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Quantitative Assessment of the Risk Linked to the Consumption of Braised Beef Meat "*Choukouya*" Contaminated with Pathogenic *Clostridium perfringens* in Côte D'Ivoire

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Abstract

Inadequate handling, processing and sales of meat products have resulted in microbial contamination responsible for most infectious human food-borne illness. The study conducted a stochastic assessment of the risk of infection linked to the consumption of braised beef meat "*Choukouya*" contaminated by *Clostridium perfringens* in Côte d'Ivoire. We conducted "*Choukouya*" consumers (n = 900) and vendors (n = 300) survey to characterize the actors behavior. 189 samples of "*Choukouya*" were collected and microbiologically analyzed according to French standard protocols NF V 08-061 and XPV 08-061. A risk model was developed and the risk of infection linked to the consumption of "*Choukouya*" was estimated by Monte Carlo simulation procedure. The consumer's surveys showed that the percentage of population consuming "*Choukouya*" was 74.4%, with an average consumption of 114.3 ± 0.5 g/person/intake. The microbiological analysis revealed the isolation of 70% of *Clostridium perfringens* in the vegetative form and 60.3% in sporulated form with an average loading of 3.7 ± 2.6 $\log_{10}$·cfu/g and 1.1 ± 1.0 $\log_{10}$·cfu/g respectively. The probability of ingesting a dose greater than 10^9 bacteria of *Clostridium prefringens* varied between 7.36% and 7.93%. The braised beef meat "*Choukouya*" sold in the streets of cities in Côte d'Ivoire represents a real risk of infection, and *Clostridium perfringens* is one of the causes. This risk could be mitigated by the establishment of good hygiene practices and adequate handling processes in this informal sector.

Keywords

Braised Beef Meat "*Choukouya*", Inadequate Handling, *Clostridium perfringens*, Risk, Simulation Monte Carlo

1. Introduction

Bovine meat is of nutritional value, given the good bioavailability of zinc, iron, essential amino acids and vitamin B_{12} [1] Its composition in these nutrients, especially in essential amino acids, gives bovine meat an important place in the nutritional balance of the human being [2]. It is eaten in several forms, the dried form *(Kilishi),* is much more commonly found in Mali, grilled, cooked with water and braised, or the barbecue type named "*Dibi*" in Senegal, "*Tchatchanga*" in Benin *and* "*Choukouya*" of meat in Côte d'Ivoire [3] [4]. The barbecue type or braised meat "*Choukouya*", is a dish made of different meats cooked outdoors by using live coals or woods. It is consumed by many populations in the world and is an important part of the diet of many developing countries [5]. In Côte d'Ivoire, this met called "*Choukouya*" is usually consumed by a large number of people during ceremonies such as weddings, baptisms and birthdays. It is much more popular during outings and night activities because of being part of foods sold on public roads also named "street food". The large proportion of this informal activity and the surveyed data on food-borne diseases associated with it, remains a threat and a major public health problem [6] [7].

Meat, in spite of its nutritional properties, is often prepared under hygienic conditions that are beyond official control, which is a source of microbiological contamination [8]. Poor selling conditions, involving frequent pre- and post-cooking operations, and inadequate cooking, contribute to the risk of contamination by pathogenic germs.

According to the National Food Safety, Environment and Labor Agency, the main microorganisms responsible for food-borne illnesses are *Staphylococcus aureus, Salmonella, Campylobacter, Yersinia enterocolitica, Clostridium perfringens* and *Bacillus cereus* [9]. In Côte d'Ivoire*, Salmonella, Clostridium, Staphylococcus and Escherichia coli* are germs found in foods [10] [11]. Thus, in the case of a protein-rich foods, *Clostridium perfringens* is the major bacteria that should be to be taken into account [12] [13]. Other authors [14] classified this species as the second most common causes of food poisoning in England [15]. Quantitative microbiological risk assessment related to various widely consumed food products has been carried out and published in different countries [16]. It was found that in Africa, and particularly in Côte d'Ivoire, very little interest is given to this sector given the tiny number of biological or chemical risk assessment studies carried out [8].

Braised beef meat "*Choukouya*" sold on the streets could be contaminated by some sporogenic germs due to its exposure to the open air on public road. This

source of microbial contamination poses a risk to the health of the consumers [17]. It is therefore necessary to know the level of risk associated with infection linked to the presence of *Clostridium perfringens* in "*Choukouya*" at the level of the exposed population. This study was initiated to assess the infection risk associated with the consumption of braised "*Choukouya*" contaminated with *Clostridium perfringens*. This assessment consists of obtaining a numerical value of the risk of the population facing the hazard [18].

2. Material and Methods

2.1. Study Area

This study was conducted from July 2013 to June 2015, in six major cities of Côte d'Ivoire namely Korhogo (North), Daloa (West), Bouaké (North-Central), Yamoussoukro (South-central), Abengourou (East), Abidjan (South and North). The sites were selected purposively, based on the importance of markets for provisioning of foodstuff to the populations and the helpfulness of actors (Vendors, consumers) of the bovine sector to participate in the study.

2.2. Questionnaire Survey

The questionnaire survey was conducted among consumers and vendors of "*Choukouya*" in the six (06) selected cities for the study. The questions included the quantity consumed, the frequency of consumption of braised beef "*Choukouya*" and the sales practices and conditions used. The interest of the survey was to gather informations to assess the impact of consumption of braised beef "*Choukouya*" on consumer health. In total, 1200 people comprising nine hundred (900) consumers and three hundred (300) vendors were interviewed.

2.3. Sampling of Braised Beef Meat "*Choukouya*"

Braised beef meat "*Choukouya*" samples were collected from 27 vendors of "*Choukouya*" on all sites. Three campaigns were carried out per site and a total of one hundred eighty-nine (189) samples were collected from the whole sites. For each campaign nine (9) samples of approximately 100 g per sample of braised beef meat were purchased from each vendor of "*Choukouya*". It should be underline that each sample bought and labeled (site, date and time of collection) is placed in a sterile bag "Stomacher". The ideal conditions to ensure the preservation of samples were a quick transport into a cooler containing cold packs, so as keeping the temperature under 5˚C. The maximum storage period of samples from point of sale to the laboratory where they undergo microbiological analyzes was 16 hours.

