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# **Quantitative Risk Assessment for**  *Salmonella* **in Lettuce (***Lactuca sativa***) Consumed in Benin, West Africa**

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## *Authors' contributions*

*This work was carried out in collaboration among all authors. Author DSD designed the study, performed the microbial risk assessment and wrote the first draft. Author VECC participated in data collection and contributed to the improvement of the first draft of the manuscript. Author PA approved the study design and contributed to the improvement of the first draft of the manuscript. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The present study aims at quantitatively assessing the risk associated with *Salmonella* in lettuce (*Lactuca sativa*) consumed in Benin (West Africa). To that end, a survey was conducted involving 550 respondents to determine the conditions under which the product is handled along the supply chain and its consumption pattern. The prevalence and concentration of *Salmonella* in lettuce were collected from the literature. The consumption data and the data on *Salmonella* concentrations in lettuce were combined to estimate the exposure to *Salmonella* using a probabilistic risk assessment method. The @Risk software package (Palisade USA) was used to run Monte Carlo simulations with 10,000 iterations. Three dose-response models were used to assess the risk of salmonellosis. Different scenarios were tested to identify factors that could influence the risk of

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salmonellosis. The results showed that lettuce is exposed to temperature abuse under inappropriate hygienic conditions. In 90% of the cases, the exposure to *Salmonella* was between 3 and 7 log CFU/serving. The risk of salmonellosis per serving varied from 7.7% to 95% depending on the dose-response used with the scenario taking into account the current handling conditions of the lettuce. In contrast, when considering the scenario where the cold chain is respected along the supply chain, the risk of salmonellosis varied from 0% to 3.3% depending on the dose response used. The study highlights the importance of the cold chain, good agricultural practices and good hygienic practices to reduce exposure to *Salmonella* through the consumption of lettuce and thus the risk of salmonellosis.

*Keywords: Food safety; salmonellosis; cold chain; vegetables; good hygienic practices.*

## **1. INTRODUCTION**

In Africa, unsafe food and water contribute noticeably to health-related matters [1]. Of foodborne diseases, salmonellosis is one of the most frequently reported worldwide [2,3]. The disease is caused by a bacterial pathogen named *Salmonella* and may be characterized by gastroenteritis, septicemia, typhoid fever, and even death [4,5].

Although *Salmonella* is most frequently associated with food from animal origin [6], there is an increase report of food from non-animal origin, especially fresh produce involved in salmonellosis [7-9]. Since fresh produce is well recognized as important parts of a nutritious and healthy diet, its consumption has increased worldwide in recent years [7]. For example, vegetables production in rainfed upland ecologies is popular in West Africa, especially for urban and peri-urban areas [10]. Unfortunately, the increased consumption of fresh produce has led to an increase in the number of outbreaks especially those caused by *Salmonella* [11,12]. For example, *Salmonella* outbreaks related to lettuce in Europe and other developed countries have been documented [13-15]. The contamination of fresh produce may occur at the production sites, during its transportation, processing or handling [8,9]. Contaminated irrigation water and manure constitute the main sources for the preharvest contamination of lettuce [16]. In many developing countries, where sanitation in general and wastewater treatment in particular remain challenges, the risk of getting fresh produce contaminated by pathogens could be high [17]. Also, lettuce is consumed uncooked and therefore, may present a higher risk of salmonellosis compared to cooked vegetables. This risk should be managed to ensure the safety of consumers.

Microbial risk assessment (MRA) provides an objective, transparent, evidence-based assessment of the health risk of (different) exposure pathways/ scenarios [18]. MRA can also explore the potential control of foodborne illness [19] by providing structured information on the effect of potential interventions on the risk that can be implemented by decision makers of public health authorities or food industry [20,21]. Quantitative microbial risk assessment (QMRA) models for pathogens in vegetables production chains have been developed in various industrialized countries. Examples are QMRA models developed by [22] in USA, [8] in the Netherlands, [23] in Australia. To our knowledge, there is a lack of QMRA models for pathogens in vegetables taking into account the conditions that prevail in West African vegetables supply chains. Therefore, this study aims at quantitatively assessing the risks related to *Salmonella* in lettuce in Benin. More specifically, the study aims at (1) determining the conditions under which lettuce is handled along the supply chain; (2) determining the exposure to *Salmonella* through consumption of lettuce; and (3) estimating the risk on illness per serving of lettuce.

