

Microbial and Parasitic Contamination on Fresh Vegetables Sold in Traditional Markets in Hue City, Vietnam

Ho Le Quynh Chau^{1,*}, Ho Trung Thong¹, Nguyen Van Chao¹, Pham Hoang Son Hung¹, Vu Van Hai¹, Le Van An¹, Ayako Fujieda², Tanaka Ueru³, Miki Akamatsu⁴

¹College of Agriculture and Forestry, Hue University, Vietnam

²Graduate School of Global Environmental Studies, Kyoto University, Japan

³Research Institute for Humanity and Nature, Kyoto, Japan

⁴Graduate School of Agriculture, Kyoto University, Japan

*Corresponding author: holequynhchau@huaf.edu.vn

Received October 29, 2014; Revised November 12, 2014; Accepted November 20, 2014

Abstract This study was conducted to evaluate microbial and parasitic contamination in twelve types of popular vegetables in Hue city. A total of 108 vegetable samples, equal numbers of young mustard greens, celery, amaranth, cilantro, water spinach, rice paddy herb, Vietnamese cilantro, basil, centella, lettuce, watercress, and iceberg lettuce were collected from three traditional markets in Hue city. All of samples were tested for total aerobic bacteria counts and *E. coli* by traditional culture-based methods. The *Salmonella* and parasites on the vegetables were detected by PCR technique and microscopic methods, respectively. All samples were highly contaminated with aerobic bacteria and *E. coli*. The aerobic bacteria counts ranged from 6.84 to 8.40 log CFU/g. *Escherichia coli* levels ranged from 5.47 – 6.88 log CFU/g. *Salmonella* was detected in 19/108 of test samples. Water spinach was found to have the highest contamination prevalence with *Salmonella* (55.56%). Contamination by multiple parasites was detected in all vegetable samples. The contamination rates of *Fasciola*, *Ascaris*, *Trichuris* and *Clonorchis sinensis* eggs were 83.33%, 85.19%, 64.81% and 16.67%, respectively. The oocysts of *Cryptosporidium*, *Isospora* and *Cyclospora* were found on the samples at rates of 47.22%, 27.78% and 27.78%, respectively. These bacteria and parasites may become potential sources of cross contamination and pose a serious risk to human health.

Keywords: aerobic bacteria, *E. coli*, parasite, *Salmonella*, vegetable

Cite This Article: Ho Le Quynh Chau, Ho Trung Thong, Nguyen Van Chao, Pham Hoang Son Hung, Vu Van Hai, Le Van An, Ayako Fujieda, Tanaka Ueru, and Miki Akamatsu, "Microbial and Parasitic Contamination on Fresh Vegetables Sold in Traditional Markets in Hue City, Vietnam." *Journal of Food and Nutrition Research*, vol. 2, no. 12 (2014): 959-964. doi: 10.12691/jfnr-2-12-16.

1. Introduction

Vegetables are essential ingredients in Vietnamese diet. Many kinds of vegetables are consumed fresh (raw) and provide benefit nutrients as vitamins, fiber and ash for human body. However, these vegetables may be a potential source of infection if they are contaminated. Evaluation of hygienic-sanitary quality of vegetables is not only based on chemical safety (pesticide and hormone residues, heavy metals) but biological safety (bacteria and parasites).

Cases of food poisoning due to vegetable consumption have been reported. Contamination with bacteria (especially *E. coli* and *Salmonella*) and parasites on vegetables have been reported in many countries ([11,16,24]). *Salmonella* was detected in 5.76% of the examined vegetables in Mexico [16]. Meanwhile, *E. coli* and *Salmonella* were found on 7.5% and 86.1% of

the vegetable samples collected from farms, a wholesale market, supermarkets and small shops in Granada, Spain [19]. Vegetables may be infected by bacteria and parasites during the planting (through contaminated fertilizer, water source and soil), transportation, post-harvest or unhygienic cooking ([5,8,12]).