2.4. Laboratory Procedures

Once at the laboratory, 10 g of each sample were homogenized in 90 mL of sterile bacteriological peptone (Oxoid, Hampshire, Eng-land). Decimal dilutions (1:10) of all samples were made in buffered peptone water (BioRad) was carried

according to the [19] method. Enumeration of *Clostridium perfringens* was carried according to the [20] method. 0.1 mL of appropriate dilution (10^{-1} to 10^{-4}) was plated into a sterile Petri dish containing 15 mL of tryptone sulphite agar with neomycin (TSN), and incubated at 37˚C for 24 hours. To count sporulated forms according to the [21] method. 20 ml of the suspension are introduced into the screw tubes. The tubes undergo a thermal treatment of 80˚C for 10 minutes and are immediately cooled in an ice water bath. This process is used to eliminate vegetative forms and creates a thermal shock in so burst in the spores. From this suspension, a dilution of 10^{-1} was made and then, 0.1 mL of the obtained solution was inoculated in micronist plates containing each of the pre-prepared TSN medium (BioRad, Paris, France) poured at a rate of 15 mL per dish and incubated at 44˚C for 24 hours. Tryptone sulphite neomycin agar (TSN) that give black colonies are characteristic of the presence of *Clostridium perfringens*. The number of *Clostridium perfringens* per gram was calculated according to the European Standard [22]. The unacceptable microbiological limits for which the ready to eat Braised beef meat "*Choukouya*" samples were considered potentially hazardous in this study was based on standards for ready to-eat foods by [23].

2.5. Quantitative Risk Assessment for *Clostridium* Sulfite-Reducing Pathogenic Species

The use of reference pathogens is an accepted practice in the field of Quantitative Microbial Risk Assessment [24] [25] to represent the potential adverse health effects of each broader microbial group. Risk assessment of infection linked to the consumption of braised beef meat "*Choukouya*" was carried out according to the *Codex Alimentarius* approach [26]. It is a scientific approach carried out in four steps: hazard identification, hazard characterization, exposure assessment and risk characterization.

2.5.1. Hazard Identification (Pathogen)

Beef meat "*Choukouya*" ranked in category of foods sold on public roads has been associated with many serious diseases [6] [17]. Beef meat, can be contaminate by bacteria representative a risk to human health [11] [27] [28]. In this study, *Clostridium perfringens* was selected according to its probable presence in the contamination of the beef meat [12]. This product is characterized by a relatively high protein content ranging from 20 to 22 g, allowing rapid growth of the germ. *Clostridium perfringens* is slightly acidic and kept at temperatures between 15˚C and 50˚C. Its optimum pH is between 6.0 - 7.0, but certain strains continue to grow at pH ranged from 5.0 to 8.3 [12]. It can end up in beef dishes due to unsanitary environment, unsanitary conditions of preparation and type of packaging used [29]. These bacteria are identified as dangerous because they cause food-borne infections in the world, particularly in Côte d'Ivoire [30] [31]. Main symptoms of the disease are fever, vomiting, diarrhea but mortalities were identified in the elderly and young children [32]. In our study, risk describes the probability an infection after consumption of contaminated beef meat "*Choukouya*".

2.5.2. Hazard Characterization

The characterization of the hazard allows to assess qualitatively and quantitatively the nature of the adverse health effects associated with the pathogen present in the food. This step provides a description of the severity of the effects resulting from the ingestion of the hazard in the food. Establishment of the dose-response relationship or the relationship between the level of the hazard exposure and the level of a toxic effect should allow knowing the infective dose or disease [33]. The maximum concentration of the bacteria in food or culinary preparations at 10^5 cfu/g, concentration from which there is likelihood of multiplication in the small intestine of the host, sporulation and enterotoxin production, knowing that the number of *Clostridium perfringens* in the digestive content is low between 10 to 10^3 bacteria per gram. According to the literature, the infectious dose which is the dose - effect is 10^8 to 10^9 bacteria per gram. Thus, the ingestion of food containing 10^8 and 10^9 or more of viable vegetative *Clostridium perfringens* cells can result in food poisoning causing electrolyte leakage and necrosis [31] [34].

2.5.3. Exposure Assessment

A stochastic methodology was used to estimate infection from pathogenic microorganisms through ingestion of product [35]. Individuals are assumed to ingest amount of product meat beef "*Choukouya*".

Representative diagram of the evaluation model

The step of exposure assessment is the quantitative or qualitative estimation of the likely ingestion of biological or chemical hazards through food [26]. The exposure estimation is based on consumption scenarios of contaminated food (**Figure 1**). The realization of these scenarios takes into account various identified parameters identified to the risk to be evaluated. After cooking, which is usually done at high temperatures between 100˚C and 120˚C, "*Choukouya*" is exposed ambient temperature (27˚C to 32˚C). In Most of cases, it is consumed immediately after cooking or after exposure time in contact with the environment which could lead to cross contamination and recontamination. All these reasons allow to suppose that the contamination of the "*Choukouya*" occurs at the time of its sale [36]. The exposure assessment consists of estimating the level of danger to which the consumer is exposed at the time of consumption of the food. Thus, the concentration *(C)* of *Clostridium perfringens* in the "*Choukouya*" could be supposed to be equal to its initial concentration (C_i) at the time of its sale. Into these conditions, the exposure assessment was to estimate the dose *(I)* of the bacteria in its vegetative and sporulated form ingested by the consumer at each "*Choukouya*" intake.

Mathematical model of ingested dose

Stochastic modeling requiring probability distributions was used for the exposure assessment. Thus, a diagram showing all the events that can lead to infection in the consumer has been established. In our study, exposure or Ingested dose (D_i) of *Clostridium perfringens* in "*Choukouya*" was estimated using

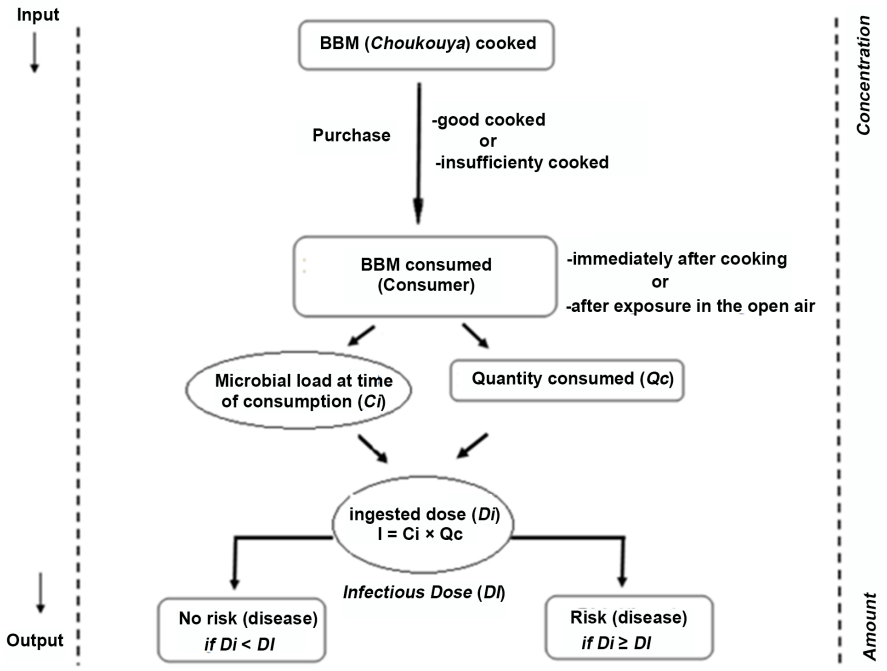

Figure 1. Fault tree on the infection risk with braised beef meat (BBM) "*Choukouya*".

