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Parasitological and Bacterial Assessment of Vegetables Commonly Sold at the Bamenda Food Market, North West Region, Cameroon

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ABSTRACT

Background and Objective: Vegetables are very important for the human diet as they provide all the nutrients needed to be healthy. However, they constitute major infectious agents. Thus monitoring of contaminants in vegetables is very crucial for human safety. This study aimed to assess common bacterial and gastrointestinal parasitic contaminants in vegetables and determine the risk factors associated with contamination of these vegetables and provide baseline information for the control of these diseases. **Materials and Methods:** A cross-sectional study was conducted on 180 local vegetables sold at the Bamenda food market. A structured questionnaire was administered to vendors and vegetable samples like carrots, cabbage, lettuce, huckleberries, etc., were collected for bacterial and parasitic assessment. Microscopy was done for parasites while culture was done for *Escherichia coli* (*E. coli*) isolation. Data was analyzed by SPSS version 25.0. **Results:** The general prevalence of parasites in vegetables collected from the food market was 53.9%. *Entamoeba histolytica* (30.6%) was the most prevalent gastrointestinal parasite. The prevalence of contamination of each vegetable ranges from Cabbage 17.19%, Carrots (14.28%), and tomatoes (14.28%). The risk factors associated with vegetable contamination were vegetable types, poor hygiene and sanitation of the vendors, and poor environmental conditions of the Bamenda Food Market. **Conclusion:** Vegetable samples collected from food market, Bamenda were highly contaminated with both *E. coli* and gastrointestinal parasites hence consumers are at risk of contracting food-borne illnesses. Proper personal and environmental hygiene should be maintained while handling these vegetables.

KEYWORDS

Gastrointestinal parasitic, bacteria, vegetable, Cameroon, environmental hygiene, food-borne illnesses

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INTRODUCTION

Being a source of vitamins, minerals, and phytochemicals, some of which include antioxidants, phytoestrogens, and anti-inflammatory agents, vegetables offer health-promoting properties[1,2]. Vegetable consumption has increased as a result of the recent rise in public knowledge of their health advantages. A low fruit and vegetable diet raises the risk of non-communicable illnesses and is associated with ill health[3]. Vegetable consumption as part of a diet helps people lose weight[4], which lowers their chance of obesity, and raises their risk of developing non-communicable diseases. To preserve their natural flavor and nutrients that are sensitive to heat, many vegetables are eaten raw in salads. Given that raw vegetables have been reported to contain parasites[5] and harmful bacteria, there is significant worry about the safety of eating raw vegetables. Contamination is a result of unsanitary manufacturing and post-harvest procedures[6,7]. The geographic spread of these infections has been facilitated by trade[8]. Studies in developed and developing countries have demonstrated the potential of raw vegetables to transmit pathogens[9,10]. Despite a rise in vegetable consumption and cultivation, there is a dearth of information on the microbiological quality of vegetables in Cameroon. This is because, in addition to providing food security, vegetable farming has turned into a source of revenue[6,11]. Vegetables are irrigated with tainted water, according to recent studies conducted in Cameroon[7,12]. Evaluating the microbiological safety of salad vegetables sold in Cameroon is therefore urgently necessary. This study investigated the microbiological quality of vegetables sold in the Bamenda food market, North West Region of Cameroon, to highlight their potential for disease transmission and provide data on risk factors associated with contamination.

MATERIALS AND METHODS

Study design and study site: A cross-sectional study was conducted from March to June, 2024 in the Bamenda food market. This study was carried out in Bamenda, Northwest Region of Cameroon. Vegetables are sold at the Bamenda food market. The food market is found in Bamenda 2 subdivision, Mezam division North West Region of Cameroon with Latitude 5°57′25.32″ and Longitude 10°8′50.5″.

Study population: It was carried out on vegetable samples purchased randomly from retailers at the Bamenda food market. A total of 180 fresh vegetable and fruit samples, including eight different types that are frequently consumed in the area were randomly purchased. The fresh raw vegetable samples used in this study included Lettuce (*Lactuca serriola*), Cabbage (*Brassica oleracea*), Carrot (*Daucus carota*), Green Pepper (*Capsicum annuum*), Okra (*Abelmoschus esculentus*), Cucumber (*Cucumis sativus*), Tomatoes (*Solanum lycopersicum*), Garden Huckleberry (*Solanum scabrum*), Garden egg (*Solanum aethiopicum*), Waterleaf (*Talinum fruticosum*), Pumpkin leaves (*Cucurbita moschata)* and Celery (*Apium graveolens*).