## **2. MATERIALS AND METHODS**

This study was conducted in three steps as follow. First step, a field survey was carried out to determine the conditions under which lettuce is handled along the supply chain and the consumption pattern of the product.

Second step, literature review was carried out to determine the prevalence and concentration of *salmonella* in freshly harvested lettuce in the region. Third step, desk work was performed by building a modular process model with various nodes taking into account the different steps of the lettuce supply chain for the risk assessment.



## **Table 1. Description and distribution of variables and models for risk assessment of** *Salmonella* **in lettuce**

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*D/R = Dose-Response*

## **2.1 Lettuce Handing Conditions and Consumption Survey**

A chain-wide survey was carried out via convenience sampling to get insight into lettuce handling practices and consumption pattern in Cotonou and Abomey-Calavi; which are the most populous cities in Benin. In total, 150 stakeholders directly involved in lettuce production or trading were interviewed. In addition, 400 informants were interviewed during the lettuce consumption survey. For the handling practices, questionnaire included washing methods (types of water used) at the field if applicable, storages conditions at the field, mode of transportation from production sites to the markets (retail points, fast-food stands…). Thermochron ibutton (ds 1921g) devices were placed in some lettuce samples to record temperature and time from the production sites to the markets and during the sale of the product.

Regarding lettuce consumption, the frequency of consumption and the quantity consumed were determined. For the quantification of food portion size, food photographs tool was used as previously described [24]. The demographic characteristics of the interviewees (name, age, gender, level of education, and contact details) were also recorded. The questionnaires were administrated by trained interviewers using faceto-face interviews.

## **2.2 Determination of** *Salmonella* **Prevalence and Concentration in Lettuce**

*Salmonella* prevalence and concentration in lettuce were retrieved from literature using various databases, namely Web of Science, Science Direct, PubMed, and Google Scholar. The following search terms were used: *Salmonella* AND lettuce AND Africa. During the literature search, we did not make any restrictions with regard to language or year of publication. All studies carried out in the same region of that of Benin (West Africa) were included.

## **2.3 Exposure and Risk Model**

A modular process model with six nodes taking into account the different steps of the lettuce supply chain was used for the risk assessment (Table 1). Collected data on *Salmonella*  prevalence, lettuce consumption pattern, and time-temperature profiles were used. Additional

literature review was performed to make relevant assumptions needed to construct the model (Table 1). The @Risk software package (Palisade USA) was used to run Monte Carlo simulations with 10,000 iterations. Based on the input data, the exposure to *Salmonella* per serving of lettuce and the risk on illness per serving were estimated.

Three scenarios were simulated and the impact on the risk on illness was evaluated. These scenarios were: (1) assuming that the cold chain was maintained along the supply chain; (2) assuming that the product was not (properly) washed before consumption; (3) assuming that Good Agricultural Practices (GAP) and Good Hygienic Practices (GHP) were followed, resulting in an initial maximum load of 2 log UFC/g of *Salmonella*.

## **2.4 Data Analysis**

Raw data were recorded in Microsoft Excel, and descriptive statistics were calculated. For the probabilistic risk assessment method, appropriate statistical distributions were used from @Risk software (Palisade USA) (Table 1). For the consumption data, the best fitting distribution was selected based on (i)  $\text{Chi}^2$  value (the lowest ones); (ii)the P/P plots of the distributions (the best straight lines); and (iii) the comparison of fitting and input data focusing on the median value ( $P_{50}$ ),  $P_{90}$  and the tails ( $P_{95}$  and  $P_{99}$ ).

## **3. RESULTS**

## **3.1 Lettuce Supply Chain and Handling Practices in Benin**

The socio-demographic profiles of the informants are shown in Table 2. The majority of respondents at the production level were men (92%), while most of the stakeholders at the trade and restaurant levels were women (64- 72%). Lettuce stakeholders in Benin were at least 20 years old. Most (> 50%) of traders and restaurateurs were between 20 and 25 years old. Most of lettuce stakeholders received formal education with at least junior secondary school level.