Using fresh stool as fertilizer is still popular in Vietnam. A previous survey implemented by Uga et al (2009) has shown that 81% (121/149) farmers who lived in suburban Hanoi, Vietnam used animal and human feces as fertilizer frequently [23]. Therefore, the risk of microbiological infection related to vegetable consumption is still high whilst using raw vegetables in daily meals continues to play a key role in Vietnamese cuisine. The objective of this study was to evaluate the contamination of vegetables in traditional wet market in Hue city, Vietnam. Information obtained from this study will help vegetable consumers understand the current situation, and provide information to help local governments to develop policy to improve food safety and safeguard public health.

2. Materials and Methods

2.1. Sample Collection

A total of 108 samples of twelve vegetables types were randomly collected from three traditional markets (Tay Loc, Ben Ngu and An Cuu) in Hue city, Vietnam between March 2013 and January 2014. For each vegetable type, three samples from three different stalls were collected. The twelve types of vegetables analyzed in this study were young mustard greens (*Brassica juncea*), celery (*Apium graveolens*), amaranth (*Amaranthus tricolor*), cilantro (*Coriandrum sativum*), water spinach (*Ipomoea aquatica*), rice paddy herb (*Limnophila aromatica*), Vietnamese cilantro (*Persicaria odorata*), basil (*Ocimum basilicum*), centella (*Centella asiatica*), lettuce (*Lactuca sativa* var. *longifolia*), watercress (*Nasturtium officinale*), and iceberg lettuce (*Lactuca sativa* var. *capitata*). Five hundred grams of each vegetable were collected, identified and placed in separate sterilized bags and immediately transported to the laboratory in an ice box.

2.2. Evaluation of Aerobic Bacteria and *E. coli* Contamination

Twenty five grams of chopped vegetable sample were aseptically transferred into a stomacher bag. Samples were homogenized for 2 min at high speed (260 rpm) with 225 ml of sterile peptone water (0.1% peptone) using a Stomacher[®]400 Circulator (Seward, UK) [6]. Serial decimal dilutions of the supernatant were made in sterile peptone water and aliquots of 0.1 ml of the appropriate dilutions were surface spread in duplicate onto appropriate media. Total aerobic bacteria was enumerated on Plate Count Agar (PCA) (Oxoid CM 325, Unipath Ltd, Basingstoke, Hampshire, England) after incubation at 30°C for 48h [6]. *Escherichia coli* contamination on vegetables was evaluated by counting all colonies exhibiting typical dark to purple red colonies with metallic sheen grown on Eosin methylene blue (EMB) agar (Conda Laboratories, S.A., Spain) after incubation at 37°C for 24h. Confirmation tests were made on *E. coli* colonies by Gram stain, catalase, oxydase and biochemical tests (API 20E strips, Biomerieux, USA) [22].

2.3. *Salmonella* Detection

Buffered Peptone Water (10.0g/l peptone, 5.0g/l sodium chloride, 3.5g/l disodium phosphate, 1.5g/l monopotassium phosphate, pH 7.2) was used for preenrichment [1]. Twenty five grams of chopped vegetable sample were aseptically transferred into a stomacher bag containing 225 ml of Buffered Peptone Water, followed by homogenized for 2 min at high speed (260 rpm) using a Stomacher[®]400 Circulator (Seward, UK) [14]. The homogenized samples were incubated at 37°C for 24h.

Bacterial chromosomal DNA was isolated using DNA-boiling method. Bacteria suspension was centrifuged at 8,000 rpm for 8 min. The supernatant was removed and the pellet was resuspended in PBS (8g/l NaCl, 0.2g/l KCl; 1.44g/l Na₂HPO₄, 0.24 g/l KH₂PO₄, pH 7.2). The suspension was boiled in water bath at 100°C for 10 min and transferred into ice box immediately. After cooling down, it was centrifuged at 3,000 rpm for 10 min. The

supernatant was collected for PCR [2]. DNA of *Salmonella enterica* subsp. *diarizonae* (ATCC[®] 12325TM) was used as positive control.

