g could be observed [31]. The bacterium's viability decreases with decreasing temperatures. However, cold cannot be used for microbial deactivation in products [14, 32–34]. *V. parahaemolyticus* is destroyed by heat beyond its maximum growth temperature (43°C) [14, 35, 36], and Twedt [37] demonstrated that treatment at 60°C for 5 min reduced about 3-4 log of *V. parahaemolyticus*.

Between 1990 and 2010, in the United States of America, more than fifty outbreaks of *V. parahaemolyticus* were reported, causing nearly 2,500 cases of disease and some cases of death due to seafood consumption [12–14]. From 1995 to 2010, *V. parahaemolyticus* was involved in 13 confirmed outbreaks of collective food poisoning in Europe and 3 suspected outbreaks [38]. In Asia, from 1990 to 2005, about 800 outbreaks, including more than 50,000 cases of *V. parahaemolyticus,* were reported [13].

Pathogenicity of *V. parahaemolyticus* results from two hemolysin genes; tdh (thermostable direct hemolysin) and trh (thermostable related hemolysin), which lyse red blood cells by destroying their plasma membrane [39]. The incidence of Hemolysin genes may appear in environmental strains at 0.2 to 2% but can go up to 15% in some ecosystems [1]. Contrary to this, clinical samples (stools) show 95% of *V. parahaemolyticus* with at least one of the hemolysin genes [24]. However, in 2006, the CDC reported cases of much more serious infections associated with noncarrier strains of the two genes [23]. This can question the use of carrier genes percentage in the quantitative risk assessment of *V. parahaemolyticus* risk. Based on these few and contradictory data, it would be preferable to use the total microbial concentration in risk analyses.

Two type III secretion systems (T3SS1 and T3SS2) and two other haemolytic components (phospholipase A and lysophospholipase) have been identified in *V. parahaemolyticus,* but the exact role of the haemolytic components in their pathogenicity is not yet known [29, 40]. There are 13O antigens and 71K antigens identified in clinical strains. New pandemic clones derived from O3: K6, which appeared in Bangladesh in 1996, have been identified around the world [1].

The foods most likely to be involved in gastroenteritis are crustaceans and fish are eaten raw or undercooked. Prevalence studies, conducted in Africa [41] and Cameroon [6, 7] in particular, have revealed the presence of *V. parahaemolyticus* in several sea products caught and sold locally and/or exported. Studies in the United States have revealed the presence of *V. parahaemolyticus* in oysters in wholesale and retail markets [42]. Though the levels in this study are below 100 microorganisms/g in most of the batches tested, they can exceed 10,000 microorganisms/g in certain regions of the world.

3.3.2. Hazard Characterisation of V. parahaemolyticus. The hazard characterisation of *V. parahaemolyticus* is presented here by dose-response curves, already used for the USFDA report [14] and several other studies that deal with risk assessment regarding this pathogen around the world [1, 12, 13].

3.3.3. Exposure Assessment of V. parahaemolyticus.
3.3.3.1. Adapting Initial Concentration of V. parahaemolyticus and Shrimp Weight to Distribution Functions. The initial concentration values of *V. parahaemolyticus* in shrimp fitted well into the Weibull 3p distribution whose function is given by the following equation:

$$f_x(x) = \frac{\alpha}{\beta}\left(\frac{x-u}{\beta}\right)^{\alpha-1} e^{-(x-u/\beta)\alpha}, \quad (8)$$

where x is the initial concentration of *V. parahaemolyticus* in shrimp; α is the slope or the shape parameter; β is the scale parameter; and μ is the the location parameter.

The values of the parameters α, β, and μ of this Weibull 3p distribution function are 0.76, 0.53, and 0.016, respectively.

The weights of the shrimp were well fitted by the log-logistic distribution, whose function is given by the following equation:

$$f_x(x) = \frac{\alpha}{\beta}\left(\frac{x}{\beta}\right)^{\alpha-1}\left(1+\left(\frac{x}{\beta}\right)^{\alpha}\right)^{-2}, \quad (9)$$

where x is the weight of the shrimp; α represents the slope or the shape parameter; and β is the scale parameter.

