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Identification of Salmonella in Market Garden Products and Irrigation Sources in the City of N'djamena

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ABSTRACT

***Objective**: Irrigation of market garden crops in the city of N'Djaména is done through wastewater often heavily soiled by human and animal faeces. The aim of this study was to identify salmonella in market garden products and irrigation sources in the city of N'Djaména.*

***Methodology and Results**: Samples of water and lettuce (Lactuca sativa L.) were taken from market gardens in 16 sites covering 7 districts (1, 3, 5, 7, 8, 9 and 10) of the city of N'Djaména. Microbiological analysis was done according to ISO 6579:2002. Salmonella strains were isolated according to the standard microbiology method and confirmed by the Api 20 E bacterium. Market gardening is practiced by 87.87% of men and 12.12% of women, most of whom are illiterate (53.03%). However, 84.84% of producers have no knowledge of biosafety; 80.30% do not know what salmonellosis is; 78.78 recognize that the use of wastewater in market gardening constitutes a danger to the health of producers and consumers. The rate of contamination of water by salmonella by district was: 94.44% in the 1st ; 91.66% in the 9th; 81.57% in the 7th; 80% in the 10th; 78.26% in the 3rd; 69.69% in the 5th and 68.96% in the 8th. According to the seasons: the prevalence was 85% in the dry season, 76% in the rainy season. The prevalence of salmonella in the different types of irrigation water was: 88.48%; 86.40%; 83.33% and 35.71% in pond water, wastewater, river water and borehole water respectively. The contamination rate in lettuce was: 93.10% in the 9th; 92.10% in the 1st; 92.% in the 7th; 91.66% in the 3rd; 90.90% in the 10th; 88.88% in the 5th, and 86.95% in the 8th. According to the seasons, salmonella contamination was 92.59% in the dry season and 89% in the rainy season. For fertilizers, the prevalence was 88% for simple dung, while for dung associated with synthetic fertilizer, it was 86%.*

Keywords

Lettuce, Salmonella, Prevalence, Vegetable crops, Chad.

Introduction

Waterborne bacterial risk has historically been the most serious and frequent. In the 19th century, several deadly epidemics around the world were transmitted by water (typhoid, cholera). Today, water remains the cause of death of 3 to 10 million people worldwide, contaminated by waterborne bacteria [1].

Irrigating crops with treated, poorly treated, diluted, and even raw wastewater is a widespread practice in urban and peri-urban areas in most developing countries [2-4]. Untreated water can contain a multitude of parasites or bacteria that cause serious illness in humans. Contamination of fruits and vegetables is one of the potential risks of infection with enterotoxigenic bacteria such as Salmonella and Escherichia coli and staphylococci [5,6]. This is from an environmental, animal or human source at the time of cultivation, harvesting or handling of plants before consumption

[7,8]. Surface waters are particularly exposed to pollution from industrial, urban and agricultural effluents. These effluents typically contain a mixture of non-pathogenic and pathogenic bacteria and are responsible for several human diseases [9].

Salmonella can be responsible for the contamination of industrial, agricultural, domestic, freshwater, drinking water, groundwater and seawater [10]. Their transmission to humans is mainly through the ingestion of contaminated water or food [11,12]. Market gardening offers poor urban populations the opportunity to access products that are essential for their survival, both for food and monetary needs [13]. However, unlike seasonal food crop production in the countryside, market gardening in urban and peri-urban areas is practiced year-round with intensive production techniques that do not guarantee the sanitary quality of the vegetables produced [14]. In Chad, microbiological quality control of raw vegetables is not part of the agenda of the competent authorities; similarly, the origin of in most cases, the cause of intoxication is still uncertain. Previously, no work had been done on the microbiological evaluation of the quality of irrigation water and the vegetables irrigated by this water in the city of N'Djaména. The objective of the present work is to look for pathogens such as salmonella contaminating water and vegetables.

Materials and Methods

Study site

This descriptive and analytical study was conducted in 16 market gardening sites in the city of N'Djamena (Figure 1). The city has a tropical arid climate of the Sahelian type, alternating a dry season (November to April) and a rainy season (May to October). The average annual temperature in the city is 28.9°C with an average rainfall of 452 mm/year. These sites are crossed by wastewater effluents, which is a mixture of runoff water discharged by the city's drainage channels and water from households.