The *invA* gene of *Salmonella* was amplified using PCR technique with primer set 139: 5'GTG AAA TTA TCG CCA CGT TCG GGC AA-3' and 141: 5'TCA TCG CAC CGT CAA AGG AAC C-3' [17]. PCR product size was 284bp. The PCR mixture contained 12.5 µl of GoTaq[®] Green Master Mix, 1.0 pmol of each primer, 4.0 µl of DNA template and nuclease-free water for a final volume of 25 µl. Amplification was performed using a gradient thermal cycler (MaxyGene, Axygen). The cycling conditions were as follows: heat denaturation at 94°C for 5 min, and then 35 cycles with heat denaturation at 94°C for 1 min, primer annealing at 64°C for 1 min, DNA elongation at 72°C for 1 min and final extension for 7 min at 72°C. Amplified products were electrophoresed in 2% agarose gel, stained with ethidium bromide (0.5 µl/ml), visualized and photographed with Gel DocTM XR+ system (Biorad).

2.4. Parasitic Contamination Detection

A 10 gram chopped vegetable sample was transferred into a stomacher bag containing 100ml of sterile distilled water and homogenized for 2 min at 260 rpm using a Stomacher[®]400 Circulator (Seward, UK). Homogenized samples were centrifuged at 1200 ×g for 10 min. The supernatant was discarded and 2 ml of the pellet was collected [24]. The next steps were done following the modified Bailenger method [3]. The pellet was suspended in an equal volume of acetoacetic buffer (1 liter of buffer contained 15g sodium acetate trihydrate and 3.6ml glacial acetic acid, pH 4.5) and two volumes of ethyl acetate or ether. The mixture was mixed thoroughly in a vortex mixer and centrifuged at 1000 ×g in 15 min. After centrifugation, the sample was separated into three phases. The bottom phase contained heavy debris, including helminth eggs, larvae and protozoa. The middle layer was buffer. The materials dissolved into ethyl ether or ether formed were in the top layer. The supernatant was discarded and 5 volumes of 33% zinc sulfate solution was used to resuspend the pellet. After, thorough mixing the sample was then tested for the presence of parasites.

Helminth eggs were detected in the sample under a light microscope using ×40 magnification and identified according to the description of Ayres and Mara (1996) [3]. Protozoa were detected by staining the specimens following modified Ziehl-Neelsen technique [4]. The sample was smeared directly onto a microscope slide and air dried. The slide was then prefixed in absolute methanol for 5-10 sec, stained in carbol fuchsin – DMSO (4g of basic fuchsin crystals, 25ml of 99% ethyl alcohol, 12g of phenol crystals, 25ml of glycerol, 25ml of dimethyl sulfoxide and 75ml of distilled water) for 5 min. The slide was rinsed under running tap water for 10-30 sec until excess solution no longer ran off. The slide was placed in decolorizer-counter-stain solution (220ml of 2% aqueous solution of malachite green, 30ml of glacial acid acetic and 50ml of glycerol) for 1 min or until a green background appeared. The slide was then rinsed under running tap water for 10 sec, drained and air dried. Observing the slide under a light microscope using ×100

magnification and identifying the parasites according to the description of Ribes et al (1996) [18].

2.6. Statistical data Analysis

The data were analyzed using general linear model (GLM) in SPSS software version 18.0. Results presented in tables are mean \pm standard deviation. Significant differences among means were separated by Tukey test with a 5% level of probability.

3. Results and Discussion

3.1. Bacterial Contamination on Vegetables

The study results showed that all vegetable samples were contaminated with aerobic bacteria and *E. coli*. The average concentrations of aerobic bacteria and *E. coli* ranged from 6.84 to 8.40 log CFU/g and 5.47 – 6.88 log CFU/g, respectively (Table 1). The *E. coli* concentrations detected were higher than the acceptable limit ($10^2 - 10^3$

CFU/g) for fresh vegetables according to Vietnamese technical regulation of microbiological contaminants in food QCVN 8-3: 2012/BYT. There was a significant difference in *E. coli* load detected in the same type of vegetable between the three markets, especially in celery, cilantro and Vietnamese cilantro (Table 1). Meanwhile, the highest variation of total aerobic bacteria concentration was detected on water spinach (7.52 – 9.45 log CFU/g), followed by Vietnamese cilantro (6.82 – 7.81 log CFU/g).

