Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Bacteriological and parasitological assessment of vegetables collected from markets in Mbouda, West Cameroon

Honorine Ntangmo Tsafack[1]*, Cédric Aimé Saah Fokou[1], Franck Robéan Wamba[2] ,Teh Exodus Akwa[1], Godfroy Rostant Pokam Djoko[1], Emile Temgoua[3]

[1]Research Unit of Biology and Applied Ecology, Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon.
[2]Department of Forestry, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.
[3]Research Unit of Soil Analysis and Environmental Chemistry, Department of Soil Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.

ARTICLE INFO

Article history:
Received 06.10.2024
Received in revised form
21.12.2024
Accepted
25.12.2024

Keywords:
Vegetables;
Parasitological quality;
Bacteriological quality;
Health risks

ABSTRACT

Implication of the consumption of vegetables in the resurgence of water-borne diseases is less explored in Cameroon. This study aimed at exploring the microbiological quality of vegetables consumed in Mbouda, which is one initial step within quantitative microbial risk assessment (QMRA). A total of 75 vegetable samples comprising different types (celery, parsley, leek, green cabbage, red cabbage, African eggplant, tomato, cucumber, and carrots) collected from markets in Mbouda were analysed bacteriologically and parasitologically. Bacteriological analysis was carried out by plating the samples on selective media after serial dilutions had been performed. The search for parasitic elements was done using the sedimentation technique on samples, followed by microscopy. Four parasitic elements or stages, which included Cysts of *Entamoeba* spp., Nematode larvae, eggs of *Ascaris* spp. and *Ankylostoma* spp., were detected in these vegetables, with prevalences of 17%, 16%, 12% and 8% respectively. Bacterial contamination was high with six bacteria species isolated (*Salmonella* spp, *Escherichia coli*, fecal coliform, *Shigella* spp., fecal *streptococci*, and *Vibrio* spp). These bacteria had loads exceeding the WHO standard (10^3 cfu/g), with *Shigella* spp. 10^8 to 10^9, *Salmonella* spp. 10^2 to 10^5, fecal coliforms 10^4 to 10^5, and *E. coli* 10^2 to 10^4 times higher. It is likely that these pathogens resulted from unsanitary conditions of the production and sale of these vegetables. One of the major risks linked to the poor quality of these vegetables is the resurgence of water-borne diseases in Mbouda. It is therefore urgent to sensitize the population on the health risks linked to the contamination of the vegetables sold in Mbouda.

Citation: Ntangmo Tsafack H, Saah Fokou CA, Wamba FR,Akwa TE, Pokam Djoko GR, Wamba FR, et al,. **Bacteriological and parasitological assessment of vegetables collected from markets in Mbouda, West Cameroon.** J Food Safe & Hyg 2024; 10 (4): 297-311.http://doi.org/10.18502/jfsh.v10i4.19394

1. Introduction

Globally, water-borne diseases are responsible for significant morbidity and mortality. The World Health Organization (WHO) estimates the number of people affected each year at 2.3 billion, and the number of deaths at 1.8 million, 90% of which are

*Corresponding author. Tel.: +237 699740686
E-mail address: tsafackhonorine@yahoo.fr

children under 5 years old, mainly in developing countries (1).

One of the major factors contributing to the emergence of these diseases is the consumption of contaminated raw, poorly washed, or poorly cooked food, vegetables and fruits (2). Numerous studies around the world have established the link between the poor health quality of consumed food and water-borne diseases (3,4). In Cameroon, Akwa and Nguimbous (5) document poor food quality and contamination as an implicating factor for the occurrence of water-borne diseases.

An increase in awareness of the health benefits of vegetables has brought about an increase in their consumption. Vegetables are considered the essential component of a healthy and balanced diet because of their richness in micronutrients as well as their ability to prevent certain chronic diseases, particularly cardiovascular diseases (6,7). However, their fresh state provides a favourable shelter for the survival of pathogens. *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, cysts of *Entamoeba histolytica* and *Giardia intestinalis*, as well as eggs of helminths such as *Ascaris lumbricoides*, *Ankylostoma* spp., and the larvae of *Strongyloides stercoralis* and *Ankylostoma* spp., are pathogens frequently detected in vegetables (8,9,10). Contamination of these vegetables can occur at various stages, including production, harvesting, and distribution (11).

In the community of Mbouda-Cameroon, 70% of the inhabitants survive mainly from agriculture. Vegetables are generally grown in the lowlands, which receive the waste produced in the city, where household waste collection services are inefficient. In addition, due to a lack of a water treatment plant in the community, water after use (waste water) from homes is channelled towards waterways, which are most often used for watering vegetables. Studies carried out by Ntangmo Tsafack et al. (12,13) in Dschang-Cameroon and Mbouda document the use of contaminated water for the irrigation of vegetables. Further investigation has shown a link between poor health quality of vegetables in Dschang and poor water quality used in its irrigation (14). However, in Mbouda, there is no data on the health quality of vegetables despite the poor water quality used for irrigation, an increase in vegetable consumption, and the intensification of market gardening (15). Health district statistics in Mbouda show that between 2011 to 2013, out of 53,281 patients treated, 5,439 (10%) suffered from water-borne diseases. Despite the interest that municipal officials and people of goodwill place on the quality of drinking water, these diseases continue to persist. It is thus possible that the persistence of these water-borne diseases can also be a result of the consumption of poor-quality vegetables sold in markets. Hence, the need to determine the health risks linked to the consumption of vegetables sold in the town of Mbouda.