the parameters of pathogen concentration in "*Choukouya*" (C_i), the quantity of "*Choukouya*" consumed (Q_c).The number of the germs ingested *(I)* by the consumers expressed as Colonies Forming Unit (cfu) was calculated as follow:

$$I = C_i \times Q_c$$

I: Ingested dose of the pathogen in braised beef meat "*Choukouya*"

C_i: Concentration of the pathogen in braised beef meat "*Choukouya*" in (cfu/g)

Q_C: Amount consumed from braised beef meat "*Choukouya*" (g/person/intake)

The use of this equation requires data on the concentration of *Clostridium perfringens* (expressed in cfu/g) in the braised beef meat "*Choukouya*" sold in the popular streets of the selected cities for the study and survey data consumption of a consumer population. The data collected on these two parameters each gave a distribution reflecting their uncertainties. The uncertainty on the parameters of the model was quantified by the bootstrap method [37]. Parameter values were estimated on the basis of laboratory and survey results. The distribution of the ingestion of the pathogen by the consumers is obtained after the calculations. Monte Carlo simulation was performed for 1500 iterations using the MATLAB R 2015a software. Following the simulations, cumulative probabilities of realizations or probabilities are obtained.

2.5.4. Risk Characterization

The characterization incorporates the results of hazard identification, hazard characterization and exposure assessment. Based on this results, the risk characterization has to quantitatively estimate the severity (*i.e.* probability) of adverse

effects caused by *Clostridium perfringens* on health of consumers.Thus, based on the distribution of the contaminant intake obtained by the Monte-Carlo method, the value of the infectious dose superior than 10^8 or 10^9 bacteria per gram is reported for the estimation of the probability or the risk of developing one infection per 100,000 inhabitants exposed [38].

2.6. Statistical Data Analysis

The sociological survey data were entered on Excel and analyzed with the Statistica software 7.1. Data collected for the microbiological quality of braised beef meat "*Choukouya*" were analyzed using analysis of variance (ANOVA) with two factors (sampling site and campaign). The difference in Factor is statistically significant at 5% level (two-tailed test) if the probability (or p-value) is less than 0.05. The comparison of means was done using LSD's post hoc test, to determine the source of the significant variations. Concerning the risk assessment, the infection risk associated with the consumption of braised beef contaminated with *Clostridium perfringens* was carried out by the stochastic method. The Bootstrap method was used to resample the input data of the exposure assessment model (i.e. the data of the concentration of *Clostridium perfringens* (C_i) in braised beef meat "*Choukouya*" and the data of consumption from the survey of household). Then the Monte Carlo simulations were performed to obtain the distribution of the exposure assessment model which is also the ingested dose of *Clostridium perfringens*. Fifteen hundred iterations were performed for each simulation creating two thousand cumulative frequency distribution curves for the ingested dose [39]. Bootstrap and Monte Carlo type simulations were performed by the software MATLAB R 2015a.

3. Results

3.1. Risk Factors for Contamination of Braised Beef Meat "*Choukouya*"

The braised beef meat "*Choukouya*" consumed on the different sites are subject to packaging. The survey identified the types of conditioning and devices used. Thus, braised beef meat "*Choukouya*" is predominantly store in cement packaging (49.3%) against 19.6% for aluminum foil (**Table 1**). At the point of sale, some customers consume this dish on the spot are often served either in plastic plates (31.3%) exposed to the open air. On the other hand, it was observed that the different points of sale were located in poorly maintained environments with the majority of the product exposed to the open air (82.5%). Other points of sale are protected in showcases (17.4%). Vendors wearing blouses accounted only for 23.3%.

3.2. Average Load of *Clostridium perfringens* of Braised Beef Meat "*Choukouya*" at Sites

The results of the microbiological analysis closed to *Clostridium perfringens* are shown in **Figure 2**. There has been a variability of *Clostridium perfringens*

Table 1. Contamination factor of braised beef meat *"Choukouya"* sold in the streets of cities in Côte d'Ivoire.

Contamination factors	Number (n = 300)	Frequency (%)
Type of utensil and packaging used		
Plastic plate	64	21.3
Aluminum plate	33	11.1
Cement packaging	148	49.3
Aluminum foil	55	18.3
Point-of-sale devices		
Exposure to the open air	248	82.5
Protection in show case	52	17.4
Wearing the blouse (Body hygiene)		
	Yes	No
	70 (23.3%)	230 (76.6)

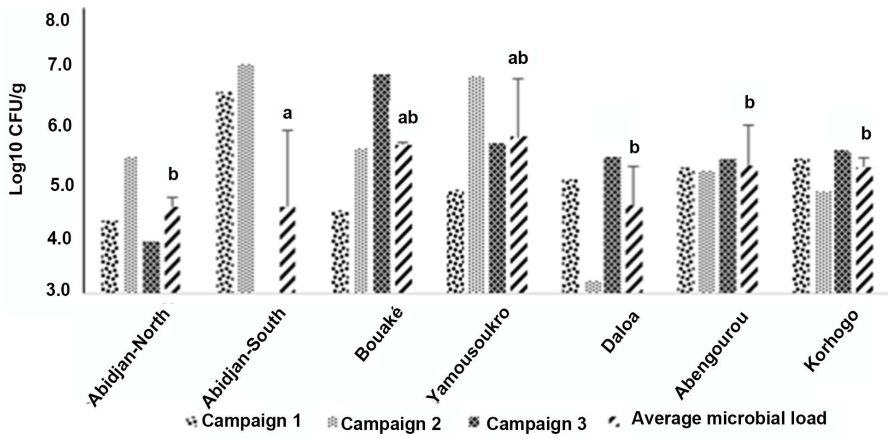

Figure 2. Average load of *Clostriduim perfringens* according to the origin of the braised beef meat *"Choukouya"*. Different letters in the same figure indicate highly significant differences ($p < 0.05$) between average microbial loads of the sites. Same letters indicate similarity between averages.

enumeration from one site to another and from one campaign to another. The average microbial loads of the bacteria are of 5.2 ± 0.3 $\log_{10}$·cfu/g for Korhogo, 5.2 ± 0.1 $\log_{10}$·cfu/g for Abengourou, 4.5 ± 1.1 $\log_{10}$·cfu/g for Daloa, 5.7 ± 1.0 $\log_{10}$·cfu/g for Yamoussoukro, 5.6 ± 1.2 $\log_{10}$·cfu/g for Bouaké, 4.5 ± 3.9 $\log_{10}$·cfu/g for Abidjan-South, 4.5 ± 0.7 $\log_{10}$·cfu/g for Abidjan-North. Analysis of the average microbial loads for the three campaigns indicates that *Clostridium perfringens* is higher than the unacceptable microbiological limit ($M = 10^4$ cfu/g) at all studied sites. The highest average loads were found at the Abidjan-South; Bouaké and Yamoussoukro while the lowest were found at Abidjan-North, Daloa, Abengourou and Korhogo respectively. The statistical analysis revealed a significant difference ($p < 0.05$) between Abidjan-South and the cities of Korho-

go, Abengourou, Daloa, and Abidjan-North which are statistically similar.