This result was higher than that in other previous reports [11,19]. The total aerobic bacteria found on leafy vegetables from small shops in Granada (Spain) was $10^4 - 10^6$ CFU/g or 4 – 6 log CFU/g [19]. In the same study, the authors also indicated a high degree of fecal contamination with 86.1% vegetable samples examined contaminated with *E. coli* [19]. Meanwhile, the concentrations of aerobic bacteria (4.5 – 6.2 log CFU/g) and *E. coli* (0.7 – 1.0 log CFU/g) were found on leafy vegetables and herbs from the southern United States [11].

Table 1. Aerobic bacteria and *E. coli* concentrations on the examined vegetables

Vegetables	Aerobic bacteria (log CFU/g)				<i>E. coli</i> (log CFU/g)			
	An Cuu market	Ben Ngu market	Tay Loc market	Average	An Cuu market	Ben Ngu market	Tay Loc market	Average
Young mustard greens	7.81 ± 0.17	7.70 ± 0.59	7.67 ± 0.06	7.73 ^{abc} ± 0.31	7.67 ± 0.77	6.73 ± 0.16	6.05 ± 0.16	6.81 ^a ± 0.81
Celery	8.59 ± 0.54	8.57 ± 0.36	7.70 ± 0.54	8.29 ^{ab} ± 0.61	8.55 ± 0.69	6.21 ± 0.41	5.89 ± 0.02	6.88 ^a ± 1.32
Amaranth	8.24 ± 0.48	8.14 ± 0.19	8.28 ± 0.08	8.22 ^{ab} ± 0.27	6.80 ± 0.07	6.34 ± 0.24	5.92 ± 0.39	6.35 ^{ab} ± 0.45
Water spinach	8.24 ± 0.24	7.52 ± 0.12	9.45 ± 0.10	8.40 ^a ± 0.85	7.07 ± 0.42	6.06 ± 0.18	5.73 ± 0.22	6.28 ^{ab} ± 0.66
Cilantro	7.73 ± 0.14	8.51 ± 0.33	7.94 ± 0.23	8.06 ^{ab} ± 0.41	8.23 ± 0.61	5.98 ± 0.65	5.93 ± 0.46	6.71 ^a ± 1.24
Rice paddy herb	7.91 ± 0.66	7.86 ± 0.47	7.06 ± 0.71	7.61 ^{abcd} ± 0.68	7.50 ± 0.53	6.05 ± 0.73	5.74 ± 0.45	6.43 ^{ab} ± 0.96
Vietnamese cilantro	6.82 ± 0.30	7.72 ± 0.08	7.81 ± 0.81	7.45 ^{bcd} ± 0.64	7.92 ± 1.24	6.67 ± 0.29	5.74 ± 0.07	6.78 ^a ± 1.14
Basil	7.66 ± 0.58	7.07 ± 0.04	6.81 ± 0.14	7.18 ^{cd} ± 0.48	6.21 ± 0.66	6.43 ± 0.22	6.09 ± 0.23	6.24 ^{ab} ± 0.39
Centella	6.55 ± 0.23	7.63 ± 0.49	6.67 ± 0.37	6.95 ^{cd} ± 0.61	6.15 ± 0.21	7.13 ± 1.02	5.84 ± 0.80	6.37 ^{ab} ± 0.88
Lettuce	7.32 ± 0.10	6.57 ± 0.25	7.09 ± 0.41	6.99 ^{cd} ± 0.41	5.36 ± 0.24	5.32 ± 0.70	6.80 ± 0.55	5.82 ^{ab} ± 0.86
Watercress	6.84 ± 0.45	6.98 ± 0.12	6.70 ± 0.25	6.84 ^d ± 0.29	5.45 ± 0.41	6.15 ± 0.25	4.80 ± 0.42	5.48 ^b ± 0.54
Iceberg lettuce	7.21 ± 0.43	7.18 ± 0.19	6.49 ± 0.31	6.96 ^{cd} ± 0.45	5.63 ± 0.48	5.80 ± 0.45	5.05 ± 0.47	5.47 ^b ± 0.66

^{a-c} Means with different superscripts within a column differ significantly ($p < 0.05$).