This study was thus aimed at investigating the bacteriological and parasitological quality of vegetables sold in markets in Mbouda, West Region of Cameroon, to highlight the relationship between contaminated vegetables and their potential in water-borne disease transmission. This provides information that could lead to the improvement of food safety and thus safeguarding public health.

2. Materials and Methods

2.1. Description of the study area

The vegetable samples analysed were taken from the market in the town of Mbouda. Mbouda (Fig. 1), located in the West Region of Cameroon, is the capital of the Bamboutos subdivision. This city is located between latitude 5°63′ North and longitude 10°25′ East, with an area of 437 km^2. The population is estimated at more than 80,000 inhabitants. The main activity of the inhabitants remains agriculture, livestock and commerce. This town has an equatorial climate, with two seasons: A short dry season from November to February and a long rainy season from March to October. Rainfall varies between 1700 and 2000 mm of water per year, with an average annual temperature of about 21.3°C (16).

2.2. Collection of vegetable samples

Three groups of vegetables consisting of leafy vegetables [celery (*Apium graveolens*), parsley (*Petroselinum crispum*), leek (*Allium porrum*), green cabbage (*Brassica oleracea*), and red cabbage (*Brassica oleracea* var. capitata f. rubra)], fruit vegetables [African eggplant (*Solanum macrocarpon*), tomato (*Solanum lycopersicum*), and cucumber (*Cucumis sativus*)], as well as carrots (*Daucus carota*) as root vegetables were chosen as part of this work. In total, 75 vegetable samples of approximately 500 grams each, in proportion of 9 samples (n = 9) per type of vegetable except tomato (n = 6) and eggplant (n = 6) were purchased from sellers chosen at random. Each sample was packaged in a sterile polyethylene bag, then placed in a refrigerated enclosure and transported to the laboratory for parasitological and bacteriological analyses.

2.3. Parasitological examination of vegetable samples

Parasitic elements (eggs, larvae and cysts) specific for causing gastrointestinal diseases were examined. The identification and characterisation of these parasites on the vegetable samples were carried out using the sedimentation technique as described by Amahmid (2). One hundred (100) grams of each vegetable sample was washed in 1 litre of tap water. The water from washing was then sieved with a coarse mesh sieve, and allowed to stand for 8 h. The sedimented residue was centrifuged at 3500 rpm for 15 min, and the pellet obtained diluted with aceto-acetic buffer, and ether was added to it. After emulsifying with vigorous stirring, the mixture was again centrifuged for 4 min at 1500 rpm. The resulting pellet was further diluted with the buffer solution and observed under a microscope, using 10X, 40X and 100X objectives successively, after being applied with a Lugol stain for cyst observation.

The identification of the parasitic elements was based on morphological criteria such as the shape, size, nature of the shell or membrane and content of the cytoplasm, which are the World Health Organization (WHO) identification keys for intestinal parasites. The quantification of parasites per gram of vegetable was determined following the formula used by Andoh (17) as follows;

$$N = \frac{AX}{PM}$$

N = number of parasites per gram of sample

A = number of parasites counted on the slide or average of parasites counted on the slides

X = volume of the final product (mL)

P = volume of sample deposited on the slide (mL)

M = mass of the initial sample (g).

2.4. Bacteriological analyses of vegetable samples

Vegetables were treated with the serial dilution method to isolate bacteria specific for causing gastrointestinal diseases and indicators of fecal contamination. Identification and characterisation of these bacteria (*Pseudomonas* spp, *Salmonella* spp, *Escherichia coli*, fecal coliform, *Shigella* spp., fecal streptococci, and *Vibrio* spp) on samples was carried out according to the method described by Amahmid et al. (2). It consisted of grinding 20 g (fresh mass) of vegetable sample in a sterile mortar, and transferring the ground material into a sterile beaker containing 180 mL of distilled water. After carrying out a series of three dilutions (0.1, 0.01, and 0.001) from the initial suspension of the stock solution, 0.1 mL of each sample was inoculated onto specific culture medium in a petri dish and incubated. The culture media that were used in this study depended on the targeted and enumerated bacteria. These culture media were previously prepared according to the manufacturer's instructions.

2.5. Isolation and identification of bacteria

For *Salmonella* spp. and *Shigella* spp., inoculation of samples was done on Salmonella-Shigella agar and incubated at 37°C for 24 h. For fecal coliform and *Escherichia coli*, inoculation was done on lactose agar medium containing Triphenyl Tetrazolium Chloride (TTC) and tergitol, and incubation was performed at 44°C for 24 h. For fecal Streptococci, inoculation was carried out on Slanetz medium and incubation at 37°C for 48 h. *Pseudomonas* spp. were tested on Pseudomonas agar medium, and *Vibrio* on Thiosulfate Citrate Bile Sucrose (TCBS) medium.

The identification of the various bacteria was based on the cultural and morphological characteristics of the colonies formed on the respective media.

Colonies of *Shigella* sp on the medium were differentiated by their colourless appearance with no blackening, while those of *Salmonella* sp were identified by their colourless appearance with black centers. For *E. coli*, a metallic burgundy colony is formed. Fecal coliforms are burgundy pink, while fecal streptococci form a distinguishing red colored colony (2). *Vibrio cholerae* forms yellow colonies on Thiosulfate Citrate Bile Sucrose (TCBS) medium. *Pseudomonas* spp are identified by their blue-green colonies on Pseudomonas agar medium (2).