Table 2 shows the probability (p-value) associated to the average loads of bacteria for each considered parameter. At the sampling sites, for braised beef meat, the p-value is less than 0.05. It was therefore deduced that there was a significant difference between average microbial loads of *Clostridium perfringens* from one sampling site to another. No significant differences in mean of *Clostridium perfringens* loads were observed between campaigns. Similarly, at the level of interactions, the analysis of p-value shows that there is no interaction effect between the sampling site and the campaigns for the enumeration of *Clostridium perfringens*.

3.3. Beef Meat Consumption Patterns

The survey revealed an average amount of 114.3 g of beef consumed per person per intake. Regarding beef meat consumption patterns, the most appreciate one is its braised form "*Choukouya*" (74.3%) against 25.6% for the cooked form in sauce. Among consumers of braised beef meat, 12.8% consume it more than three times a week. The highest proportion of consumers (47.5%) was the young people (20 - 30 years). However, braised beef meat "*Choukouya*" is consumed by all age classes Table 3.

3.4. Risk of Infection LInked to the Consumption of Braised Beef Meat "*Choukouya*" Sold on Public Roads According to the Stochastic Method

3.4.1. Proportion of *Clostridium perfringens*

Clostridium perfringens was isolated in braised beef meat. The samples contained vegetative forms and sporulated forms in proportions of 70% and 60.3%, respectively with average loads of 3.7 ± 2.6 $\log_{10}$·cfu/g for vegetative forms and 1.1 ± 1.0 $\log_{10}$·cfu/g for the sporulated form (Table 4). These loads of contamination are superior to 10^5 cfu/g, the critical threshold for ready-to-eat foods.

3.4.2. *Clostridium perfringens* Concentration

The results of the distribution of the different concentration levels of *Clostridium perfringens* in the braised beef samples are shown in Figure 3. The levels of concentration varied from 10^2 cfu/g to 10^8 cfu/g. The analysis of Figure 2 shows that 69.84% of the 189 samples have levels of contamination higher than 10^5 cfu/g, which is the maximum concentration in ready-to-eat foods. Thus, about 70 portions contain doses of *Clostridium perfringens* which, after

Table 2. Tests of significance giving the probability (p-value) to accept means equality

Parameters	*Clostridium perfringens*
Sites	0.045
Campaigns	0.252
Sites* Campaigns	0.104

Table 3. Consumption patterns of braised beef meat "*Choukouya*".

Variable	Characteristic	Frequency (%)
Consumption mode	Meat cooked in braised	217 (25.6)
	Meat cooked in sauce	630 (74.3)
Frequency of consumption of braised meat	Occasionally (one time/month)	265 (42)
	Every 2 weeks	146 (23.1)
	Weekly	142 (22.5)
	2 or 3 to 4 times/week	81 (12.8)
Person who consume purchased meat	youngest (>20 years)	41 (6.5)
	Young people (20 - 30 years)	299 (47.5)
	Adults (30 - 40 years)	271 (43)
	Elderly (50 years and over)	21 (3.3)
Average quantity of braised meat consumed per person per part (g)		
114.3 g/person/intake		

Table 4. Proportion of braised beef meat *"Choukouya"* contaminated with *Clostridium perfringens.*

Clostridium perfringens.	BBM "*Choukouya*" contaminated	
	vegetative form	sporulated form
Number and percentage (%) of contaminated samples	132/189	114/189
Contamination rate	70%	60.3%
Average load (log_{10}·cfu/g)	3.7 ± 2.6.	1.1 ± 1.0.

ingestion, can lead to a multiplication and thus enterotoxin production. Therefore, for 100 servings of "*Choukouya*" consumed, 70 servings may present a risk of food poisoning from *Clostridium perfringens* to human health (**Figure 3**).

3.4.3. Consumption of Serving of Braised Beef Meat "*Choukouya*"

Nine hundred (900) individuals were interviewed during the consumer survey. The results of the evolution of the distribution of the consumption of "*Choukouya*" by the population of the different sites were treated and then expressed in frequency (%) for the different classes of serving during consumption per intake (**Figure 4**). The minimum serving size ranges from 50 to 100 g. The maximum serving weight ranges from 1000 g to 1050 g. Thus, the size of the most consumed portion which is the mode is between 100 and 150 grams per person per intake and corresponding to a frequency of 20.8%.

3.4.4. Exposure Assessment

The result of the Monte Carlo simulation obtained the distribution of the exposure assessment model represented by a cumulative frequency distribution curve for the ingested doses. One thousand five hundred (1500) simulations were

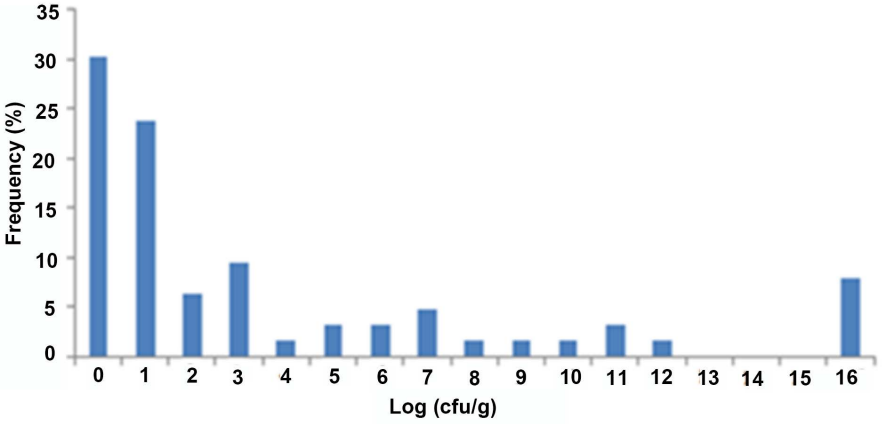

Figure 3. Distribution of different concentrations of *Clostridium perfringens* inservings braised beef meat "*Choukouya*".