The values of the parameters α and β of this log-logistic distribution function are 5.40 and 67.67, respectively.

The summary statistics for the fitted distributions are presented in Table 5.

Table 5: Summary statistics for fitted distributions of shrimp samples by Weibull 3p distribution (initial concentrations) and log-logistic distribution (weight) used in the exposure assessment.

Statistics	Value	Percentile	Value
V. parahaemolyticus concentration in shrimp (cells/g)			
Size	87	Min	0.016
Range	5.746	5%	0.029
Mean	0.665	10%	0.045
Variance	0.776	25% (Q1)	0.127
Stand. deviation	0.881	50% (Median)	0.415
Variation coef	1.324	75% (Q3)	0.892
Stand. error	0.094	90%	1.534
Kurtosis	3.124	95%	2.444
Skewness	13.487	Max	5.762
Shrimp weight (g)			
Size	87	Min	29.11
Range	106.54	5%	36.766
Mean	71.478	10%	42.204
Variance	450.96	25% (Q1)	56.9
Stand. deviation	21.236	50% (Median)	68.8
Variation coef	0.297	75% (Q3)	85.2
Stand. error	2.277	90%	98.13
Kurtosis	0.355	95%	112.37
Skewness	0.277	Max	135.65

3.3.3.2. Assessment of the Meal Size Consumed per Day and the Average Number of Meals Consumed per Year. Based on the results of the survey, it was possible to simulate distributions of the average daily consumed meal sizes (Figure 3) and the average number of meals per year (Figure 4). It was observed that for the average size of the daily meal, 90% of the values were between 279.58 g and 629.97 g (Figure 3), with an average of 407.4 g. Regarding the average number of meals per year, 90% of the values were between 3.65×10^7 and 8×10^7 meals (Figure 4), with an average of 5.9×10^7 meals.

3.3.3.3. Evaluation of the Daily Exposure to V. parahaemolyticus. Several possible scenarios were performed to illustrate minimal, average, and maximum predicted risk conditions. This exposure is, in general, proportional to the size of the meal, and the storage time but inversely proportional to the cooking time (Table 6). It was observed that 90% of the predicted conditions for the minimum exposure were between 14.73 cells/d and 23.38 cells/d, and those associated with predicted conditions for maximum exposure were between 159.86 cells/d and 228.80 cells/d, while for the average scenarios, the values were between 47 and 181.05 cells/d.

Figure 5 represents the simulation of the exposure trend of the consumers with modal behaviour. These predicted simulations were based on the modal values of the survey. It was observed that 90% of the predicted exposure values were between 73.10 and 109.38 cells/d with a mean of 86.03 cells/d.

3.3.4. Risk Characterisation of V. parahaemolyticus. The probabilities of infection were calculated based on the exposure degree of the different scenarios (Table 6). It was observed that 90% of the simulated values for the minimum and maximum risk were between 2.41×10^{-7} and 3.80×10^{-7}