2.6. Enumeration of bacterial colonies

Identified colonies were enumerated using a colony counter. The results of bacteriological enumeration were expressed in Log cfu/g of vegetable using the following formula:

$$N = \frac{(\sum c)}{V(n_1 + 0.1n_2)d_1}$$

N = number of cfu/g of initial product;

$\sum c$: sum of the colonies in the boxes

V: volume of the solution deposited (mL)

n_1: number of boxes considered at the first dilution retained

n_2: number of boxes considered at the second dilution retained

d_1: dilution factors (d=1 for undiluted samples, 0.1 for those diluted to 10^{th},…….)

2.7. Statistical analysis

All data collected in the study were entered in Microsoft Excel 2013 and then imported to SPSS version 22 software for analysis. ANOVA was carried out to compare the degree of bacterial contamination on vegetables. The Tukey HSD test was used as a post hoc test. Additionally, the Chi-square test was used to compare the prevalence of vegetable contamination. Kruskal-Wallis test was also used in the analysis to compare the population of parasites on vegetables. For all analyses used, differences were considered significant for p values < 0.05.

3. Results

3.1. Parasite contamination on vegetables

The results of contamination of vegetables by parasites are presented in Table 1. Twenty-six (26) vegetable samples contained at least one parasite type, that is, a general prevalence of 34.66%. Among the 9 types of vegetables analysed, celery was the most contaminated (67%), followed by parsley (56%), eggplant (50%), carrots (44%), cucumber (33%), tomatoes (33%), green cabbage (22%), and leeks (11%). No parasite was found on the red cabbages (Table 1).

Cysts of *Entamoeba* spp., eggs of *Ascaris* spp., eggs of *Ankylostoma* spp., and nematode larvae were also found on these analysed vegetables (Fig. 2). Cysts of *Entamoeba* spp. were the most prevalent parasite (17%), followed by nematode larvae (16%), eggs of *Ascaris* spp (12%), then eggs of *Ankylostoma* spp. (8%). There was a significant variation in the degree of contamination of the different vegetables by *Entamoeba* spp. cysts (p=0.001).

Table 2 shows the mean counts of parasites found per 100 grams of vegetable. Their population ranged between 0 to 193 parasites per 100 grams of vegetable. Eggs of *Ascaris* spp., *Ankylostoma* spp., *Entamoeba* spp. Cysts and nematode larvae were detected in the vegetable samples, with a concentration of 6 eggs, 4 eggs, 36 cysts, and 11 larvae in 100 g of samples, respectively. The degree of contamination of vegetables by *Entamoeba* cysts varied significantly from one vegetable to another (p=0.001). Celery had the highest occurrence of *Entamoeba* cysts (193 cysts per 100 grams).

3.2. Bacterial contamination on vegetables

All the vegetable samples analysed were contaminated with bacteria. However, the degree of contamination of vegetables by bacteria varied significantly from one vegetable to another (p=0.0001). Six bacteria species (*Salmonella* spp, *Escherichia coli*, fecal coliform, *Shigella* spp., fecal streptococci, and *Vibrio* spp) were detected in the vegetable types. *Pseudomonas* spp. was absent on all the vegetable types (Fig. 3).

Comparing the results of each vegetable sample by bacterial contamination, it was observed that Eggplant was highly contaminated by *Shigella* (9.127 Log cfu/g and least by *Vibrio* spp (0.981 Log cfu/g). Carrots also had the highest contamination by *shigella* but no contamination was recorded by *Streptococcus*, *Vibrio* and *Pseudomonas*. Red cabbage was highly contaminated by *Shigella* (7.857 Log cfu/g) and least by *Vibrio* (1.672 Log cfu/g). Similar to red cabbage, high contamination by fecal coliform and *Shigella* was also observed in green cabbage. Cucumber was also greatly contaminated by *Shigella* (9.237 Log cfu/g) and least by *Vibrio* (1.464 Log cfu/g). Leeks had the highest

contamination with fecal coliform (8.287 Log cfu/g) and *Shigella* (9.127 Log cfu/g), but absence of *Vibrio*. Parsley had higher levels of *E. coli*, fecal coliform, and *shigella*, but no contamination was recorded by *Vibrio*. Tomato also recorded higher contamination levels of *Shigella* but no contamination with *Vibrio*.

All the vegetables were shown to be contaminated with *Salmonella* though at lower levels compared to fecal coliform, *E. coli*, and *Shigella*. Although *Vibrio* was absent in carrots, celery, parsley, leeks, and tomatoes, it had higher values recorded in green cabbages (5.580 Log cfu/g). In general, *Shigella* was the most frequently isolated bacterium in all the vegetables analysed.

Table 1. Prevalence of parasites on vegetable samples.