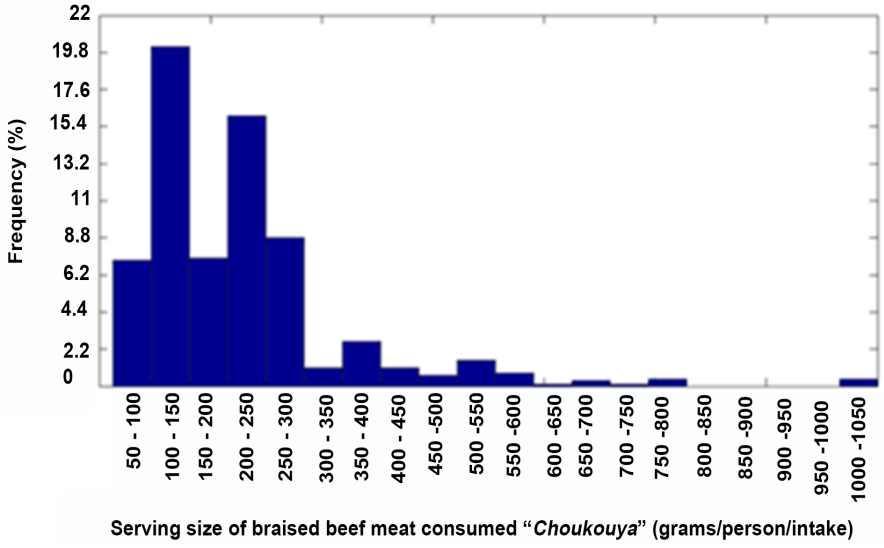

Figure 4. Distribution of consumption of braised beef meat "*Choukouya*".

carried out, ie one thousand five hundred (1500) curves of ingestion doses (**Figure 5**). This figure gives the probability Y, in an uncertainty range, with an ingested dose of *Clostridium perfringens* (expressed in cfu/g) below a limit value X. The infectious dose of *Clostridium perfringens* which can cause food poisoning by ingesting an amount of braised beef meat "*Choukouya*" is estimated to be more than 10^8 cfu/g or 10^9 cfu/g. Assuming 10^9 bacteria as infectious dose, the probability of developing food poisoning due to *Clostridium perfringens* by a consumption of serving of braised beef meat "*Choukouya*" sold in streets of cities in Côte d'Ivoire is at minimum 7.36% and maximum 7.93%.

3.4.5. Risk Characterization of *Clostridium perfringens*

The critical threshold for infection is more than 10^8 bacteria/g. The risk of developing food poisoning due to *Clostridium perfringens* by a consumption of serving of braised beef meat "*Choukouya*" being at minimum 7.36% and

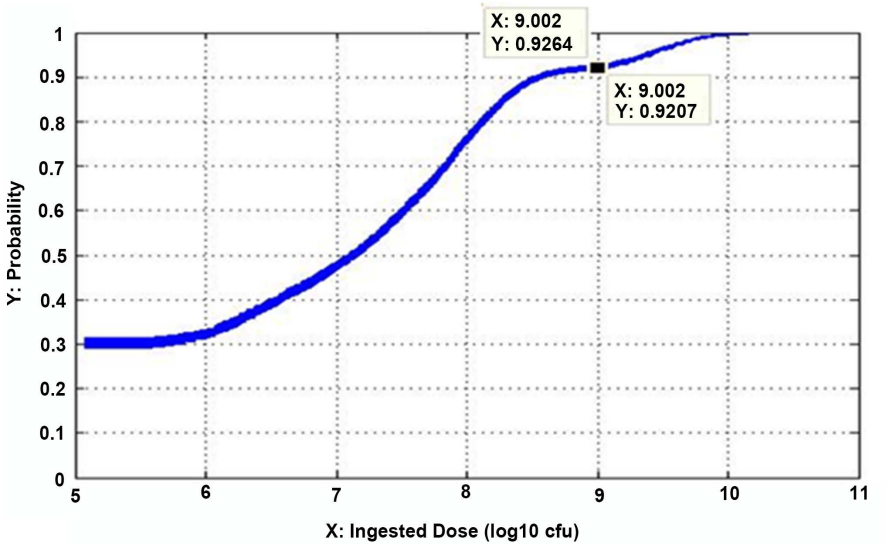

Figure 5. Cumulative probability of ingested doses of *Clostridium perfringens.*

maximum 7.93% allows us to deduce 7360 and 7930 cases of food-borne infection due to *Clostridium perfringens* for 100,000 inhabitants consuming contaminated braised beef meat "*Choukouya*". This risk estimate represents the quantitative estimate of the probability and severity of adverse effects sequel the consumption of braised beef meat "*Choukouya*".

4. Discussion

The evaluation of the risks associated with the consumption of braised beef meat "*Choukouya*" sold in the streets of the cities of Côte d'Ivoire was carried out through analysis of the contamination of beef braised by *Clostridium perfringens* and consumer behavior of food. Various factors involve in the contamination of the "*Choukouya*". Packagings, unhygienic utensils, point-of-sale devices, wearing blouses. The majority of sellers of "*Choukouya*" (49.3%) used inadequate packaging such as cement packaging. The latter constitutes a risk of multiplication of germs. The latter constitutes a risk of multiplication of germs including some pathogen such as *Clostridium perfringens* before and after cooking. Concerning the wearing of the blouse by the vendors, only 23.3% of the vendors of "*Choukouya*" respected this rule. All these actions result in intensive and direct contact with the product. Thus, the possibilities for cross contamination between packaging, utensils, vendor hands and product is unavoidable [40]. The practices of vendors at the point of sale are at the origin of the contamination, the growth and the bacterial alteration of the product [41] [42]. The level of contamination by *Clostridium perfringens* in the braised beef meat "*Choukouya*" sold on streets of Côte d'Ivoire differs according the selected cities for the study. This variability would reflect poor practices in these areas and could show that the sampling sites have different environments that promote contamination. All sampling sites had average loads of *Clostridium perfringens* beyond unacceptable microbiological limits 10^4 cfu/g. indeed *Clostridium perfringens* are