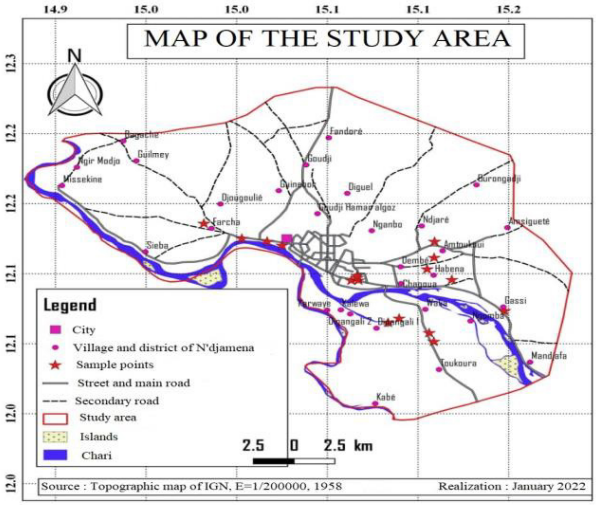

Figure 1: Location of market garden sites in the city of N'Djamena.

Field Survey

A descriptive study was conducted on market gardening sites in the city of N'Djamena in November 2020 and in February 2021. Sociological approaches such as questionnaires and direct observation were used to obtain information on cultivation practices at 66 randomly selected market gardeners. The main information collected concerned: Socio-demographics (age, gender, social status and main activity), description of agricultural practices (types of vegetables grown, sources of water supply, irrigation systems), perceptions of market gardeners on water and vegetable quality and finally adaptation strategies of market gardeners in relation to climate change (water management). The information collected from the respondents was expressed as percentages.

Water Sampling

According to the method described by [15], water samples for microbiological analysis were taken in 500 ml sterile bottles from a wastewater collection tank. These bottles were placed in a sterile insulated cooler containing ice cubes.

The microbiological analyses were conducted on 208 water samples taken from 7 of the 10 districts in the city of N'Djamena, i.e., from 16 market gardening sites. These sites were chosen in order to cover the entire territory of the city of N'Djamena and to include the different types of neighborhoods (Figure 1). The water samples analyzed were taken at the source, respecting aseptic conditions and sterilization of the sampling equipment [16].

Sampling of Lettuce Leaves

Lettuce leaves were sampled in order to obtain a representative sample of the plots studied. Using sterile forceps, lettuce leaves (inner and outer leaves of the plant) were removed from different plants and then put in sterile plastic bags. The samples were then stored in an isothermal cooler with ice cubes.

Bacteriological Analysis of Irrigation Water

The search for Salmonella consists of four steps [10]: Preenrichment in double-concentrated buffered peptone water and incubation at 37°C for 6 to 18 hours. Enrichment in rappaport broth at 42°C for 18 to 24 hours. Isolation on selective medium which consists in seeding Hektoen medium from the enrichment broth, and incubation at 37°C for 24 to 48 hours. Identification in which the suspect colonies on Hektoen medium, lactose negative with black center, are plated on Kligler medium in tubes and incubated at 37°C for 24 hours. In our case, we proceeded to the biochemical identification of the suspected Salmonella strains by the conventional gallery and the API 20E gallery whose biochemical characteristics are comparable to those sought in the conventional methods of identification, but the reading of the tests is faster.

Bacteriological Analysis of Lettuce Leaves

A 10 g test sample of lettuce leaves, crushed with a grinder, is put in a sterile dilution bag (plastic bag) by adding aseptically 90 ml of buffered peptone water:

1. **Pre-enrichment:** Lettuce leaves are crushed and put in buffered peptone water (nutrient broth) at a dilution of 1/10th. Incubation is at 37°C for 18 to 24 hours.
2. **Enrichment:** The broth is inoculated with 0.1 ml of nutrient broth and incubated at 44°C for 24 hours.
3. **Isolation:** Hektoen medium (selective medium) is inoculated from the rappaport broth and incubated at 37°C for 18 to 24 hours.
4. **Identification:** The identification of Salmonella in lettuce leaves was performed following the same protocol for identification of Salmonella in irrigation water described above.