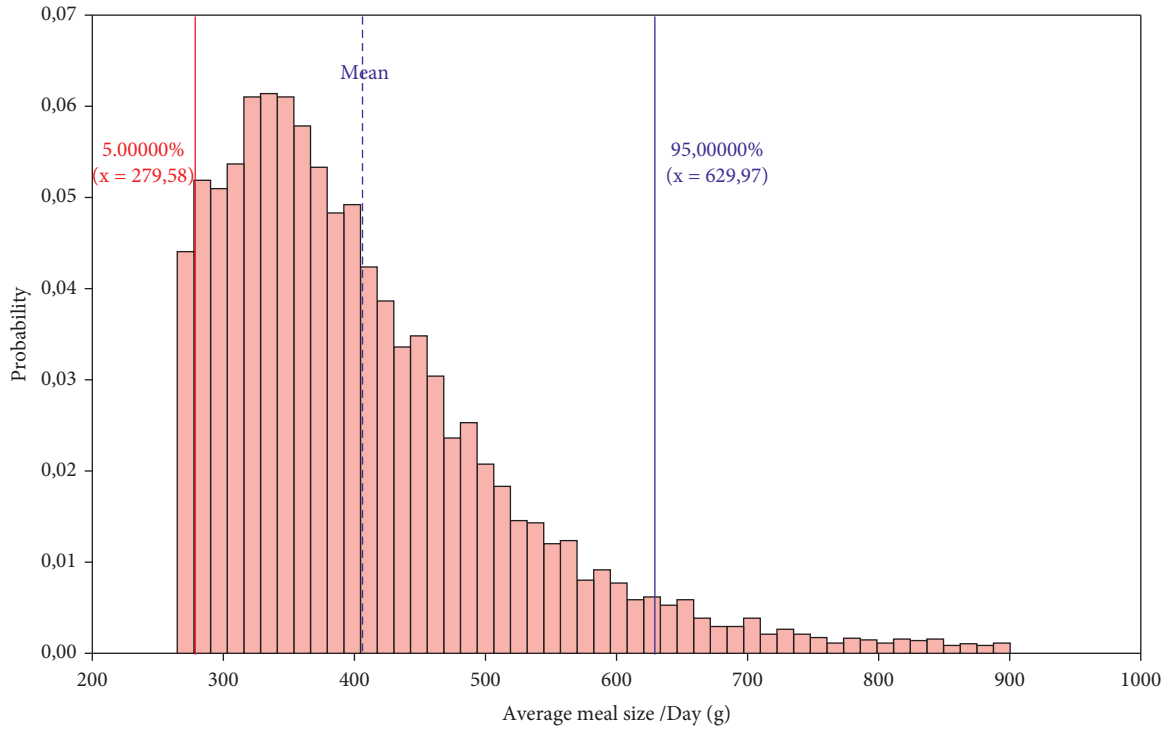

Figure 3: Simulated distribution of the daily average meal size.

