

Short Communication

Water used to moisten vegetables is a source of *Escherichia coli* and protozoan parasite contamination at markets in Hanoi, Vietnam

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ABSTRACT

The study was done to assess the level of fecal (*Escherichia coli*) and protozoan parasite (*Cryptosporidium* spp. and *Giardia* spp.) contamination in water used by traders to moisten vegetables at markets in Hanoi, Vietnam. A total of 200 splashing water samples from markets located within eight districts were analyzed for *E. coli* and *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts. *Giardia* cysts were found in 17 splashing water samples and *Cryptosporidium* oocysts in nine samples, with median values of 20 cysts ml⁻¹ and 10 oocysts ml⁻¹, respectively. *E. coli* was found with a median concentration of 636 cfu ml⁻¹ and its occurrence was negatively correlated with the numbers of protozoan parasites. The splashing water was kept in buckets that were rarely cleaned and often used for handwashing. The finding of these pathogens in splashing water is likely to represent real food safety hazards.

Key words | *Cryptosporidium* spp., foodborne, *Giardia* spp., splashing water, waterborne

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INTRODUCTION

Cryptosporidium and *Giardia* (oo)cysts are recognized as common waterborne pathogens found in surface water (lakes, streams, rivers, and reservoirs) and effluent from wastewater treatment plants (Khouja *et al.* 2010; Julio *et al.* 2012; Duris *et al.* 2013; Gallas-Lindemann *et al.* 2013; Xiao *et al.* 2013). These protozoan parasites have been detected on vegetables and in irrigation water used for crop production (Thurston-Enriquez *et al.* 2002; Vuong *et al.* 2007). Cryptosporidiosis and giardiasis are perceived more as waterborne than as foodborne diseases. (Oo) cysts are resistant to most disinfectants normally used for water treatment (Baldursson & Karanis 2011). Thus, contaminated water appears to be a major source of protozoan parasites contamination of fresh produce (Chai-dez *et al.* 2005; Amoros *et al.* 2010). *Cryptosporidium* spp.

and *Giardia* spp. might subsequently cause illness when fresh produce is consumed raw or with minimal heat treatment.

Fresh produce may become contaminated with *Cryptosporidium* spp. and *Giardia* spp. along the entire chain from field to kitchen, for example, when vegetables are harvested, transported, stored, and sold. In Vietnam, vegetables are often washed in different types of water after harvest. At rural and urban markets, traders keep a bucket of water at their stall for splashing vegetables to keep them fresh and moist. Water from taps located within the market area, taps at public toilets or concrete water storage tanks are stored in buckets that are rarely cleaned and the water may also be used for hand washing. This study therefore aimed to assess the level of fecal (*Escherichia coli*) and protozoan parasites

(*Cryptosporidium* spp. and *Giardia* spp.) contamination in water used by traders at markets in Hanoi, Vietnam.

METHODS

Markets and collection of water samples

The survey was conducted during 5 weeks from February 2011 to March 2011 at markets in Hanoi, Vietnam. A total of 200 water samples used to moisten vegetables (termed splashing water below) were randomly collected from traders at 20 markets located in eight districts of Hanoi city. A market trader was defined as one having a permanent stall from where vegetables were sold. All traders at the markets had a bucket which was filled with water collected either from the trader's home or taps at the market. A schematic drawing was made of each market, showing the outline of the stalls, the location of traders, the location of toilet(s), streets surrounding the market, and the location where traders were selling vegetables outside the market. The number of traders was counted as a measure of market size. A market included both the actual market buildings and nearby streets with food traders.

The market area used for selling vegetables was identified at each market. Traders were chosen randomly by dividing this area into separate sections on the schematic drawing and choosing an equal number of traders in each section. Ten traders were randomly selected at each market area to provide a water sample. A questionnaire interview was conducted with the market traders to collect information about water splashing activities. Information about hygiene conditions at the market was obtained through observations. During each interview a 2,000 ml water sample was collected in sterile screwed-cap bottles by pouring it from the water bucket at each stall. Water samples were handled using sterile gloves and transported to the laboratory in an insulated ice-box and analyses initiated the day of sampling.