Sample size: The 180 vegetable samples were collected in this study. The sample size was calculated using the Cochran formula[9] at 95% confidence interval and subjects were chosen at random:

$$N=\frac{Z^2p\,(1-p)}{d^2}$$

Where:

N = Required sample

Z = Confidence level at 95% (standard value 1.96)

p = Estimated prevalence of the infection of vegetables under investigation

d = Margin of error at 5%

The prevalence of parasites was 22.8% obtained from studies carried out by Akoachere *et al.*[13] bacterial and parasitic contaminants of salad vegetables sold in markets in Fako division, Cameroon:

$$P=23.65/100=0.2365\ Z=1.96,\ d=5/100=0.05$$

Hence:

$$N = \frac{(1.96^2)(0.2365)(1-0.2365)}{0.05^2} = 200$$

Hence, a total of 200 samples of vegetables were collected from different vegetable types.

Inclusion and exclusion criteria: This study includes vegetable vendors at the Bamenda food market and vendors who agree to sign the consent form. This study excludes all vendors who do not reside in the Northwest Region.

Data collection: A pre-tested structured questionnaire was used to collect data about pre-disposing factors for bacteria and gastrointestinal parasitic contamination of vegetables at local markets. The questionnaire was prepared in English and translated to Pigine (local language). Approximately equal amounts of vegetables were collected in properly labeled sterile plastic bags and transported to the laboratory for analysis.

Parasitological analysis: The method described by Fitsum *et al.*[14] was used for parasitological analysis. Briefly, each vegetable and fruit was washed separately in 250mL of normal saline to detach the parasitic stages (eggs, cysts, and oocysts) of helminths and protozoan parasites commonly assumed to be associated with vegetable contamination. The filtrate was allowed to Sediment for about 30 min, and 5 mL of each sediment from each washed sample was centrifuged for 5 min at 3000 rpm. The supernatant was discarded and the sediment was examined microscopically.

Bacteriological analysis: The filtrate from the washed vegetable was vigorously shaken and homogenized. The 1 mL of aliquots of each stock solution were poured into the MacConkey agar-prepared plates and allowed for 2 days at 37°C. For the isolation of *E. coli*, pink colonies on MacConkey agar were gram-stained and observed. Indole test was also used for confirmation of *E. coli* colonies.

Ethical consideration: Ethical consideration was obtained from the University of Bamenda Ethical Review Committee/Institute Review Board, the Faculty of Health Sciences Ethical number: 51755044.

Statistical analysis: The logistic regression was performed to identify the main risk factors associated with contamination of vegetables. The chi-square test of independence was used to test the association between the different risk factors and the prevalence of *E. coli.* The significance threshold was set at 95% Odd Ratio>1: The association is positive the risk factor is higher in exposed subjects than in unexposed subjects equals to risk factor. OR<1 the association is inverse-the risk of disease is lower in exposed subjects than in unexposed subjects. OR = 1, there is no association between exposure and disease.

RESULTS

Socio-demographic characteristics of the study population: Table 1 shows the socio-demographic characteristics of vendors at the food market in Bamenda. A total of 180 vegetable samples were collected from 52 vendors. Of the vendors interviewed, 33.3% of the vendors were between the ages of 46-55 years, 27.1% were 31-45 years, 22.9% were 15-30 years, and 16.7% were 56 years and above. The majority of the vendor population were females (85.4%) while the minority were males (14.6%). Vendor's educational status was as follows; 35.4% had no formal education, 34.5 % had primary education, 27.1% had secondary education and 2.1% of them had university education. 68.8% of vendors were traders, while 27.1% were farmers, and 4.2 % had other occupations.

General Prevalence of gastrointestinal parasites in vegetables: The general prevalence of parasites in the 180 vegetables samples collected was 53.9% contamination.