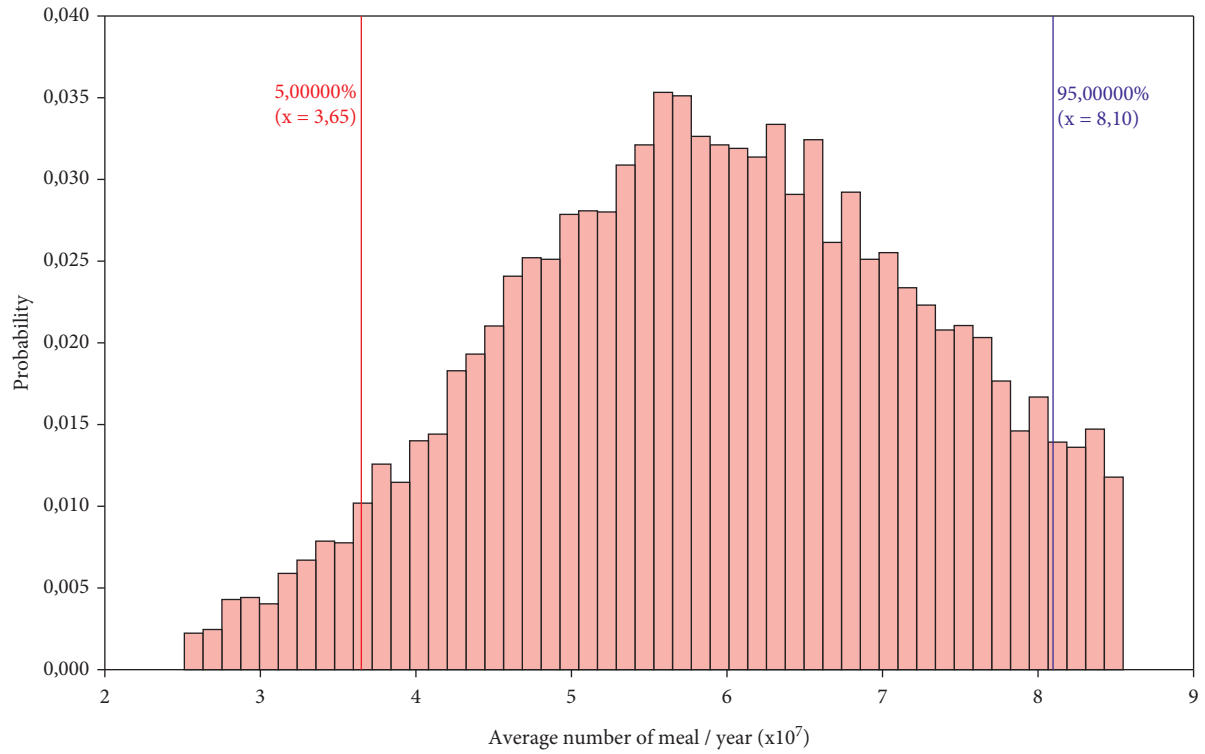

Figure 4: Simulated distribution of the average number of meal per year.

and between 2.62×10^{-6} and 3.76×10^{-6}, respectively, with mean values of 2.95×10^{-7} and 2.96×10^{-6}, respectively. It was also observed that an increase in storage time from 1 to 3 h (conditions 1 and 3, respectively) increased the risk of infection per day by 1.97 times. Increasing the quantity of food per day as well (number of shrimp/plate and number of plates/day) in conditions 11 and 12, the risk of infection per day is multiplied by 1.28 times.

Table 6: Different combinations reflecting the minimum, average, and maximum exposure and probability of infection scenarios during daily consumption.

	Conditions	Number of shrimp/plate	Number of plates/day	Cooking time (min)	Storage time (h)	Intake/day (cells)	Risk of inf/day
Minimal risk condition	1	2	1	30	1	17.15	$3.41E-07$
	2	3	1	30	1	26.87	$3.83EE-07$
	3	2	1	30	3	32.15	$5.75E-07$
	3	2	2	30	1	33.71	$6.57E-07$
	5	3	1	30	4	37.65	$6.31E-07$
Average risk condition	6	2	2	20	4	47.76	$8.61E-07$
	7	4	1	20	5	62.91	$1.03E-06$
	8	3	2	20	5	76.56	$1.31E-06$
	9	3	2	20	6	88.80	$1.54E-06$
	10	3	2	20	7	95.84	$1.90E-06$
Maximal risk condition	11	5	2	10	9	121.18	$1.20E-06$
	12	7	3	10	9	171.40	$3.15E-06$
	13	6	3	10	10	192.74	$2.27E-06$
	14	7	3	10	10	204.92	$3.35E-06$
	15	7	3	10	11	219.47	$3.18E-06$