ubiquitous bacteria spread in the environment, contamination followed by proliferation of this bacteria could be inevitable. Cooking and reheating after cooking is a mean of preserving the microbiological quality of the food. However, they constitute risk factors for contamination. Thus, *Clostridium perfringens* is present in the vegetative form in 70% of the samples analyzed and 60.3% in sporulated form. These results would be close to the work of [43] on cooked skewers sold in streets of Abidjan. During their studies, these authors found *Clostridium perfringens* in vegetative (76.40%) and sporulated (83.14%) forms. Indeed, braised beef insufficiently cooked or badly reheated favors the development of spores of *Clostridium perfringens* [44]. Also, the dust generated by the circulation could constitute a major vector of contamination by *Clostridium perfringens* [45], because it possesses telluric species that can contaminate food [46]. The survey revealed 74.3% of people who preferred to eat beef meat in the braised "*Choukouya*" form. In this form, beef meat is consumed three to four times a week by 12.8% of people but much more by young people 47.5% followed by 43% of adults. Indeed, this dish is highly appreciated by the Ivorian population with an average consumption of 114.3 ± 0.5 g per person per intake. This strong appreciation is due to the many entertainment activities, the presence of refreshments and other relaxation sites observed in the main cities of the country. These activities are supported by the strong demographic growth observed in these cities [47]. In view of the above consideration, a quantitative microbiological risk assessment was carried out in accordance with the approach of the *Codex Alimentarius* method [48]. Thus, *Clostridium perfringens* was chosen as a danger because of its sporulation ability and virulence. The quantitative risk assessment carried out in this study was based on a probabilistic approach for assessing exposure which was also the ingested dose for *Clostridium perfringens* in braised beef meat "*Choukouya*" consumption. This probabilistic method requires probability distributions to represent the variability or uncertainty of the parameters and leads to a probability distribution of risk and refines the interpretation of the results of the model [18] [49]. Thus, using Monte-Carlo simulations, it is possible to perform variable probability distributions when the model of each variable is determined [33] [50]. The model of the evaluation, was defined based on actual data collected on 2 parameters: the distribution of the concentration of *Clostridium perfringens* (C_i) and the distribution of the serving sizes (Q_C) of braised beef meat "*Choukouya*" consumed at each meal per day per individual. This method has been described by [36] in his study on the risk assessment of attiéké consumption. Then the concentration levels of *Clostridium perfringens* range from 10^2 cfu/g to 10^8 cfu/g with 69.84% servings of braised beef meat "*Choukouya*" higher than 10^5 cfu/g which is the maximum concentration of *Clostridium perfringens* in ready-to-eat foods. At this level of contamination, there is a possibility of multiplication of the contaminant with the production of enterotoxin [31] by the spores in the small intestine of the consumer. The consumer could therefore be exposed to a food-borne infection [51]. For 10^9 ingested bacteria, the individual risk of food poisoning due to *Clostridium per-*

fringens for consumption of portions of braised beef meat "*Choukouya*" per intake is obtained. Thus, after the Monte Carlo simulations, the results of the assessment of the infection risk associated with the consumption of "*Choukouya*" contaminated by *Clostridium perfringens* revealed that the risk varies between 7.36% and 7.93%, *i.e.*, 7360 and 7930 cases of *Clostridium perfringens* disease per 100,000 inhabitants. These results are high than those obtained by [36] in attièke sold on the open market. This risk appears relatively real and high compared to the risk assessment for certain germs found in other food matrices in developed countries. In their study on the assessment of the risk of *Clostridium perfringens* in hospital catering [52], by applying the microbial models, the simulations allowed to predict a low intake of *Clostridium perfringens* by the patients, which translates into a very weak risk. In Denmark, in 2001, at a concentration of *Campylobacter jejuni* between 0.5 and 1.5 log·cfu/g in a chicken meal, the individual risk was estimated to be 7.0×10^{-5} for the general population [53]. This high probability of developing a disease can be explained by the high proportion of samples exceeding the acceptable limits set by quality standards. Thus, the high bacterial loads of the "*Choukouya*" observed at the point of sale, the quantity of "*Choukouya*" consumed and the frequency of consumption are potential risk factors [54]. This risk can increase with the possibility of cross-contamination caused by the poor quality of packaging and utensils used for consumption in addition to its exposure to the open air. As a result, it has been noticed that in several outlets, the recurrent use of plastic utensils to rough surfaces and uncleaned with the presence of dust. In this case, a nest of microorganisms in general and *Clostridium perfringens* in particular, capable of forming biofilms on inert surfaces [55]. The association between braised beef "*Choukouya*" considered as food sold on public streets "street food" and food infections have been reported by several authors [6] [17] [56] [57]. Other authors have particularly cited *Clostridium* sulfite-reducing in certain food infections [58] [59]. Bacterial infections are frequently met in developing countries [31]. In Côte d'Ivoire, it was observed that consumers do not make a link between the food consumed and their pain felt. However, according to [50] diarrheal syndrome-related disorders lead to food-borne illness, which is similar to food infection caused by *Clostridium perfringens*. Therefore, the consumption of "*Choukouya*" under the conditions presented in our study could lead to consequences on the health of consumers. Informal sector actors need to be aware of the threat represented by the poor processing of meat dishes and good hygiene practices that are not respected at the point of sale. For this reason, the inspection of the public health authorities must be carried out in order to improve the conditions of sale and the quality of the products of this sector.

5. Conclusion

The study made it possible to highlight the poor hygienic quality of braised beef "*Choukouya*" sold in the popular streets of several cities in Côte d'Ivoire. The average microbial load of *Clostridium perfringens* of braised beef meat differs

significantly from one site to another. This dish, which is highly prized by the Ivorian population, represents a health risk for the consumer due to its high concentration found in the samples analyzed. Thus, the study evaluating the risk of infection with *Clostridium perfringens* linked to the consumption of braised beef meat "*Choukouya*" appears real and high. This risk can cause between 7360 and 7930 cases of disease per 100,000 inhabitants consuming the "*Choukouya*". These results should draw the attention of the authorities to an awareness of good hygiene and product manufacturing practices.

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References

[1] Duchène, C., Gérard, P. and Prigent, S. (2010) Les viandes aujourd'hui: Principales caractéristiques nutritionnelles. *Cahiers de nutrition et de diététique*, **45**, 44-55.

[2] Patureau, M.P. and Remond, D. (2001) Viande et nutrition protéique: Une place confortée par les nouvelles connaissances. *Viandes Produits Carnés*, **22**, 103-107.

[3] Dibi, E.A.D.B., N'goran, Z.E.B.A., Akmel, D.C., Tano, K. and Assidjo, N.E. (2017) Risques microbiens liés à la consommation de la viande bovine braisée "*Choukouya*" en Côte d'Ivoire. *International Journal of Innovation and Applied Studies*, **19**, 496-507.

[4] Huand, L. (2003) Estimation of Growth of *Clostridium perfringens* in Cooked Beef under Fluctuating Temperature Conditions. *Food Microbiology*, **61**, 4441-4447.

[5] Miller, M., Gravel, D., Mulvey, M. and Talor, G. (2010) Health Care-Associated *Clostridium difficile* Infection in Canada: Patient Age and Infecting Strain Type Are Highly Predictive of Severe Outcome and Mortality. *Clinical Infectious Diseases*, **50**, 194-201. https://doi.org/10.1086/649213

[6] OMS (2010) Mesures de base pour améliorer la sécurité sanitaire des aliments vendus sur la voie publique. Note d'information INFOSAN N 3/2010 Sécurité sanitaire des aliments vendus dans la rue, Genève, suisse, 6 p.