Identification on API 20 E gallery

Rod-shaped, catalase-positive and Gram-negative bacteria were identified using API 20 E (BioMérieux, France) galleries for Enterobacteriaceae. Gallery and inoculum preparation followed the manufacturer's instructions. After incubation at 37°C for 24 hours, the results were analyzed using the APIWEB identification software.

Data Analysis

For statistical analysis of the data, XLSTAT 7.5.2, SPSS software was used. The analysis of variances (ANOVA) was performed to compare the mean values of the different variables using Fisher's tests at the probability threshold p= 5%.

Results

The results of our investigations (Table 1) show that market gardening is carried out mainly by men (87.87%), while women are less represented (12.12%). 68.18% of them are married, compared to 31.81% who are single. 53.03% of market gardeners have no education, compared to 27.27% who have primary education, and only 19.69% have secondary education. The majority of market gardeners (84.84%) stated that they had no knowledge of biosafety, compared to 15.15%. In addition, 80.30% do not know what salmonellosis is, 19.69% claim to know. Young people are the most represented on the sites (37.5% '30; 47.5% for the 30-50 age group); older people are less represented (15.27%). Among the 66 market gardeners surveyed, 78.78% (52/66) recognize that the use of wastewater in market gardening constitutes a health risk for producers and consumers.

Table 1: Socio-demographic characteristics of market gardeners.

Boroughs	1 er	3 éme	5 éme	7éme	8 éme	9 éme	10 éme	Total	Proportions (%)
Sex of the market gardeners									
- Male	9	4	14	10	9	6	6	58	87,87
- Female	1	1	1	2	1	0	2	8	12,12
Marital status									
- Married	7	2	10	9	6	6	5	45	68,18
Single	3	4	2	3	4	2	3	21	31,81
Level of study									
-Primary	3	3	2	3	2	3	2	18	27,27
-Secondary	2	1	1	3	2	2	2	13	19,69
-Not instructed	5	4	4	6	5	7	4	35	53,03
Biosafety knowledge									
-Yes	0	0	4	2	3	0	1	10	15,15
-No	10	5	11	10	7	6	7	56	84,84
Knowledge of salmonellosis									
-Yes	0	0	4	2	4	1	2	13	19,69
-No	10	5	11	10	6	5	6	53	80,30

The rate of contamination in pond water according to the districts is respectively in decreasing order (Figure 2) 94.44% for district 1; 91.66% in district 9; 81.57% for district 7; 80% in district 10; 78.26% for district 3; 69.69% for district 5, and finally 68.96% for district 8. The prevalence rates according to season are as follows (Figure 5): dry season with a prevalence of 85.18% and 76% for the rainy season.

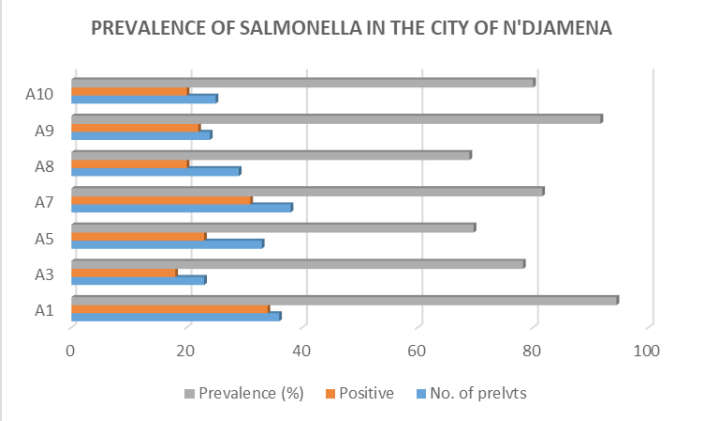

Figure 2: *Salmonella* prevalence in water from market garden sites by district.

The prevalences in the different types of irrigation water (Figure 3) are as follows: pond water with a rate of 88.48%, wastewater 86.40%, river water 83.33% and well water with a rate of 35.71%.