Lettuce supply chain in the cities of Cotonou and Abomey-Calavi is depicted in Fig. 1. After harvest, lettuce is delivered mainly to wholesalers who in turn supply mainly retailers or restaurateurs with the product. Consumers then



## **Table 2. Sociodemographic profiles of the respondents**



**Fig. 1. Lettuce supply chain in urban areas in Benin**

buy the product from restaurateurs at restaurants, hotels, schools etc. or from retailers for home consumption. However, in some cases, some people act as an intermediary between the farmers and the wholesalers. These intermediate traders buy the lettuce from farmers before the harvest time and sell it to wholesalers when the product is ready to be harvested. Also, it should be noted that sometimes restaurateurs can be provided with lettuce by retailers.

The survey revealed that 100% of the lettuce farmers (*n=*50) use poultry manure as fertilizer (data not shown). In addition to poultry manure, most of farmers (96%) use chemical fertilizers (urea or NPK) or other organic fertilizers (cow dung or compost) (data not shown).

Regarding lettuce handling practices, after harvest, surface water is used to wash the product by up to 16% of the informants (Table 3).

The most holding materials were clothes, wooden baskets and mosquito nets. The most common means of lettuce transportation from the growing areas to selling points are motorbikes (86% of the informants). The transportation lasts between 10 and 60 min (data not shown). Lettuce is kept at ambient temperature (26  $\pm$  3°C) along the supply chain by most stakeholders (approximately 90%). Only restaurateurs at hotels and at some restaurants store the product

in a refrigerator ( $7 \pm 2$ °C). To keep the product fresh during storage at ambient temperature, water is regularly sprayed on the product by most of the informants (64%). According to the lettuce stakeholders interviewed, the shelf-life of the product is 48-72h when it is kept at ambient temperature while under refrigerator storage it is between five and seven days.

Before using the lettuce to make salad dishes, the product is sometimes treated with chemical additives acting as sanitizers such as potassium permanganate (KMnO4), vinegar (acid acetic), sodium hypochlorite (NaClO) or lemon juice.

## **3.2 Exposure Assessment**

Our survey showed that the portion size of lettuce per serving ranged approximately between 60 g and 200 g with  $P_{50}$  value of 101.2 g and an average value of 110.5 g (Table 4). The consumers weight varied between 61 kg and 122.3 kg with  $P_{50}$  value of 58 kg and an average of 61.4 kg (Table 4). The frequency of consumption ranged from 6 times per week (0.3% of informants *n =*400) to 1 time per year (1.8% of informants *n* =400). The most cited frequencies were: two times per month (24.5% of informants *n =*400), once a week (21.8% of informants *n =*400) and once a month (21.3% of informants *n =*400).



#### **Table 3. Lettuce handling conditions in urban areas in Benin**



#### **Table 4. Portion size of lettuce and weight of consumers (***n***=400)**





*nr = not reported*





*Values in x axis are in log CFU/serving*

Table 5 shows the prevalence and concentration in freshly harvested lettuce in some West African countries. The prevalence of *Salmonella* in the region ranged from 3% to 50%. Data on *Salmonella* concentration in lettuce from the region is scanty. Concentration up to 6 log UFC/g was reported (Table 5).

Combining lettuce consumption data, *Salmonella*  prevalence and concentration data and other data from the survey and literature in a modular process model (Table 1), the exposure to

*Salmonella* per serving was estimated (Fig. 2). In 90% of cases, consumers of lettuce were exposed to a dose ranging from 3 to 7 log UFC/serving. The average exposure and P50 dose were 4.8 log UFC/serving and 4.6 log UFC/serving, respectively.

#### **3.3 Risk on Illness Estimation**

The risk on illness per serving according to the dose response models is depicted in Table 6.