[7] Nyenje, M.E., Odjadjare, C.E., Tanih, N.F., Green, E. and Ndip, R.N. (2012) Foodborne Pathogens Recovered from Ready-to-Eat Foods from Roadside Cafeterias and Retail Outlets in Alice, Eastern Cape Province, South Africa: Public Health Implications. *International Journal of EnvironmentalResearch and Public Health*, **9**, 2608-2619. https://doi.org/10.3390/ijerph9082608

[8] Glazy, P. and Guiraud, J. (2003). L'analyse microbiologique dans les industries agroalimentaires. Ed de l'usine nouvelle, France: 223 p.

[9] ANSES (2013) Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. Mis à jour le 14/03/2013 site

https://www.anses.fr/fr/system/files/BIORISK2012sa0005.pdf Les toxi-infections alimentaires collectives (TIAC). Consulte le 06/01/2014.

[10] FAO/OMS (2005) Food and Agriculture Organization/World HealthOrganization. "Système national de securite sanitaire des aliments et ses impacts socio-economiques et sanitaires (préparé par la Cote d'Ivoire)". Document de séance 16, Conférence régionale sur la sécurité sanitaire des aliments pour l'Afrique. Harare, Zimbabwe, 3-6 October 2005, 1-6.
http://www.fao.org/docrep/meeting/010/a0215f/A0215F21.htm

[11] Koffi-Nevry, R., Judicael, A.C.B., Assemand, E.F., Wognin, A.S. and Koussemon, M. (2012) Origine de contamination fécale de l'eau d'arrosage de la laitue cultivée dans d'Abidjan. *Journal of Applied Biosciences*, **52**, 3669-3675.

[12] ANSES (2010) Fiche de description de danger microbien transmissible par les aliments: *Clostridium perfringens* Famille des Clostridiaceae.

[13] Rosset, P.H., Morelli, E., Noel, V. and Poumeyrol, G. (2011) Approche quantitative de l'évolution des dangers Comment évaluer les potentiels de croissance/destruction bactériennes. *Viande et Produits Carnes*, **28**, 15-20.

[14] Lindström, M., Kiviniemi, K. and Korkeala, H. (2006) Hazard and Control of Group II (Non Proteolytic) *Clostridium botulinum* in Modern Foodprocessing. *International Journal of Food Microbiology*, **108**, 92-104.

[15] Mari, N., Hielm, S., Lindstrom, M., Horn, H., Koivulehto, K. and Korkeala, H. (2002) High Prevalence of *Clostridium Botulinum* Types A and B in Honey Samples Detected by Polymerase Chain Reaction. *International Journal of Food Microbiology,* **72**, 45-52.

[16] Chen, Y., Jackson, K., Chea, F. and Schaffer, D. (2001) Quantification and Variability Analysis of Bacterial Cross-Contamination Rates in Commonfood Service Tasks. *Journal of Food Protection*, **64**, 72-80.
https://doi.org/10.4315/0362-028X-64.1.72

[17] Bendech, M.A.G. (2013) Alimentation de rue à Bamako au Mali: Problématique et approches d'intervention. *MSP*, **3**, 116-118.

[18] Delhalle, L., Saegerman, C., Farnir, F., Korsak, N. and Daube, G. (2008) L'évaluation quantitative du risque microbiologique: Revue de trois modèles liées à *Salmonella* dans les aliments. *Annales De Médecine Vétérinaire*, **152**, 116-129.

[19] AFNOR NF V 08-010 (1996) Microbiologie des aliments—Règles générales pour la préparation des dilutions en vue de l'examen. Analyse microbiologique recueil de normes françaises'. 6th Edition, Paris, 67-75.

[20] AFNOR Norme NF V 08-061 (2009) Microbiologie des aliments en anaérobiose des bactéries sulfito-réductrice. Decembre.

[21] AFNOR Norme NF XPV 08-061 (2009) Méthode de routine pour le dénombrement après incubation à 37˚C des Anaérobies Sulfito-réducteurs dans les produits destinés à la consommation humaine. 105 p.

[22] ISO 7218 (2007) Microbiology of Food and Animal Feeding Stuffs-General Requirements and Guidance for Microbiological Examinations. 3rd Edition, 66 p.

[23] CECMA (2009) Lignes directrice et normes pour l'interprétation des résultats analytiques en microbiologie. Comité sur l'élaboration des critères microbiologiques dans les aliments (CECMA), Quebec.

[24] Roser, D. and Ashbolt, N. (2007) Source Water Quality Assessment and the Management of Pathogens in Surface Catchments and Aquifers. Research Report 29, Cooperative Research Centre for Water Quality and Treatment.

[25] Schoen, M.E., Soller, J.A. and Ashbolt, N.J. (2011) Evaluating the Importance of Faecal Sources in Human-Impacted Waters. *Waterresources*, **45**, 2670-2680. https://doi.org/10.1016/j.watres.2011.02.025

[26] Codex Alimentarius Commission (2007) Principes et directives pour la gestion des risques microbiologiques. Food and Agriculture Organization of the United Nations, World Health Organization, Geneva, 12 p.

[27] Anonyme 1 (2010) Plan d'Action National de Sécurité Sanitaire des Aliments Ministère de l'Agriculture de la République de Côte d'Ivoire (MINAGRI). 1-48.

[28] Barro, N., Aly, S., Tidiane, O.C. and Sababenedjo, T.A. (2006) Carriage of Bacteria by Proboscises, Legs, and Feces of Two Species of Flies in Street Food Vending Sites in Ouagadougou, Burkina Faso. *Journal of Food Protection*, **69**, 2007-2010. https://doi.org/10.4315/0362-028X-69.8.2007

[29] Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. and Ablordey, A. (2002) Street Foods in Accra, Ghana: How Safe Are They? *Bulletin of the World Health Organization*, **80**, 546-554.

[30] Dosso, M., Coulibaly, M. and Kadio, A. (1998) Place des diarrhées bactériennes dans les pays en développement. *Bulletin de la Société de Pathologie Exotique*, **5**, 402-405.

[31] Johnson, E.A., Summanen, P. and Finegold, S.M. (2007) Clostridium. ASM Press, Washington DC, 889-910.

[32] Anonyme 2 (2011) Critère microbiologiques applicables aux denrées alimentaires: Lignes directrices pour l'interprétation. Ministère de la santé direction de la santé: Service de la Sécurité Alimentaire. Grand-Duche de Luxembourg, 49 p.

[33] Cornu, M., Bergis, H., Miconnet, N., Delignette-Muller, M.L. and Beaufort, A. (2003) Appréciation des risques microbiologiques. Vol. 1032, 33-42.