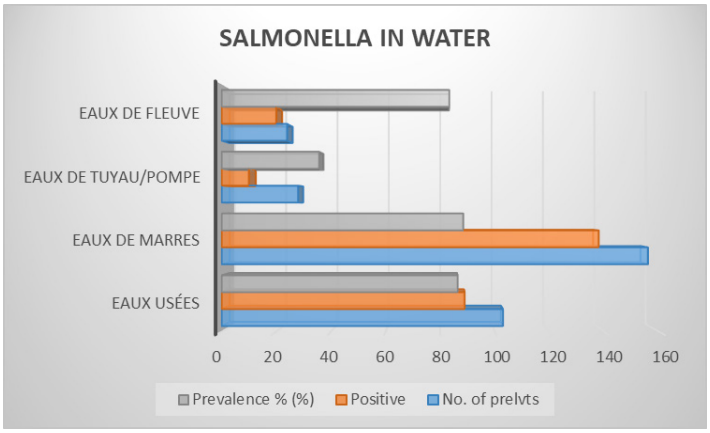

Figure 3: Variation of *Salmonella* contamination rate in different irrigation waters.

The contamination rate of lettuce according to the districts are respectively in decreasing order (Figure 4) 93.10% for district 9; 92.10% in district 1; 92% for district 7; 91.66% in district 3; 90.90% for district 10 ; 88.88% for district 5, and finally 86.95% for district 8. Those according to the seasons are as follows: dry season with a prevalence of 92.59% and 89% for the rainy season.

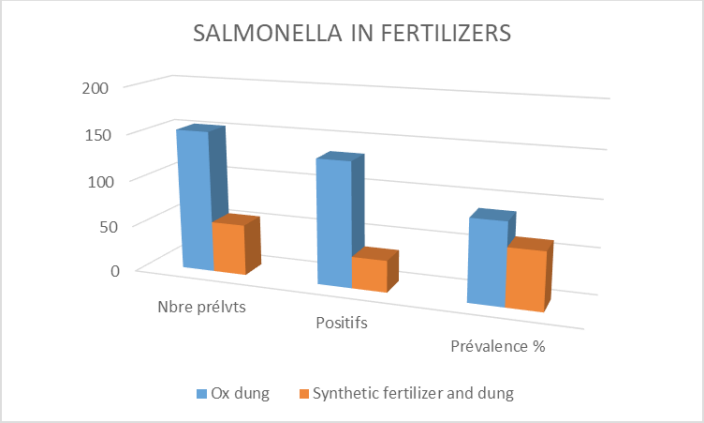

Figure 4: Variation of salmonella in lettuce according to boroughs.

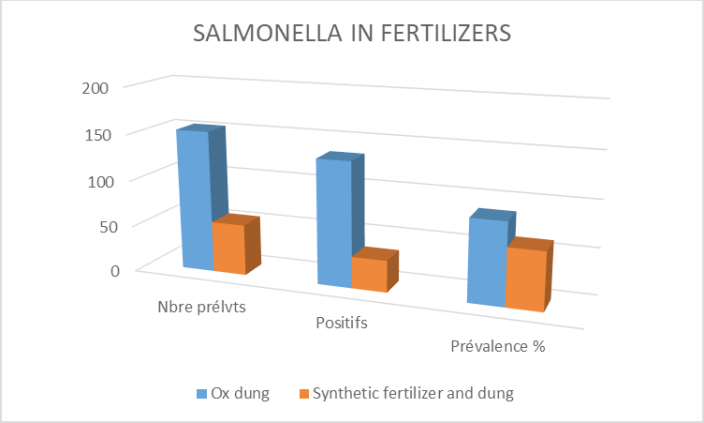

Figure 5: Seasonal variation of Salmonella in water.

Discussion

Survey

Market gardening is in fact developing for self-subsistence purposes or to supply local markets. The low representation of women (12.12%) in market gardening would be due to the lack of means or to the arduousness of this work. These results corroborate the work of [5,17,18] in Abidjan, Yaoundé and Niger respectively. Market gardening in these countries is practiced mostly by men (77%, 83.33% and 93.33%). In contrast, in Maroua, Cameroon, 100% of market gardening is done by women [19]. The low level of education of market gardeners could be due to the fact that market gardening is an activity that does not require specific skills. In search of subsidies, the idle engage in this activity. The high level of illiteracy could justify the behavior of producers who have no knowledge of biosecurity related to vegetable production. More than 80% with primary level of education are engaged in this activity. Similar results have been reported in other studies by authors such as [5,20].