Table 2. Aerobic bacteria and *E. coli* contamination on the vegetables between markets

Market	Number of samples	Aerobic bacteria (log CFU/g)	<i>E. coli</i> (log CFU/g)
An Cuu	36	7.58 ^a ± 0.70	6.88 ^a ± 1.19
Ben Ngu	36	7.62 ^a ± 0.65	6.24 ^b ± 0.62
Tay Loc	36	7.47 ^a ± 0.89	5.79 ^c ± 0.60
Total	108	7.56 ± 0.75	6.30 ± 0.95

^{a-c} Means with different superscripts within a column differ significantly ($p < 0.05$).

There was no significant difference in total aerobic bacteria count on vegetable samples between the three markets (Table 2, $p > 0.05$). However, the concentrations of *E. coli* detected on the samples differed significantly

between Tay Loc, An Cuu and Ben Ngu markets. The lowest degree of *E. coli* contamination on vegetables was found at Tay Loc market (5.79 log CFU/g). Conversely, the highest contamination with *E. coli* was detected on vegetables collected from An Cuu market (6.88 log CFU/g).

Being able to get results quickly is one of the advantages of using PCR technique on *Salmonella* detection, compared to traditional culture method. Moreover, the dead *Salmonella* cells are can be detected using PCR. Amplification of *invA* gene sequence of *Salmonella* has been being used for detecting *Salmonella* in poultry, meats and dairy products, and in vegetables and fruits [9]. The result of *invA* gene amplification is presented in Figure 1.

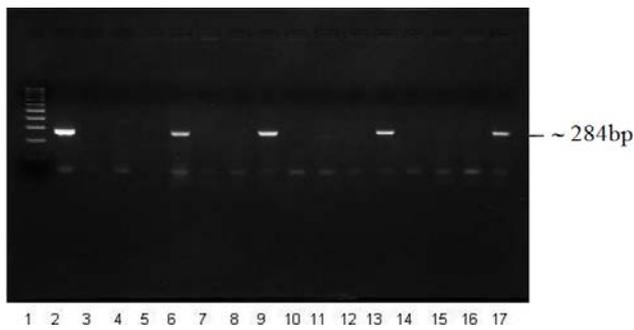


Figure 1. Detection of *Salmonella* by PCR from the examined vegetables

Lane 1: 100bp DNA ladder, lane 2: positive control – *Salmonella enterica* subsp. *diarizonae* (ATCC® 12325™), lane 3: negative control – nuclease-free water, lane 4: *E. coli*, lanes 5 to 17: examined vegetables

Eighteen percent (19/108) of samples were contaminated with *Salmonella* (Table 3). The percentage of *Salmonella*-positive samples from each market were Tay Loc 7/36 (19.44%), Ben Ngu 5/36 (13.89%) and An Cuu 7/36 (19.44%). Most vegetable types were contaminated with *Salmonella* except cilantro, Vietnamese cilantro, basil and centella. The highest prevalence of *Salmonella* contamination was found on water spinach (55.56%). A previous study carried out by Ruiz et al (1987) has shown that *Salmonella* was detected in 7.5% of the examined vegetables in Granada (Spain). Furthermore, the study result of Quiroz-Santiago et al (2009) showed that 98/1700 vegetable samples collected in Mexico was contaminated with *Salmonella* [16]. The prevalence of *Salmonella* contamination in each type of vegetables ranged from 1% to 12% [16].