#### **Table 6. Estimation of the risk on illness for various scenarios using three dose response models**

Based on the current lettuce handling practices (baseline scenario), the risk on illness ranged between 7.7% and 95%. Assuming that the cold is maintained along the supply chain, the risk on illness reduced tremendously ranging between 0% and 3.3% according to the dose response model. Also, assuming that appropriate measures are taken (good agricultural practices and good hygienic practices) to limit the maximum initial concentration of *Salmonella* to 2 log UFC/g, the risk of illness reduced by 89.6 and 14.7% with dose response models 1 and 2, respectively (Table 6). In contrast, assuming that the washing step of lettuce before its consumption currently taken into account on the baseline scenario is not adequately performed, the risk on illness per serving varied between 49.6% to 100% according to the dose response model, thus an increase of 84.9% with dose response model 1

## **4. DISCUSSION**

In this study, lettuce handling practices along the supply chain in urban areas in Benin was investigated and the health risk associated with the consumption of the product was assessed.

Like in many previous studies [38-40] this study shows that in Benin, men are more involved in raw food production while women are specialized in the processing and trade of these products. After harvest, lettuce is washed to get rid of sand and other debris attached to the leaves. However, the quality of water used may constitute a source of microbiological contamination of the product. Indeed, various studies have reported the presence of fecal indicator organisms and specific foodborne pathogens including *Salmonella* especially in surface water used to irrigate or wash vegetables [34,41,42].

In addition to water used for lettuce irrigation or washing, another potential source of *Salmonella*  contamination is the poultry manure widely used as fertilizer. It is in fact well documented that poultry manure can play an important role in contaminating soil and root vegetables with *Salmonella* for a relatively long time [43-45]. Moreover, cross-contamination along the supply chain due to the poor handling practices observed during the survey could be an important cause of *Salmonella* contamination in lettuce in the region as previously reported in other studies [46,47]. Although the use of sanitizers can play an important role in controlling pathogens associated with vegetables, they are not always effective against the targeted microorganisms and they cannot eliminate cross-contamination [46,48].

Our study reveals that in most cases, lettuce is kept at ambient temperature along the supply chain (from farmers to consumers). This practice in a tropical region where ambient temperature is above 20ºC all year long, is in violation of Codex Alimentarius recommendations which stipulate that fresh vegetables should be maintained at low temperatures at all stages to minimize microbiological growth [49].

Similar observations of temperature abuse and inappropriate handling practices of vegetables have also been reported in other West African cities including Ouagadougou (Burkina-Faso) and Accra (Ghana) [50].

This temperature abuse could explain the relatively high prevalence and concentration of *Salmonella* in lettuce from the region as demonstrated by [51]. In a study on systematic review and meta-analysis of *Salmonella* spp. and *Escherichia coli* O157:H7 prevalence and levels on lettuce from various countries across the globe, [52] found that the highest prevalence of *Salmonella* spp. on lettuce (50%) was from Burkina-Faso, which is a neighboring country of Benin.

Although, relatively high *Salmonella* prevalence and concentration on lettuce were reported in West Africa (Table 5), little is known about the health risk incurred by the population in the region. In this study, based on lettuce consumption data in Benin the health risk associated with the contamination of the product by *Salmonella* was assessed using a probabilistic risk assessment method. As expected, the risk per serving was relatively high, especially with dose response models addressing low *Salmonella* doses. However, this health risk can be significantly reduced when the cold chain is maintained along the supply chain. This study highlights the need to maintain temperature control along the lettuce supply chain. Good Agricultural practices and good hygiene practices including appropriate washing step with potable water of the lettuce before consumption are also important to reduce the health risk associated with this product.

## **5. CONCLUSION**

This study shows that like in other West African countries, lettuce is exposed to temperature abuse under inappropriate hygienic conditions in Benin. This leads to high prevalence and concentration of *Salmonella* on lettuce in the region. The median portion size of lettuce was 101.2 g and in 90% of cases, consumers are exposed to a dose of *Salmonella* ranging from 3 to 7 log UFC/serving. The estimated health risk depends on the type of dose response model used varying between 7.7% and 95% per serving. However, this risk can be significantly reduced (0% to 3.3%) if cold chain is implemented and maintained along the supply chain preventing *Salmonella* from growing. Reducing the initial concentration of *Salmonella t*hrough Good Agricultural Practices and Good Hygienic Practices, and appropriate lettuce washing step before consumption are also important to minimize the health risk. It is therefore important to train lettuce stakeholders in Good Agricultural Practices and safe food handling practices. In addition, affordable and energy efficient pre-cooling and cold storage facilities for stakeholders should be promoted.

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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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