Prevalence of various parasites: Table 2 shows the prevalence of each parasite species. *Entamoeba species* was the most common parasites found with a prevalence of 30.6%, followed by *Giardia lambia* (22.8%), *Ascaris lumbricoides* (11.7%), *Strongyloides stercoralis* and *Trichuris trichiura* (6.1%) and the least prevalent parasites found were hookworm and *cryptosporidium* with 1.1%, respectively.

Prevalence of contamination of vegetables with respect to species of parasites: Table 3 showed the prevalence of contaminated vegetables for species of parasites, it follows from the analysis that 15 samples of each vegetable type were collected and analysed and the results show that, Cabbage was the most contaminated vegetable type with 17.19% contamination, carrots, and tomatoes had 14.28% contamination, celery (12.03%), lettuce (11.27%), okra and pumpkin leaf had (6.77%) each, water leaf (5.26%), huckelberry leaves (4.51%), green pepper (3.76%), garden eggs (3.01%), and cucumber, which was the least contaminated (0.75%).

Table 1: Socio-demographic characteristics of vendors

Variable	Modalities	Effectiveness	Percentage
Age	15-30	11	22.9
	31-45	14	27.1
	46-55	17	33.3
	56>	8	16.7
Sex	Female	42	85.4
	Male	8	14.6
Highest level of education	No formal education	17	35.4
	Primary education	13	35.4
	Secondary education	17	27.1
	University education	1	2.1
Occupation	Farmer	13	27.1
	Traders	33	68.8
	Others	2	4.2

Table 2: Prevalence of gastrointestinal parasites concerning gastrointestinal parasites

	Sample size: n = 180	
Number of parasites examine	Number of positive	Prevalence (%)
Giardia lamblia	41	22.8
Entamoeba histolytica	55	30.6
Ascaris lumbricoides	21	11.7
Trichuris trichiura	11	6.1
Strongyloides stercoralis	11	6.1
Hookworm	2	1.1
Cryptosporidium parvum	2	1.1

Table 3: Prevalence of contamination of vegetables concerning species of parasites

	Parasites identified in vegetables (number of parasites = 133)							
Variable	*G. lamblia*	*E. histolytica*	*A. lumbricoides*	*T. trichiura*	*S. stercoralis*	Hookworm	*C. parvum*	Total (%)
Cabbage	5	9	4	2	2	0	1	23 (17.19)
Carrot	5	5	4	2	2	1	0	19 (14.28)
Celery	7	7	2	0	0	0	0	16 (12.03)
Cucumber	0	1	0	0	0	0	0	1 (0.75)
Garden eggs	1	2	0	0	0	0	1	4 (3.01)
Green pepper	2	3	0	0	0	0	0	5 (3.76)
Huckleberry leaves	4	4	0	1	1	0	0	6 (4.51)
Lettuce	5	6	2	1	2	0	0	15 (11.27)
Okra	1	5	1	1	1	0	0	9 (6.77)
Pumpkin Leaves	4	2	2	1	0	0	0	9 (6.77)
Tomatoes	5	7	3	3	1	0	0	19 (14.28)
Waterleaf	2	4	1	0	0	0	0	7 (5.26)
p-value	0.056	0.049	0.091	0.360	0.428	0.438	0.520	-

Prevalence of *E. coli*: Out of the 180 samples collected from the different vendors, 49.4% of samples were reported positive while 50.6% negative.

Prevalence of *E. coli* concerning vegetable types: Figure 1 shows the frequency of contamination of vegetables by *E. coli*. It follows from the analysis of this figure that, all cabbage vegetable samples tested positive for *E. coli,* followed by carrots (14 out of 15), tomatoes (11 out of 15), lettuce (10 out of 15), celery (8 out of 15), garden eggs, pumpkin leaves and huckleberry (6 out of 15 each), green pepper, water leaf, and okra (4 out of 15 each), and last 1 out of 15 cucumbers which was the least contaminated.