Table 3. Prevalence of *Salmonella* contamination on the examined vegetables

Vegetables	Number of samples	<i>Salmonella</i> positive samples			Prevalence of <i>Salmonella</i> contamination (%)
		Tay Loc market (n = 3)	Ben Ngu market (n = 3)	An Cuu market (n = 3)	
Young mustard greens	9	1	1	0	22.22
Celery	9	1	0	2	33.33
Amaranth	9	0	1	0	11.11
Cilantro	9	0	0	0	0.00
Water spinach	9	1	2	2	55.56
Rice paddy herb	9	0	0	1	11.11
Vietnamese cilantro	9	0	0	0	0.00
Basil	9	0	0	0	0.00
Centella	9	0	0	0	0.00
Lettuce	9	3	0	0	33.33
Watercress	9	1	0	2	33.33
Iceberg lettuce	9	0	1	0	11.11
Total	108	7	5	7	17.59

Bacteria can contaminate vegetables in different ways and at different points in time. The study result of Enabulele and Uraih (2009) indicated that the source of *E. coli* contamination on vegetables sold in Benin (Nigeria) was contaminated irrigated water [5]. This contaminated water had come from contact with grazing cattle and cross contamination from the vehicles that were used for animal transportation prior to carrying vegetables. In addition to this, *Salmonella*-contaminated wastewater used for irrigation in Marrakesh city (Morocco) caused contamination on vegetables was also reported [13]; and a study on the microbiological quality of carrots used for fresh juice preparation in India showed that there was a large increase in total aerobic bacteria and *Staphylococcus* on carrots as they travelled further through the distribution chain [8]. The total aerobic bacteria and *Staphylococcus* counts detected on carrots increased from 10^4 to 3.6×10^7 CFU/g and from 1.9×10^4 to 7.7×10^6 CFU/g, respectively during the transportation from the central distribution site to street vendors. It is posited that unhygienic handling, cross contamination and water quality caused the contamination of the carrots [8].

3.2. Parasitic Contamination on Vegetables

All vegetable samples were contaminated with parasites. The parasites most commonly detected were *Fasciola* (eggs, 83.33%), *Ascaris* (eggs, 85.19%) and *Trichuris* (eggs, 64.81%) (Table 4). *Clonorchis sinensis* eggs was

also detected with the frequency of 16.67%. In addition, oocysts of some protozoa as *Cryptosporidium*, *Isospora* and *Cyclospora* were found on the test samples. The *Cryptosporidium* contamination ratio on 12 types of examined vegetables ranged from 22.22% to 66.67%. The number of samples contaminated with *Isospora* and *Cyclospora* were relatively low. Cilantro was *Cyclospora*-free. Of twelve types of examined vegetables, watercress was the vegetable most infected by parasites, followed by basil and lettuce.

The parasitic contamination on vegetables in Vietnam was also reported [23]. There were 26% vegetable samples collected from a suburban market in Hanoi (Vietnam) contaminated with helminth eggs. Leafy vegetables had the highest contamination ratio (31%; [23]). The five main types of parasite eggs were found in that study were *Ascaris* sp., *Trichuris* sp., *Toxocara* sp., *Taenia* sp. and *Ascaridia galli* [23]. Furthermore, Klápec and Borecka (2012) also reported the contamination of vegetables, fruits and soil on organic and conventional farms with parasite eggs (*Ascaris*, *Trichuris* and *Toxocara*) in south-eastern Poland [12]. The situation of contamination with parasites on vegetables in other countries was also reported, for example in Iran [7], Nigeria [10], Semnan [15], Egypt [20] and Philippines [21]. The reasons of parasitic contamination on vegetables were probably contaminated from fertilizer ([23], [12]), wastewater source [24], soil [12].