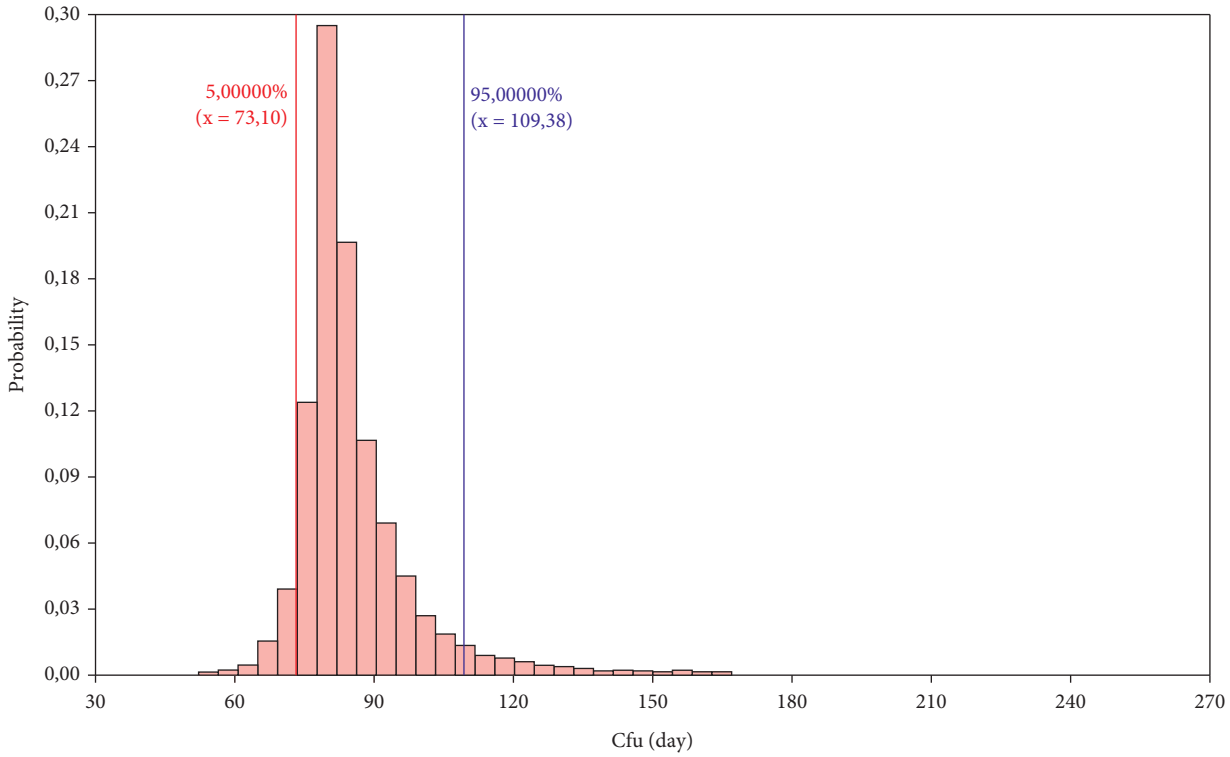

Figure 5: Simulated distribution for exposure trend of the majority of households.

Figure 6 represents the distribution simulation of the risk of infection to which consumers with modal behaviours are exposed. It was observed that 90% of the predicted risk values were between 11.96×10^{-7} and 17.79×10^{-7} with an average of 1.41×10^{-6}. In general, an average of 14 people in a ten million is likely to contract gastroenteritis following the ingestion of their meal.

Annual distributions of disease cases were simulated at minimum, maximum, and modal trend behaviour scenarios. Ninety percent of the simulated disease cases per year for minimum, maximum, and general risk scenarios are between 0.36 and 0.90 cases (minimum risk scenario), between 2.33 and 23.6 cases for maximum risk scenario, and between 1.74 and 4.28 cases for modal risk scenario (Figure 7). From the average value of the distribution, it can be noted that about 3 consumers are likely to suffer from gastroenteritis in a year.

4. Discussion

According to the literature we could access, no data related to *V. parahaemolyticus* toxi infection has yet been reported in Cameroon, unlike in other African countries