Table 4: Risk factors associated with contamination of vegetables

Variable	Modalities	Prevalence	p-value	Odd ratio
Vegetable type	Leafy	18	0.98	5.429
	Root	6	0.99	4.694
	Fruit	7	-	-
Washing	No	22	0.96	4.69
	Yes	9	-	-
Source of wash water	Pipe	1	0.99	0.001
	River	6	0.99	-
	Well	24	-	-
Safety procedure	No	22	0.982	4.0
	Yes	9	-	-
Complaints	No	24	0.995	-
	Yes	7	-	-
Training	No	25	0.992	-
	Yes	6	-	-
Risk awareness	No	15	0.930	-
	Yes	16	-	-
Sources of contamination	Farms	10	0.981	1.56
	Local	21	0.881	9.217
	Others	0	-	-
Handling	No	10	0.982	1.07
	Yes	21	-	-
Means of display	On the floor	19	0.981	-
	On tables	5	0.995	0.052
	On wheelbarrow	7	-	-
Fingernails status	Untrimmed	1	0.952	4.21
	Trimmed	30	-	-
Vendors surrounding	Unclean	0	0.969	2. 53
	Clean	31	-	-

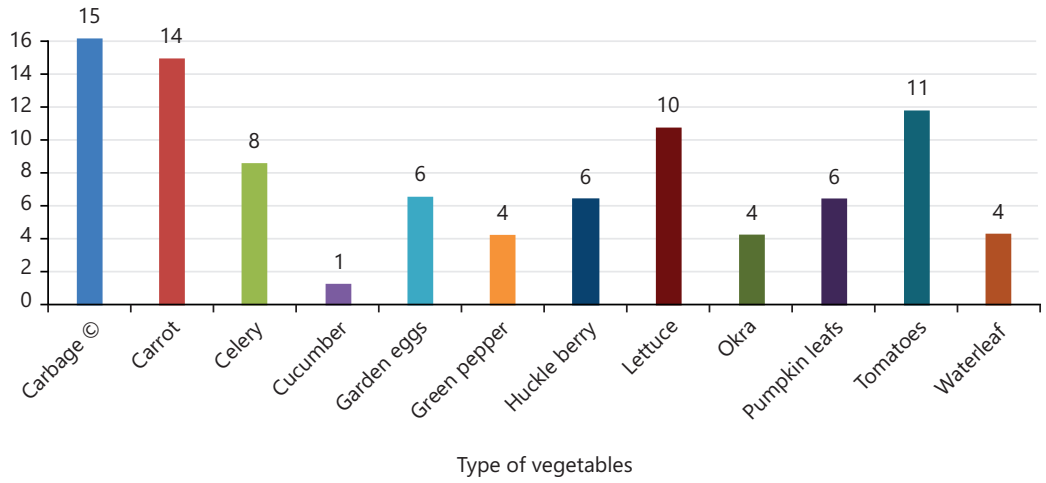

Fig. 1: Frequency of contamination of vegetables by *E. coli*

Risk factors associated with contamination of vegetables: Table 4 shows the risk factors associated with the contamination of vegetables sold at the food market in Bamenda. It follows from the analysis of this table that, leafy and root vegetable type, the absence of washing, the absence of safety procedure, farms and local sources of contamination, the absence of handling, untrimmed fingernails, and unclean vendors surrounding were statistically non-significant risk factors.

DISCUSSION

The overall prevalence of parasites in all vegetable samples collected at the food market in Bamenda was 53.9%. These results were similar to research carried out on the frequency of parasites in lettuces and cabbages sold in some selected markets in Maputo city, the capital of Mozambique[15], and parasitic contamination of commonly sold vegetables in Ghana (52.4%)[16,17]. However, the results obtained were higher than those carried out on the contamination of lettuce Mfoundi division, Cameroon (28.21%)[18] and lower than research carried out in Yemen (76.9%). The differences may be attributed to the different sample populations used, diagnostic tests, and environmental and geographical differences. In addition, the difference in this study and others might also be due to the type of soil and water used for agriculture and poor hygienic conditions during the transportation and marketing of these vegetables. Under these conditions, it appears that certain cases of gastrointestinal infections and other waterborne diseases, from which the population suffers in Bamenda town, are certainly in connection with the consumption of fresh products contaminated by watering water.