Irrigation water quality

The water analyzed is more polluted in the dry season (85.18%) than in the rainy season (76%). This work corroborates with those of [21] in Bawéra in Benin, as temperature is one of the parameters that influence the growth of microorganisms. The increase in the number of pathogens in dry periods would be mainly due to the increase in water temperature [22]. Stagnant water, i.e. pond water used for watering significantly showed a higher prevalence of Salmonella (88%) compared to wastewater, river or borehole water respectively 86%, 83% and 36%. These stagnant waters are exposed to contamination with manure (animal feces used as fertilizer). They are also subject to the effects of human activities. On the banks of the Logone and Chari rivers, the water is exposed to all kinds of human and animal activities. Due to the flow of water, the prevalence of salmonella is lower than that of wastewater and ponds. These different problems have been raised in other studies [5,17,18,23]. The increase in the rate of contamination in districts 7, 1 and 9 can be is explained by the fact that they are the largest districts in the city and therefore the market gardening extends over a very large area.

Quality of lettuce

Our work reveals that the prevalence of salmonella in lettuce varies according to the season: 89% in the rainy season and 92.59% in the dry season. Our results are higher than those obtained by [5] which is 0% to 56%. Other studies conducted in lettuce found prevalences of 50% in Burkina Faso [24], 22% in Sokoto, Nigeria [25] and 16% in Maiduguri, Nigeria [26] respectively. This difference is thought to be related to the educational levels of vegetable farmers and lack of awareness of biosecurity in lettuce cultural routes. According to the WHO [27], no raw vegetables for consumption should contain salmonella. Lettuce grown in the urban areas investigated did not meet the WHO guidelines. With a very high prevalence of Salmonella isolated from lettuce the risk of salmonellosis contamination among consumers is high.

Influence of Irrigation Water on Lettuce

The contamination of these crops is probably intimately linked to the pollution of irrigation water (wastewater) and soil. According to [28], rain and runoff are responsible for the dissemination of pollutants in the environment. However, contrary to this observation, the results of the analyses showed an increase in the contamination rate of Salmonella during the dry season for both irrigation water and vegetables. The higher contamination of vegetables in the dry season could be explained by the fact that the most used irrigation water comes from city wastewater, which is the most contaminated. Influence of fertilizers on lettuce: For a dung, used as a fertilizer in the soil fine, the prevalence was 88% while for the same dung associated with synthetic fertilizer, this prevalence was 86%. These results show that the itineraries have an influence on the contamination of vegetables (Figure 6).

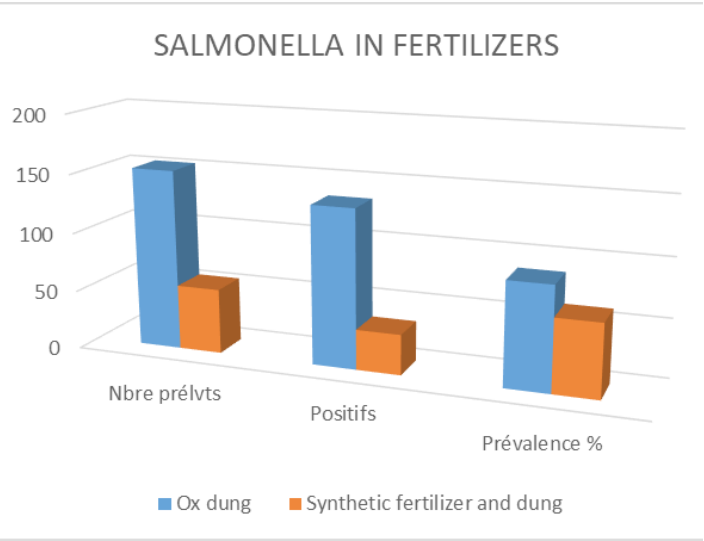

Figure 6: Variation of salmonella levels in fertilizers.

Conclusion

The identification of Salmonella in lettuce leaves and in irrigation water, and in the same sampling, confirms the direct contamination of lettuce leaves by irrigation water. The prevalence of Salmonella isolated in lettuce and irrigation water is very high, which leads to a very high risk of contamination. The presence of Salmonella in lettuce leaves is a real danger for the health of the consumer, especially when it is consumed raw. The lettuce grown in the city of N'Djaména is not suitable for consumption. Preventive measures must be taken and awareness raised among market gardeners to avoid the proliferation of infectious diseases such as typhoid fever, gastroenteritis and dysentery.

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