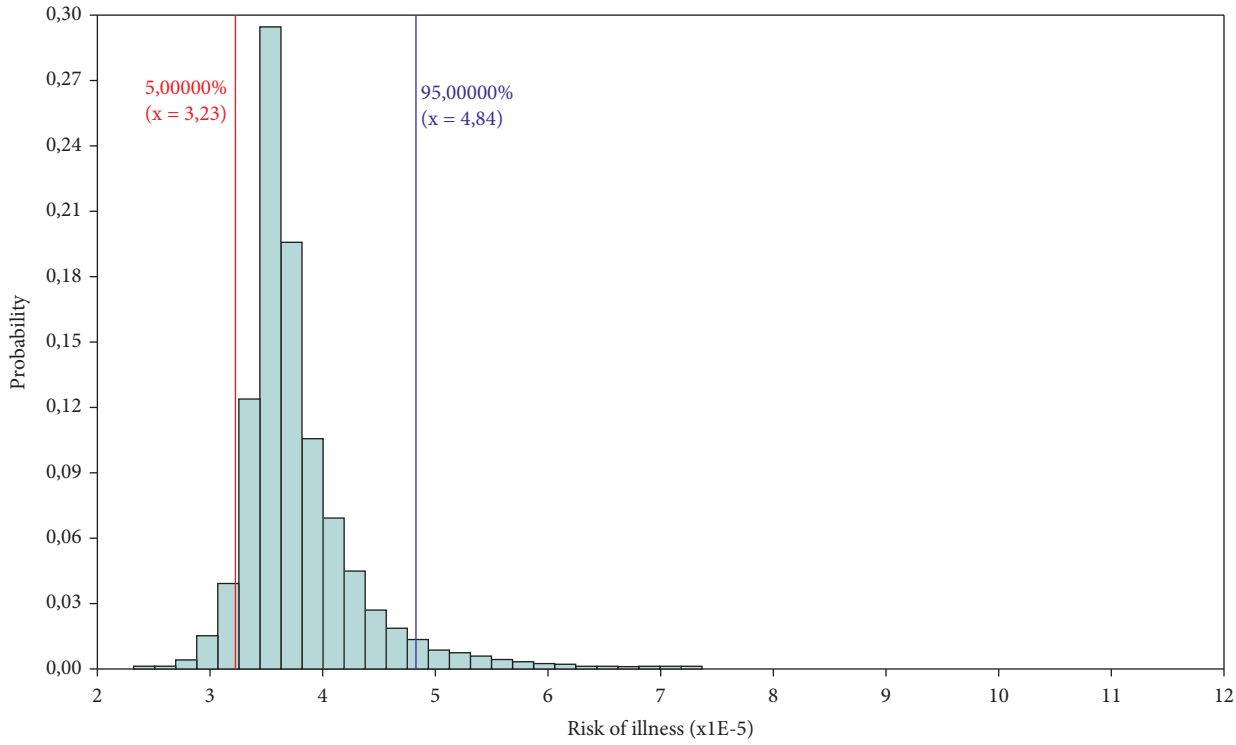

Figure 6: Simulated distribution for risk of illness for consumers having modal behaviors regarding the factors studied.

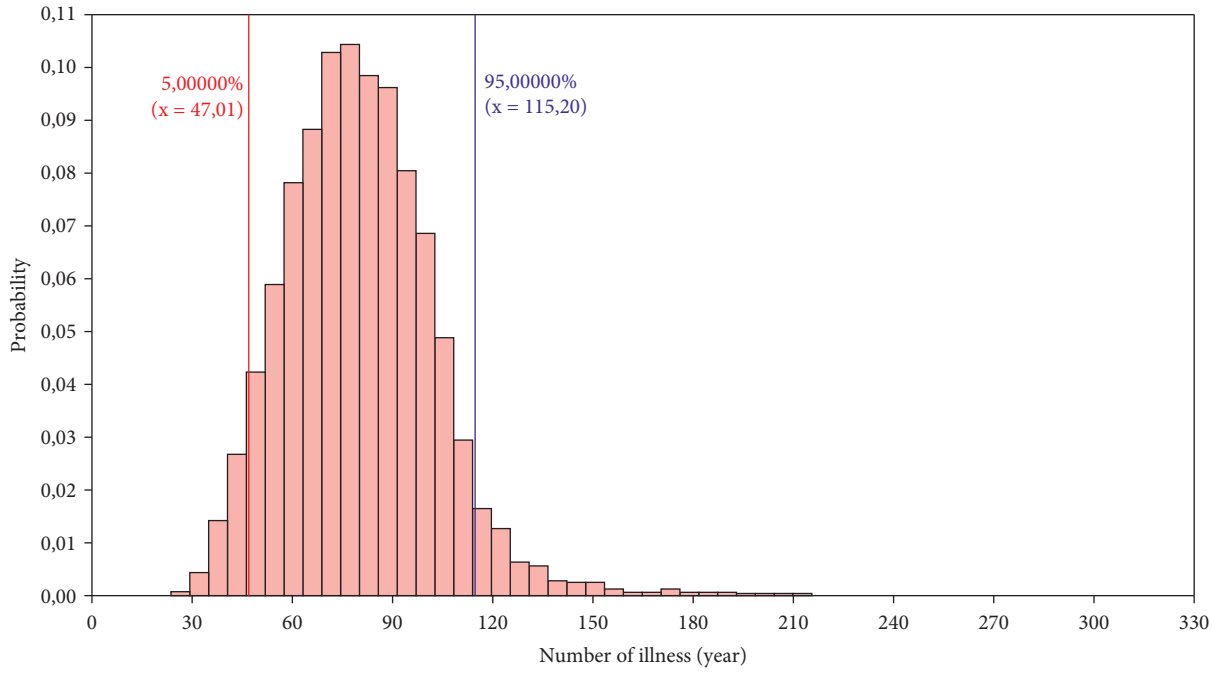

Figure 7: Simulated distribution for the number of illness per year for Cameroonian.