Entamoeba histolytica, was the most prevalent protozoan (30.6%) which is consistent with research carried out in Gaza, Palestine (37.5%)[19]. Also, research in different cities in Ethiopia, Debre town and Bahir city show *Entamoeba* as the most common parasite with lower prevalence of 13.0 and 12.8%, respectively[20]. This is because, cysts of *Entamoeba* can be found in water, soil, and environment and their ability to withstand harsh environmental conditions is quite high the high prevalence of *Entamoeba* is a public health concern, as *Entamoeba* is the 3rd most common cause of parasitic mortality worldwide. *Ascaris lumbricoides* was found to be the most prevalent intestinal helminth, with a prevalence of 11.7% this aligns with studies carried out in Brazil with a prevalence of 12.7%[21].

The high prevalence of Ascaris in these vegetable samples is because Ascaris has 3 layers of skin which makes it resistant to harsh environmental conditions. The egg of this parasite is also resistant to chemical disinfectants. Ascaris produces about 200,000 eggs each day, which are present in feces and can contaminate the environment[22]. The differences in these results when compared with other studies are due to differences in environmental conditions, economic conditions, and different laboratory techniques in identifying these parasites. The contamination of these cleaning waters with these parasites can be explained either by the origin of the water used for washing or the contamination of previously washed vegetables. These results obtained during the studies are close to the prevalence of 23.3% obtained[23] in Sudan on the parasitological quality of washing water used by traders.

For the contamination trends of all vegetable types, the order of contamination ranges from Cabbage>Carrot>tomatoes>lettuce>celery>garden eggs huckleberry and pumpkin, leaves> green pepper, waterleaf and okra>cucumber. Cabbage was the most contaminated vegetable, while cucumber was the least contaminated. The frequency of contamination was due to the proximity of the plant to the soil and the large rough surfaces of the leaves of cabbage plants, carrots, and lettuce which facilitate the attachment of different parasitic stages to their surfaces. Cucumber on the other hand has a smooth-surface which makes it difficult for attachments, and easy to wash and peel before eating.

The overall prevalence of *E. coli* was 49.4% from vegetables collected from the food market in Bamenda. Other similar studies carried out in Jashore Bangladesh show a prevalence of 46%[24]. In contrast, Turkey shows a higher prevalence of 62.5%[25] and research carried out in Bangkok, Thailand shows a, lower revalence of 32% *E. coli* isolated from fresh vegetables. These differences in results may be due to differences in sample size of the different researchers, different techniques in the enumeration of *E. coli* strains, and economic and environmental standards of the study population. While most *E. coli* strains are harmless, pathogenic *E. coli* strains produce Shiga toxins which cause severe food-borne illnesses.

The high prevalence of *E. coli* in this study is a call for concern because infection with pathogenic *E. coli* may cause symptoms varying from mild diarrhea, and vomiting to severe symptoms like hemorrhagic colitis, hemolytic uremic syndrome in children, and thrombotic thrombocytopenic purpura in elderly which are very deadly[26]. The presence in this water of high concentrations of bacteria from fecal contamination makes it possible to consider the infections of bacterial origin in enterotoxin *Escherichia coli* (gastroenteritis), Cabbage was the most contaminated vegetable sample followed by carrots, this ties in with the fact that these 2 vegetables are close to the ground, the rough and uneven surface of these vegetables also contributed, hence susceptible to contamination by *E. coli*. The least contaminated samples were cucumber which is far from the ground surface and has a smooth surface reducing susceptibility.

CONCLUSION

The study suggests that unsanitary handling practices of the vendors, inadequate washing and disinfection, poor agricultural practices, cross-contamination from untrimmed nails, and dirty markets were significant risk factors for bacterial and parasitic contamination of vegetables sold at the food market. Proper personal and environmental hygiene should be maintained while handling these vegetables such as; cleaning their sales environment, using pipe-borne water to wash vegetables, and wearing gloves when touching these vegetables to avoid cross-contamination from other sources

SIGNIFICANCE STATEMENT

This study aimed to assess common bacterial and gastrointestinal parasitic contaminants in vegetables and determine the risk factors associated with contamination of these vegetables to provide baseline information for the control of these diseases. The general prevalence of parasites in vegetables collected from the food market was 53.9%. *Entamoeba histolytica* (30.6%) was the most prevalent gastrointestinal parasite. Proper personal and environmental hygiene should be maintained while handling these vegetables. The general public should properly wash vegetables with acetic acid (vinegar) OR cooking salt to kill bacteria and remove parasitic eggs and wash all vegetables under running tap water.

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