	Prevalence n(%)										
	Egg plant	Carrots	Cellery	Red cabbage	Green cabbage	Cucumber	Parsley	Leeks	Tomato	Total	P value
	n=6(%)	n=9(%)	n=9(%)	n=9(%)	n=9(%)	n=9(%)	n=9(%)	n=9(%)	n=6(%)	n=75(%)	
Parasites detected											
Eggs of *Ascaris* spp.	0(0.00)	0(0.00)	3(33.33)	0(0.00)	1(11.11)	2(22.22)	3(33.33)	0(0.00)	0(0.00)	9(12.00)	0.14
Eggs of *Ankylostoma* spp.	2(33.33)	0(0.00)	1(11.11)	0(0.00)	0(0.00)	1(11.11)	1(11.11)	1(11.11)	0(0.00)	6(8.00)	0.27
Cyst of *Entamoeba* spp.	1(16.66)	4(44.44)	5(55.55)	0(0.00)	0(0.00)	1(11.11)	1(11.11)	0(0.00)	1(16.66)	13(17.33)	0.001*
Nematode larva	1(16.66)	1(11.11)	4(44.44)	0(0.00)	1(11.11)	0(0.00)	3(33.33)	1(11.11)	1(16.66)	12(16.00)	0.22
Occurrence	4(66.66)	5(55.55)	13(144.44)	0(0.00)	2(22.22)	4(44.44)	8(88.88)	2(22.22)	2(33.33)	40(54.66)	-
Positive samples	3(50.00)	4(44.44)	6(66.66)	0(0.00)	2(22.22)	3(33.33)	5(55.99)	1(11.11)	2(33.33)	26(34.66)	-

Table 2. Mean counts of parasites per 100 g of a vegetable samples

	Mean counts of parasites per 100g of vegetable sample (mean ± SE)			
Vegetable	Eggs of *Ascaris* spp.	Eggs of *Ankylostoma* spp.	Cyst of *Entamoeba* spp.	Nematode larva
Egg plant	0±0.00	25±0.39	5±0.12	5±0.12
Carots	0±0.00	0±0.00	130±1.72	5±0.12
Cellery	10±0.15	6±0.20	193±4.29	53±0.94
Red cabbage	0±0.00	0±0.00	0±0.00	0±0.00
Green Cabbage	16±0.50	0±0.00	0±0.00	3±0.10
Cucumber	13±0.30	3±0.10	6±0.20	0±0.00
Parsley	13±0.21	3±0.10	3±0.10	23±0.49
Leeks	0±0.00	6±0.20	0±0.00	3±0.10
Tomatoes	0±0.00	0±0.00	10±0.30	6±0.20
Promedio	6±0.22	4±0.16	36±1.63	11±0.39
P value	0.104	0.347	0.001*	0.184

*= Significant difference

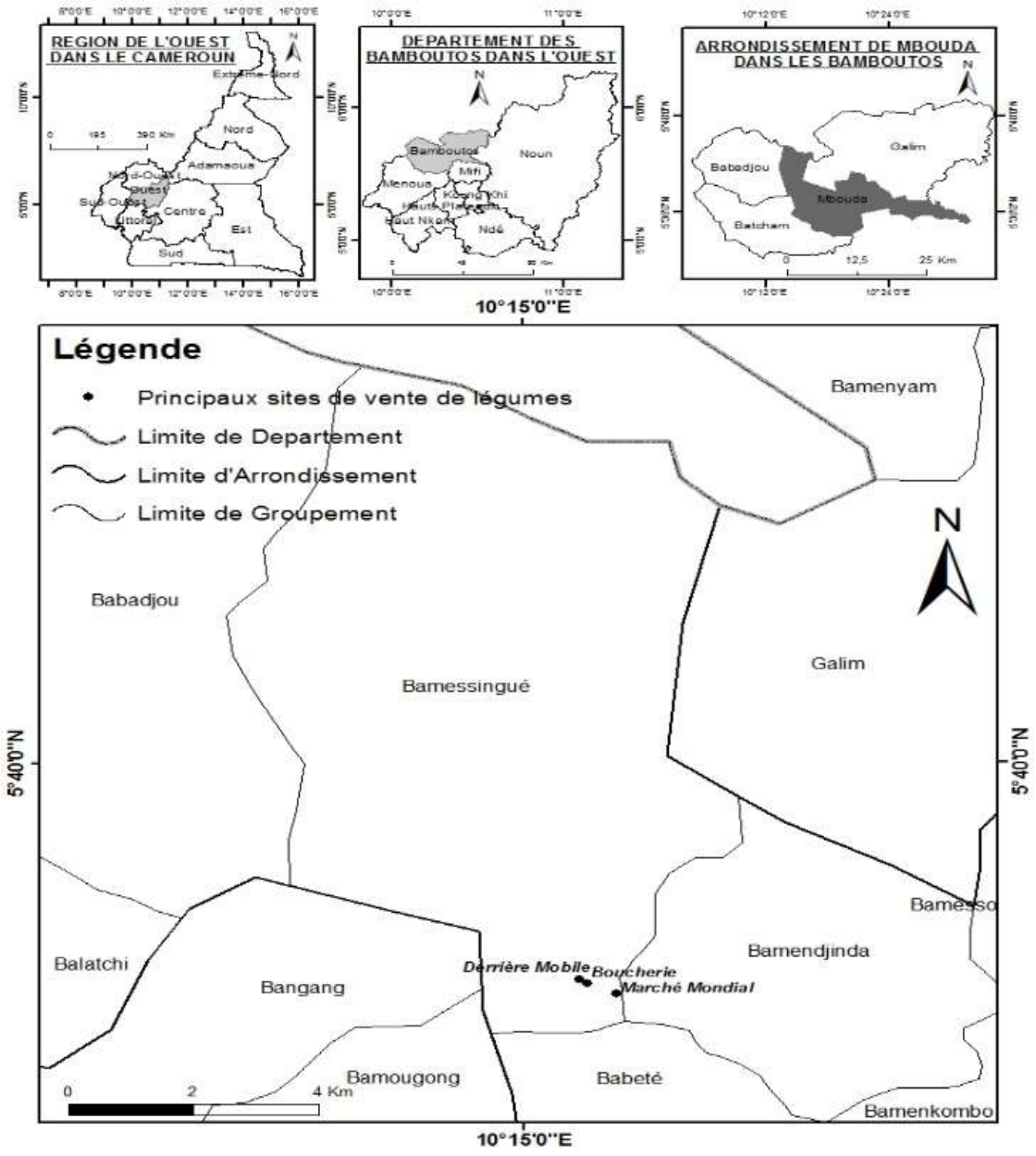


Figure 1. Location of study area (Mbouda)