[9–11] and the world [1, 13, 14, 38]. This does not exclude the fact that the problem may exist. The risk quantification in this work is based on the results of the survey and the predictive mathematical model proposed with several scenarios representing minimum, average, maximum, and modal-consumer behaviours. The values obtained by simulation on the basis of 10,000 iteration risk show that the exposure is proportional to the initial load, the size of the meal, and the storage time but inversely proportional to the cooking time. In the same light, the risk of infection and the number of gastroenteritis cases follow these same trends. In fact, the higher the exposure, the higher the risk of infection, and the greater the number of disease cases [1, 12].

In this study, it was generally noted that the Cameroonian population consuming the product has an average exposure of 86 cells/d with an average risk of infection of 1.41×10^{-6} and a number of annual gastroenteritis cases estimated at 3 cases/year based on annual shrimp production. This shows that the risk is low on the Risk Ranking scale proposed by Summer and Gallagher [43], and thus, there is a low incidence of the disease. In Australia in 2009, *V. parahaemolyticus* was downgraded from the second category (intermediate) of the Risk Ranking for the hazards associated with seafood [43] to the first category (low) [44]. Abdullah et al. [12] also observed a low risk associated with cooked black shrimp in Malaysia. Indeed, to induce a disease with a 100% probability, an exposure value of 10^6 CFU/meal is required [14], which is not achieved even in the maximum exposure scenarios of this study. As indicated in the hazard identification part of this study, the choice of using total *V. parahaemolyticus* was deliberate. For comparison's sake, simulation based on the same worst scenario (Table 6, condition 15), taking into consideration total *V. parahaemolyticus,* 8%, and 15% pathogen species incidences, has led to the following infection risk: 3.18×10^{-6}, 2.16×10^{-7}, and 2.58×10^{-7}, respectively. These simulation results indicate that extrapolating haemolytic carrier percentage species obtained from other regions in this context would result in a ten-fold reduction of the risk. The influence of variables on the risk of infection per day and the observed shifts due to modulation of the studied factors indicate that these are possible ways of implementing mitigation policies. The risk model obtained can be implemented in a simple excel sheet in order to help decisions-makers simulate different patterns of cooking and handling of shrimp, and this will orientate risk communication.

This study was performed while accepting some limitations. Firstly, the use of the Baranyi and Robert model to adapt our growth data with respect to the 3 isolates of *V. parahaemolyticus* and the use of the estimated parameters to predict their initial concentration in shrimp before enrichment. In a dilemma, the choice that could lead to little overestimation of the risk was preferred. Secondly, the dose-response relationship of *V. parahaemolyticus* was based only on previous studies on a small number of volunteers (20 people) and modified to fit US CDC statistics. The bacteria load was administered to the volunteers with a pH-neutralizing buffer rather than with a food matrix [21]. Thirdly, the exposure assessment model was predicted from total *V. parahaemolyticus* (with and without virulent genes) in roasted shrimp.

5. Conclusion

For the Cameroonian population consuming roasted *Penaeus monodon*, the mean estimated exposure trend for most households is 86 cells/day. 90% of the predicted values are between 73 and 110 cells/day. 90% of the risk of infection to which most households are exposed falls between 11.96×10^{-7} and 17.79×10^{-7} with an average of 1.41×10^{-6}. The estimated number of annual disease cases based on annual production is between 1 and 10 cases.

This hazard characterisation indicates that roasted shrimp have a very low incidence of *V. parahaemolyticus* health effects on populations. This hazard could be further reduced by controlling roasting temperature as well as the storage conditions before consumption.

Data Availability

The data used to support the findings of this study are included within the article. Untransformed data are available on demand to the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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Supplementary Materials

Graphical abstract: the time of roasting and conservation of shrimp is very variable in Cameroon. This work evaluates the risk of illness caused by *Vibrio parahaemolyticus* after eating roasted *Peneaus monodon*. The estimated number of annual disease cases based on annual production is between 1 and 10 cases. (*Supplementary Materials*)

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