Table 4. Prevalence of parasitic contamination on vegetables

Vegetables	Number of samples	Positive ratio (%)						
		<i>Fasciola</i> egg	<i>Ascaris</i> egg	<i>Trichuris</i> egg	<i>Clonorchis sinensis</i> egg	<i>Cryptosporidium</i> oocyst	<i>Cyclospora</i> oocyst	<i>Isospora</i> oocyst
Young mustard greens	9	100.00	77.78	77.78	0.00	44.44	22.22	33.33
Celery	9	88.89	77.78	77.78	0.00	33.33	33.33	33.33
Amaranth	9	88.89	77.78	55.56	0.00	22.22	33.33	22.22
Water spinach	9	100.00	88.89	66.67	0.00	55.56	33.33	11.11
Cilantro	9	100.00	88.89	66.67	0.00	33.33	0.00	22.22
Rice paddy herb	9	100.00	77.78	66.67	0.00	55.56	11.11	33.33
Vietnamese cilantro	9	100.00	100.00	66.67	0.00	44.44	22.22	11.11
Basil	9	77.78	77.78	66.67	44.44	55.56	33.33	22.22
Centella	9	55.56	88.89	44.44	33.33	66.67	22.22	22.22
Lettuce	9	77.78	88.89	66.67	33.33	55.56	22.22	33.33
Watercress	9	44.44	100.00	77.78	66.67	44.44	55.56	33.33
Iceberg lettuce	9	66.67	77.78	44.44	22.22	55.56	44.44	55.56
Total	108	83.33	85.19	64.81	16.67	47.22	27.78	27.78

4. Conclusion

Contamination with bacteria and parasites was detected on a range of vegetables at the three big, traditional markets in Hue city. All of examined vegetables samples were contaminated with multiple bacteria and parasites. Contamination levels of *E. coli* and *Salmonella* on most of vegetables examined were higher than acceptable limits for fresh vegetables according to Vietnamese technical regulations on microbiological contamination in food. Vegetables contaminated in this way can become potential sources of cross contamination and pose a serious risk to human health. Therefore, with the aim of reducing the risk of cross contamination and food poisoning, washing vegetables after harvest and before using is very important. Studies aimed at reducing pathogen contamination in the field, during transportation, and to limit or remove pathogens on vegetables after harvest are necessary to supply safe vegetables to consumers.

Acknowledgement

We would like to acknowledge the financial support of the Graduate School of Global Environmental Studies, Kyoto University, Japan. We also thank Dr. Mary Kathryn Finlay-Doney for her support in English writing.

Statement of Competing Interests

The authors have no competing interests.