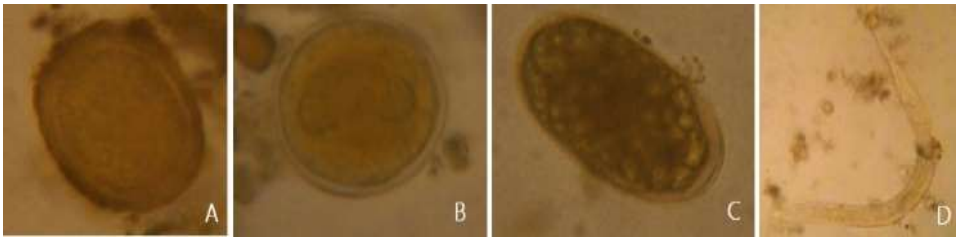
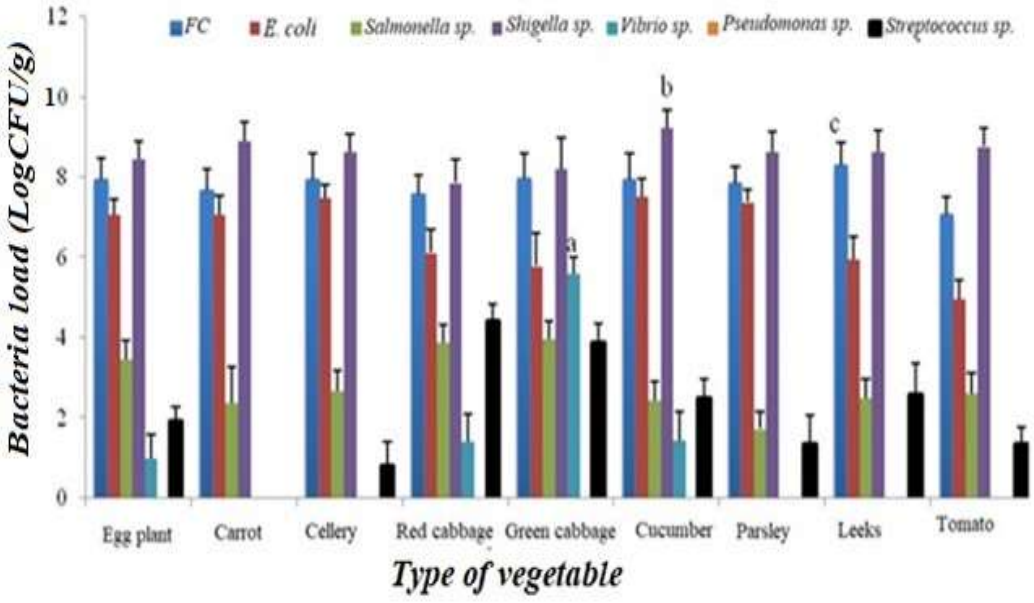


Figure 2. Images of parasites observed under the microscope from vegetable sample A) Egg of *Ascaris* spp. B) Cysts of *Entamoeba* spp. C) Egg of *Ankylostoma* spp D) Nematode larvae.



FC= Fecal coliform, *E. coli*= *Escherichia coli*, a, b, c =Significant difference of bacteria load

Figure 3. Distribution of bacteria isolates in vegetable samples

4. Discussion

4.1. Vegetables sold in the market of Mbouda are of poor parasitological quality

One of the objectives of this study was to investigate the potential parasitological contamination of fresh vegetables sold in markets of Mbouda in the west region of Cameroon. *Ankylostoma* spp, *Ascaris* spp. Eggs, *Entamoeba* spp. Cysts,Cysts and nematode larvae have been found. A single parasite (egg, cyst, or larva) present in food is capable of causing serious health problems in humans if ingested. According to the World Health Organization standard, vegetables intended for human consumption must not contain any parasites. with this standard, the vegetables sold in the market of Mbouda are of poor quality.

Indeed, 35% of vegetables collected were contaminated by at least one parasite, with concentrations ranging from 0 to 196 parasites per 100 grams of vegetable. These parasites come from the use of animal droppings for crop amendment, the poor quality of irrigation water or the poor treatment of vegetables during transport and in the market. Studies by Ntangmo Tsafack et al (14) identified parasites such as eggs of *Ascaris* spp., cysts of *Entamoeba* spp., and nematode larvae in the water used to irrigate vegetables in the lowlands of Mbouda. This prevalence of 35% is identical to that obtained in Nakhon in Thailand (1). However, this value is lower than what was recorded in Casablanca (63%), Morocco (52%), Cape Coast in Ghana and Jequitinhonha in Brazil (51%) (18,19,20). In contrast, it remains higher than that obtained in Burdur-Turkey (6%) and Dschang- Cameroon (8 %) (21,22). The differences observed could be linked to the size of the sample, the type of vegetable analyzed, the sampling period and the level of sanitation in each city.