[34] Malintrop Afrique (2002) Manuel de maladies infectieuses pour Afrique. Edition John-Libbey Eurotext, Paris.

[35] Soller, J.A. and Eisenberg, J.N.S. (2008) Anevaluation of Parsimony for Microbial Risk Assessment Models. *Environmetrics*, **19**, 61-78. https://doi.org/10.1002/env.856

[36] Akmel, D.C., Aw, S., Montet, D., Assidjo, N.E., Degni, M.L., Akaki, D., Moretti, C., Elleingand, E., Brabet, C., Baud, G., Mens, F., Yao, B., Michel, T., Durand, N., Berthiot, L., Hubert, A. and Tape, T. (2017) Quantitative Assessment of the Microbiological Risk Associated with the Consumption of Attieke in Côte d'Ivoire. *Food Control*, No. 81, 65-73. https://doi.org/10.1016/j.foodcont.2017.05.035

[37] Efron, B. and Tibshirani, R.J. (1993) An Introduction to the Bootstrap. Chapman and Hall. https://doi.org/10.1007/978-1-4899-4541-9

[38] Dromigny, E. (2012) Les critères microbiologiques. Règlementation Agents microbiologiques—Autocontrôle. Technique et Documentation Lavoisier, Paris, 231 p.

[39] Food and Agriculture Organization/World Health Organization FAO/OMS (2004) Évaluations des risques liés à S*almonella* dans les œufs et dans les poulets de chair. Résumé interprétatif. Département de l'agriculture, Rome, 1-20.

[40] Ministère à la Santé Animale et de l'Inspection des Aliments MSAIA (2009) Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire.

[41] Barro, N., Sangare, L., Tahita, M.C., Ouattara Cheik, A.T. and Traore, A.S. (2005) Les principaux agents du péril identifiés dans les aliments de rue et ceux des cantines et leur prévalence en milieu hospitalier. Mémoire de Master, Université

d'Ouagadougou, 73 p.

[42] Daube, G. (2002) Micro-organismes pathogènes et viande: La traçabilité alliée de la sécurité. *Bulletin de la Société Royale des Sciences de Liège*, **71**, 11-30. http://popups.ulg.ac.be/0037-9565/index.php?id=1031&file=1&pid=1022

[43] Kouassi, K.A., Dadie, A.T., Nanga, Z.Y., Dje, K.M. and Loukou, Y.G. (2011) Prevalence of Sulfite Reducing *Clostridium* Species in Barbecuedmeat in Abidjan, Cote d'Ivoire. *Journal of Applied Biosciences*, **38**, 2518-2522.

[44] Vijay, K., Juneja, A., Huang, L., Harshvardhan, A. and Thippareddi, H. (2006) Predictive Model for Growth of *Clostridium perfringens* in Cooked Cured Pork. *International Journal Food Microbiology*, **110**, 85-92. https://doi.org/10.1016/j.ijfoodmicro.2006.01.038

[45] Simong, C. and Mwarkurudza, C. (2008) *Clostridium difficile* in Broiler Chickens Sold at Market Places in Zimbabwe and Their Antimicrobial Susceptibility. *International Journal of Food Microbiology*, **124**, 268-270. https://doi.org/10.1016/j.ijfoodmicro.2008.03.020

[46] Simango, C. (2006) Prevalence of *Clostridium difficile* in the Environment in a Rural Community in Zimbabwe. *Transactions of the Royal Society of Tropical Medecine and Hygiene*, **100**, 1146-1150. https://doi.org/10.1016/j.trstmh.2006.01.009

[47] Zoro, E.G. (2001) Apports de l'information géographique dans l'élaboration d'un indicateur de développement urbain: Abidjan et l'île de Montréal. Thèse Université de sherbrooke, 123 p.

[48] AFSCA (2005) Terminologie en matière d'analyse des dangers et des risques selon le Codex Alimentarius. PB 05-I 01-REV 0-2005-30, 46.

[49] Assidjo, E., Aw, S., Akmel, C., Akaki, D., Elleingand, E. and Yao, B. (2013) L'analyse des Risques: Outil innovant d'amélioration de la sécurité sanitaire des aliments. Revue Africaine de sante et production animales, Dakar.

[50] Thrusfield, M. (2007) Veterinary Epidemiology. 3rd Edition, BlackwellPublishers, Oxford, 610.

[51] Novak, J.S. and Yuan, J.T.C. (2004) In Creased Inactivation of Ozone-Treated *Clostridium perfringens* Vegetative Cells and Spores on Fabricated Beef Surface using Mild Heat. *Journal of Food Protection*, **67**, 342-346. https://doi.org/10.4315/0362-028X-67.2.342

[52] Jaloustre, S. (2011) Appréciation quantitative des risques pour l'évaluation de mesures de maitrise sanitaire dans une filière Agro-alimentaire. Application à *Clostridium perfringens* en restauration hospitalière. Thèse de doctorat, Université de Lyon Instituts des Sciences et Industries du vivant et de l'environnement (Agro Paris Tech) 276 Présentée et soutenue publiquement le 24 Octobre 2011, Paris.

[53] DVFA (Danish Veterinary and Food Administration) (2001) Risk Assessment on *Campylobacter jejuni* in Chicken Products. Janvier.

[54] Traoré, S.G. (2013) Risques de contraction des affections à *Vibrio*sp. et à *Paragonimus*sp. Liés à la consommation des crabes et des crevettes vendus sur les marchés d'Abidjan et de Dabou. Thèse de doctorat unique, Université NanguiAbrogoua, UFR des Sciences et Technologies des Aliments, 198 Soutenue le 23/07/2013, Abidjan, Côte d'Ivoire.

[55] Ankolekar, C. and Labbé, R.G. (2010) Physical Characteristics of Spores of Food-Associated Isolates of the *Bacillus cereus* Group. *Applied and Environmental Microbiology*, No. 76, 982-984. https://doi.org/10.1128/AEM.02116-09

[56] Cohen, N., Ennaji, H., Bouchrif and Karib, H. (2003) La qualité des viandes produites sur la grande Casablanca. 10 p.

[57] Jonkuvienė, D., Salomskienė, J., Zvirdauskienė, R. and Narkevicius, R. (2012) Determining Differences in Characteristics of *Bacillus cereus* Isolated from Various Foods. *Veterinarijairzootechnika*, **60**, 22-29.

[58] Food Safety and Inspection Service/Department of Agriculture FSIS/USDA (2002) *Clostridium perfringens* Spore in Raw Ground Beef Study. FSIS Docket No. 04-00IN. U.S. Department of Agriculture, Washington DC, 57 p.

[59] Songer, J.G. (2010) Clostridia as Agents of Zoonotic Disease. *Veterinary Microbiology*, **40**, 399-404. https://doi.org/10.1016/j.vetmic.2009.07.003