References

- Adjah, Y., Karou, D.S., Djéri, B., Anani, K., Soncy, K., Ameyapoh, Y., de Souza, C., Gbeassor, M., "Hygienic quality of commonly consumed vegetables. and perception about disinfecting agents in Lomé", *International Food Research Journal*, 18(4), 1499-1503, 2011.
- Ahmed, M.M., Hossain, Md.S., Mahbub, K.R., Khaleque, H.N., Hossain, Z., Fakruddin, Md., Chowdhury, A., Hossain, Md.N., Alam, Md.Z., "Performance analysis of multiplex in chicken egg samples", *Singapore Journal of Scientific Research*, 2, 25-32, 2012.
- Ayres, R.M., Mara, D.D., *Analysis of wastewater for use in agriculture - A laboratory manual of parasitological and bacteriological techniques*, World Health Organization, Geneva, Switzerland, 1996.
- Bronsdon, M.A., "Rapid dimethyl sulfoxide-modified acid-fast stain of *Cryptosporidium* oocysts in stool specimens", *Journal of Clinical Microbiology*, 19(6), 952-953, 1984.
- Enabulele S.A., Uraih N. (2009), Enterohaemorrhagic *Escherichia coli* O157:H7 prevalence in meat and vegetables sold in Benin City, Nigeria, *African Journal of Microbiology Research*, 3 (5), pp. 276-279.
- Francis, G.A., O'Beirne, D., "Effects of vegetable type and antimicrobial dipping on survival and growth of *Listeria innocua* and *E. coli*", *International Journal of Food Science and Technology*, 37, 711-718, 2002.
- Gharavi, M.J., Jahani, M.R., Rokni, M.B., "Parasitic contamination of vegetables from farms and markets in Tehran", *Iranian Journal of Public Health*, 31, 83-86, 2002.
- Ghosh, M., Ganguli, A., Mudgil, S., "Microbiological quality of carrots used for preparation of fresh squeezed street vended carrot juices in India", *Journal of Food Agriculture and Environment*, 2(2), 143-145, 2004.
- Guo, X., Chen, J., Beuchat, L.R., Brackett, R.E., "PCR detection of *Salmonella enterica* serotype Montevideo in and on raw tomatoes using primers derived from *h1A*", *Applied and Environmental Microbiology*, 66, 5248-5252, 2000.
- Idahosa, O.T., "Parasitic contamination of fresh vegetables sold in Jos markets", *Global Journal of Medical Research*, 11(1), 20-25, 2011.
- Johnston, L.M., Jaykus, L., Moll, D., Martinez, M.C., Anciso, J., Mora, B., Moe, C.L., "A field study of the microbiological quality of fresh produce", *Journal of Food Protection*, 68(9), 1840-1847, 2005.
- Klapeć, T., Borecka, A., "Contamination of vegetables, fruits and soil with geohelminths eggs con organic farm in Poland", *Annals of Agricultural and Environmental Medicine*, 19 (3), 421-425, 2012.
- Melloul, A.A., Hassani, L., Rafouk, L., "*Salmonella* contamination of vegetables irrigated with untreated wastewater", *World Journal of Microbiology and Biotechnology*, 17, 207-209, 2001.
- Mukherjee, A., Speh, D., Dyck, E., Diez-Gonzalez, F., "Preharvest evaluation of coliforms, *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers", *Journal of Food Protection*, 67(5), 894-900, 2004.
- Nazemi, A., Raei, M., Amiri, M., Chaman, R., "Parasitic contamination of raw vegetables in Shahroud, Semnan, Zahedan", *Journal of Research in Medical Sciences*, 14(8), 84-86, 2012.
- Quiroz-Santiago, C., Rodas-Suárez, O.R., Carlos R.V., Fernández, F.J., Quiñones-Ramírez, El., Vázquez-Salinas, C., "Prevalence of

- Salmonella* in vegetables from Mexico", *Journal of Food Protection*, 72 (6), pp. 1279-1282, 2009.
- [17] Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galán, J.E., Ginocchio, C., Curtiss, R.III., Gyles, C.L., "Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*", *Molecular and Cellular Probes*, 6(4), 271-279, 1992.
- [18] Ribes, J.A., Seabolt, J.P., Overman, S.B., "Point prevalence of *Cryptosporidium*, *Cyclospora* and *Isospora* infections in patients being evaluated for diarrhea", *American Journal of Clinical Pathology*, 122, 28-32, 2004.
- [19] Ruiz, B.G., Vargas, R.G., Garcia-Villanova, R., "Contamination on fresh vegetables during cultivation and marketing", *International Journal of Food Microbiology*, 4(4), 285-291, 1987.
- [20] Said Said, D.E., "Detection of parasites in commonly consumed raw vegetables", *Alexandria Journal of Medicine*, 48, 345-352, 2012.
- [21] Sia Su, G.L., Mariano, C.M.R., Matti, N.S.A., Ramos, G.B., "Assessing parasitic infestation of vegetables in selected market in Metro Manila, Philippines", *Asian Pacific Journal of Tropical Disease*, 51-54, 2012.
- [22] Thenmozhi, M., "Isolation of potentially pathogenic *Escherichia coli* O157:H7 from the water sources", *International Journal of Pharma and Bio Sciences*, 1(4), B84-B88, 2010.
- [23] Uga, S., Hoa, N.T.V., Noda, S., Moji, K., Cong, L., Aoki, Y., Rai, S.K., Fujimaki, Y., "Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam", *Nepal Medical College Journal*, 11(2), 75-78, 2009.
- [24] Vuong, T.A., Nguyen, T.T., Klank, L.T., Phung, D.C., Dalsgaard, A., "Faecal and protozoan parasite contamination of water spinach (*Ipomoea aquatica*) cultivated in urban wastewater in Phnom Penh, Cambodia", *Tropical Medicine & International Health*, 12(Suppl 2), 73-81, 2007.