The parasites observed in their proportions were eggs of *Ascaris* spp. (12%), eggs of *Ankylostoma* spp. (8%), cysts of *Entamoeba* spp. (17%) and nematode larvae (16%). The concentrations of eggs, cysts, and larvae in 100g of vegetable sample were: 6 eggs/100g for *Ascaris* spp., 4 eggs/100g for *Ankylostoma* spp., 36 cysts/100g for *Entamoeba* spp. CystCyst and 11 larvae/100g for nematode larvae. The presence of these parasites on vegetables, particularly *Entamoeba* cysts, represents a real risk to consumers. Indeed, cysts of *Entamoeba* spp. are without latency time and have a low infective dose. Although Ascaris eggs have a long latency period, their presence on vegetables is also a real risk, as they do not require an intermediate host (14). Therefore, they are likely to cause illness in the consumer if the vegetables are poorly washed or poorly cooked. The extent of the disease would be even more serious if 36 *Entamoeba* spp. Cysts and 6 roundworm eggs were consumed in 100g of vegetables. This suggests that these vegetables sold in Mbouda are partly responsible for intestinal parasitosis in the city. The presence of these parasites on vegetables would not only be linked to climatic conditions that would favor their survival in the environment, but also to their ability to resist extreme environmental conditions. Indeed, Ascaris eggs and *Entamoeba* spp. Cysts can persist for more than 16 weeks in the soil (23), and approximately 30 and 10 days respectively on crops (12,24). This relatively long tracking time in the environment increases the chances of consumer infestation.

It is seen from this study that cysts of *Entamoeba* spp. were more prevalent (17%) on vegetables sold in Mbouda. This could be linked to the fact that this pathogen is cosmopolitan and affects millions of people

per year. As Benazzou (25) reports, Entamoeba affects more than 50 million individuals annually. The poor hygiene of the city's inhabitants would be partly responsible for their dominance over vegetables. This prevalence is higher than that obtained in Kenya (7.6%), but lower than that detected in Nigeria (26%) on vegetables collected in markets (26,27).

Nematode larvae were detected on 16% of the vegetables analyzed. This could be explained by the exposure of vegetables to stagnant water and watering with wastewater. This prevalence is higher than that of 8.6% observed in Morocco (19). However, it is lower than that of 36.5% observed in Brazil (20). Due to the morphological similarities, it was not possible within the framework of this study to make a specific identification of these larvae. However, attention should be drawn to the possible presence of larvae of *Ankylostoma duodenale*, *Necator americanus*, and/or *Strongyloides stercoralis* among these larvae. These parasites are of great clinical and epidemiological interest, particularly in people suffering from malnutrition or immunosuppression, who are vulnerable to forms of serious infestations linked to these parasites.

Among the vegetables analyzed, celery and parsley were the most contaminated by parasites. This would be linked to irregularities (leaf undulations) and leaf density, which would create a micro-habitat favorable to the survival of pathogens. In addition, the leaves of these vegetables are generally in direct contact with the soil, which would increase the risk of contamination. These results corroborate those of several studies, which revealed a high contamination rate on leafy vegetables (28, 29), but also contrast with some studies (30), which observed high contamination rates on root vegetables (carrot). On the other hand, fruit and vegetables, apart from the absence of contact with the ground in certain cases, have a smooth structure; therefore, few parasites adhere to their surface.

The occurrence of these parasitic stages on vegetables sold in the town of Mbouda poses a real health risk for consumers, since it only takes one parasite to cause a disease, and could possibly be responsible for the resurgence of waterborne diseases in the town of Mbouda.

4.2. Vegetables sold in the market in Mbouda have bacteria loads higher than health standards

Bacteriological analysis of sampled vegetables sold in the town of Mbouda revealed the presence of six bacterial isolates: Fecal coliform, *Vibrio* spp., fecal streptococci, *Shigella* spp., *Salmonella* spp., and *Escherichia coli,* which represent a real danger for consumers. The presence of these bacteria would be favored by poor hygiene in production sites (watering with wastewater), lack of personal hygiene of vendors, vectors within the markets, and cross-contamination when vegetables are manipulated within the site when displayed. These results are similar to those obtained by Vouffo (22) who indicated that unhygienic production and sales conditions are the cause of the contamination of the vegetables sold. Likewise, the work of Ntangmo Tsafack (30,14) documented the use of polluted water for irrigation in the bacteriological contamination of vegetables.

The presence of fecal streptococci and *Escherichia coli,* coupled with high concentrations of fecal coliforms on collected vegetables raises concerns, as they indicate

fecal contamination of vegetables. This raises questions about the personal hygiene of sellers and the sanitary state of vegetable production, sale and storage sites. Indeed, the presence of these indicators of fecal contamination would be linked to the application of untreated human and animal waste in agricultural plantations, the visit of storage places by animals carrying pathogens and the persistence of these pathogens on vegetables. Very often, these pathogens can survive on crops for several weeks (24).

In addition, the presence of *Vibrio* spp., *Salmonella* spp., and *Shigella* spp. results from exposure to open air, washing and refreshing of vegetables with dirty water. It could also be linked to the use of agricultural plantations as toilets by the population. This practice increases the occurrence of these bacteria and other gastroenteritis pathogens on vegetables. *Vibrio* spp. and *Salmonella* spp. have also been reported on vegetables sold in the town of Maroua in Cameroon (23). The microbiological quality of a food is considered satisfactory if pathogens such as *Salmonella* spp., *Vibrio* spp., *Shigella* spp., and *Escherichia coli* O157: H7 are not detected in 25 grams of food products (31,32,33).

This study also revealed that the identified bacteria in vegetables had loads exceeding or many times higher than WHO standards (10^3 cfu/g), with *Shigella* spp. 10^8 to 10^9, *Salmonella* spp. 10^2 to 10^5, fecal coliforms 10^4 to 10^5, and *E. coli* 10^2 to 10^4 times higher. with health standards, vegetables sold in the town of Mbouda are very dangerous for health and therefore unfit for consumption. Although confirmatory biochemical tests have not been carried out, the presence of bacteria indicative of fecal contamination, with loads well above the recommended health threshold, suggests that vegetables sold in the town of Mbouda could be a risk factor for the persistence water-borne diseases. This is a real public health problem in this city, taking reference to statistics from the Mbouda Health District (10% of cases reported between 2011 and 2013). These results are similar to those obtained from analyzed vegetables sold in markets in Fako – Cameroon, Mettu -Ethiopia, and in Sango Ota- Pakistan (28,34,35).

Under these conditions, it appears that certain cases of gastrointestinal infections and other waterborne diseases, from which the population suffers in Mbouda town, are certainly in connection with the consumption of fresh vegetables.

5. Conclusion

Fecal coliform, *Vibrio* spp., fecal streptococci, *Shigella* spp., *Salmonella* spp., and *Escherichia coli* were detected in vegetables sold in the town of Mbouda with loads above the health threshold. Cysts of *Entamoeba* spp., Nematode larvae, eggs of *Ascaris* spp. and *Ankylostoma* spp. were also observed in these vegetables with prevalences of 17%, 16%, 12% and 8% respectively. These pathogens make them potential vectors for the transmission of bacteria and parasitic infections. Additionally, some of these bacteria, such as *Salmonella* spp., *Shigella* spp., *E. coli*, *Vibrio* spp., and parasites such as *Entamoeba* spp. isolated from these market vegetable samples are pathogens associated to water-borne diseases. It is therefore possible that vegetables sold in markets in Mbouda could be a cause of the persistent cases of water-borne diseases observed in this community.

Also, Mbouda is a medium-sized city in Cameroon, and its health situation is identical to that of the majority of cities in the country, making it a representative sample

of the situation in the majority of cities in Cameroon. Therefore, the results obtained in Mbouda would allow the prediction of the situation in the large cities of Cameroon.

Promoting methods to prevent occurrence and transmission of these pathogens, such as respecting hygiene rules during the production and sale of vegetables, washing, disinfection, and cooking before consumption, would be necessary in order to reduce health risks.

Funding

Authors received no funding or financial support for this study.

Author contributions

Conceptualization - Honorine Ntangmo Tsafack and Cédric Aimé Saah Fokou; Methodology - Honorine Ntangmo Tsafack and Cédric Aimé Saah Fokou

Formal analysis - Honorine Ntangmo Tsafack, Cédric Aimé Saah Fokou, Teh Exodus Akwa, Godfroy Rostant Pokam Djoko, Franck Robéan Wamba, and Emile Temgoua

Writing-original draft - Honorine Ntangmo Tsafack, Cédric Aimé Saah Fokou, Teh Exodus Akwa, Franck Robéan Wamba, and Emile Temgoua.

All authors read and approved the final manuscript

Conflict of interest

Authors declare no competing interests in the publication of this article

Data availability

Data will be made available upon reasonable request.

Acknowledgement

We thank the Applied Biology and Ecology Research Unit of the University of Dschang for permission and monitoring of this research work.

References

1. Punsawad C, Phasuk N, Thongtup K, et al. Prevalence of parasitic contamination of raw vegetables in Nakhon Si Thammarat Province, Southern Thailand. B.M.C.l Public Health. 2019; 34:1-7.
2. Amahmid O. Health impact of the reuse of wastewater in agriculture and the effect of its treatment on protozoan cysts and on the hygienic quality of crops. Master's thesis. 2004; Cadi Ayyad University, Faculty of Sciences, 54
3. Ferguson S, Roberts C, Handy E, Ferguson S, Roberts C, Handy E, Sharma M., et al. Lytic bacteriophages reduce *Escherichia coli* O157: H7 on fresh cut lettuce introduced through cross-contamination. Bacteriophage. 2013; 3(1):e24323.
4. Little C, Gillespie I. Prepared salads and public health. J. Applied Microbiol. 2008; 6:1729-1743.
5. Akwa TE, Nguimbous SP. Investigation of typhoid fever and its associated risk factors in children attending "Deo Gratias" Hospital in Douala, Littoral, Cameroon. Eur J Med Educ Technol. 2021; 14(2): em2107.
6. Atchibri A, Yapi AY, Yapo TM, et al. Microbiological evaluation and origins of contamination of 4th range products sold on the markets of Abidjan, Ivory Coast. Eur. Sci. J. 2016; 36.
7. Eni AO, Oluwawemitan IA, Solomon OU. Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. Afr J Food Sci. 2010; 5:291-296.
8. Amoah P, Drechsel P, Abaidoo RC. Irrigated urban vegetable production in Ghana: Sources of pathogen

contamination and health risk elimination. J Water Health. 2007; 1:49-61.

9. Kozan E, Gonenc B, Sarimehmetoglu O, et al. Prevalence of helminth eggs on raw vegetables used for salads. Food Control. 2012; 16:239-42.
10. Buck JW, Walcott RR, Beuchat LR. Recent trends in microbiological safety of fruits and vegetables. Plant Health Prog. 2003; 10:1094-1103.
11. Beuchat LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microb. Infect. 2002; 4:413-423.
12. Ntangmo Tsafack H, Temgoua E, Njine T. Persistence of *E. coli*, helminth eggs and protozoan cysts contained in urban wastewater from irrigation of market garden crops in Dschang, West Cameroon. Int J Biol Chem Sci. 2019a; 5:81-90.
13. Ntangmo Tsafack H, Meli TA, Robean WF, et al. Consequences of hygiene and sanitation limitations on physicochemical, bacteriological and parasitological quality of water for vegetable irrigation purposes in the Mbouda lowlands, Cameroon, western highlands. Irrigation and Drainage. 2023;1–14.
14. Ntangmo Tsafack H, Temgoua E, Njine T. Sanitary quality of irrigated vegetables in the swampy lowlands of Dschang city, Cameroon, Irrig. Drain. 2019; 68(5):961-8.
15. Asongwei AG, Yerima PK, Tening AS. Vegetable production and the livelihood of farmers in Bamenda Municipality, Cameroon. Int. J. Curr. Microbiol. App. Sci. 2014; 12:682–700.
16. Tangwa S. The causes of the recurring water shortage in the city of Mbouda. The mirror of Western Cameroon and the diaspora. 2012; Menola N°20, 12 P.
17. Andoh lA. Helminth contamination of lettuce and associated risk factors at production sites, markets, and street food vendor sites in urban and peri-urban Kumasi. Master Thesis. 2006; Kwame Nkrumah University of Science and Technology, Kumasi, Faculty of Bioscience, 87p.
18. Dankwa K, Owusu SD, Kofi OE, et al. Parasitic profile of fresh vegetables sold in selected markets of the Cape Coast Metropolis in Ghana. An Res Rev Bio. 2019; 6:1-7
19. Hajjami K, Ennaji MM, Amdiouni H, et al. Parasitic contamination on fresh vegetables consumed in Casablanca city (Morocco) and risk for consumers. Int J Sci Tech. 2013; 7: 543-549.
20. Luz JG, Barbosa MV, Carvalho AG, et al. Contamination by intestinal parasites in vegetables marketed in an area of Jequitinhonha Valley, Minas Gerais, Brazil Rev Nutri Camp. 2017; 1:127-136.
21. Adanir R, Tasci F. Prevalence of helminth eggs in raw vegetables consumed in Burdur, Turkey. Food Control. 2013; 31(2):482-4.
22. Vouffo TA. Parasitological and bacteriological quality of vegetables sold in Dschang (West, Cameroon). Masters Thesis. 2017; University of Dschang, Faculty of Science, 71P.
23. Maïwore J, Baane MP, Tatsadjieu NL, et al. Microbiological quality of lettuce (*Lactuca sativa*) consumed on the streets of Maroua (Cameroon): Effect of disinfecting agents used by some vendors. Int J Microbiol Res. 2017; 8:913-918.
24. Feachem RG, Bradley DG, Garrelick H, et al. Sanitation and diseases: Health aspects of excreta and wastewater management, John Wiley, 1983; 1:51.
25. Benazzou N. Intestinal parasitoses. Parasitology-Mycology Laboratory. 2010; 68p.
26. Akinseye JF, Ayuba SB, Adewuyi IK, et al. Isolation of intestinal parasites in vegetables sold in major markets in Akure, Ondo State, Nigeria. Int J Health Sci Res. 2017; 6:2249-9571.
27. Habtu M. Parasite contamination of raw vegetables from selected farms and markets in Addis Abeba, Ethiopia.

Master thesis. 2011; Addis Ababa University, Faculty of Life Sciences, 66 P.

28. Akoachere KJ, Fossi TB, Mbapngong NJ. Bacterial and parasitic contaminants of salad vegetables sold in markets in Fako Division, Cameroon, and evaluation of hygiene and handling practices of vendors. BioMed. Central Res. Note. 2018; 100:1-7.
29. WHO. Environmental factors are the cause of 24% of diseases. 2006; http://www.futura-science.com
30. Ntangmo Tsafack H. Practices and health risks of the use of urban wastewater in agriculture: The case of the city of Dschang-Cameroon. Doctoral thesis. 2015; University of Yaoundé I / Faculty of Sciences. 206p.
31. FSANZ (Food Standards Australia New Zealand). Compendium of microbiological criteria for Food. 2018; 51 P.
32. HPA (Health Protection Agency). Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market. 2009; 34p.
33. Health Canada. Guidelines on microbial risks associated with ready-to-eat foods. 2010; 9 P.
34. Eshetu C, Tsegaye B, Yohannes L, et al. Parasitological survey and antimicrobial susceptibility patterns of microbial isolates from pre-washed fruits and vegetables collected in Mettu Town, Southwest Ethiopia. Indo Americ J Pharmac Sci. 2018; 11:13040-13049.
35. Hannan A, Rehman R, Saleem S, et al. Microbiological analysis of ready-to-eat salads available at different outlets in Lahore, Pakistan. Int Food Res J. 2014; 21(5):1797.