Enumeration of *E. coli* and protozoan parasites

E. coli enumeration was done on Brilliance *E. coli*/coliform Selective Agar (CM1046, Oxoid) following incubation at 37 °C for 24 h. Briefly, 1 ml of water sample was aseptically

transferred to a sterile tube containing 9 ml peptone saline broth (1:10). The sample was mixed well and appropriate ten-fold serial dilutions were prepared. Volumes of 100 µl of each dilution were surface spread onto duplicate agar plates. Agar plates showing growth of dark purple to indigo blue colonies were determined positive for *E. coli*. The total number of *E. coli* (cfu/ml) was calculated based on number of colonies enumerated on duplicate plates using standard formula.

Giardia spp. and *Cryptosporidium* spp. were enumerated using an immunofluorescent method (Vuong *et al.* 2007). Each water sample was vigorously shaken and dispensed into twenty 50 ml sterile test tubes which were centrifuged at 1,500 g for 5 min. After the supernatant was aspirated from the first batch of water samples, each test tube was refilled and their contents were re-centrifuged at the same speed and time. The contents of each set of twenty test tubes were pooled together after the supernatant was discarded, and re-centrifuged under the same conditions. The supernatant was again discarded, and the remaining sediments were subjected to flotation steps where 10 ml of sample volume was underlaid with 5 ml of flotation fluid (NaCl and glucose added per liter, diluted 1:1 with distilled water to the final density of 1.13). Larger debris was removed after centrifugation at 100 g for 1 min. The sample was washed three times with distilled water to dilute the flotation liquid out of the suspension. After the last wash, the supernatant was aspirated leaving 2 ml processed sample suspension in the tube for subsequent detection of (oo)cysts. A volume of 200 µl of sub-sample was placed on a Teflon-coated diagnostic slide and dried at room temperature. The sample was fixed with acetone for 5 min and air dried. Anti-crypto/giardia fluorescent dye was added to the well (Crypto/Giardia CEL; Cellabs Pty Ltd, Australia). The slide was placed in a humidity chamber and incubated for 1 h at 37 °C. Surplus dye was removed and the slide was washed with 1× PBS. The slide was read at 400× magnification in a Nikon Eclipse E600 fluorescent microscopy equipped with a UV-filter block (500 nm excitation, 630 nm emission). All oocysts and cysts were counted and estimated for 1 ml of water.

Statistical analyses

Data were analyzed with SPSS software (Statistical Package for Social Sciences) version 11.5 (SPSS Inc., Chicago,

Illinois, USA). Concentrations of *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts and *E. coli* in each sample were compared using the Spearman correlation coefficient ($p < 0.01$; two-tailed). Significant differences in the concentrations of *E. coli* and protozoan (oo)cysts between districts were compared using the Kruskal–Wallis test. *P*-values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Among the 200 splashing water samples analyzed, *Cryptosporidium*, *Giardia*, and *E. coli* were found in 4.5%, 8.5%, and 47.5%, respectively, with median concentrations of 10 oocysts, 20 cysts, and 636 cfu ml⁻¹, respectively (Table 1). Since ingestion of a single (oo)cyst is sufficient to establish infection (Dillingham *et al.* 2002), splashing water at Hanoi markets represent important sources of protozoan parasites that may be transmitted to vegetables.

All traders recorded splashing vegetables with tap water obtained either from the market or from their private home (Table 2). Water sources at markets included taps located in the actual market area, taps in public toilets, and concrete water storage tanks. Most traders kept water in a bucket at their vegetable stall for all day use. To keep vegetables moistened, vegetables were mainly submerged in a bucket (66.0%) or traders used the wetted vegetables to splash water on other vegetables (30.5%). On several occasions traders were observed to use their hands for scattering water onto vegetables, either by pouring water onto their hands using a plastic bottle with small holes in the cap or by dipping the hands in a water bucket. Unfortunately, we did not collect any information about hand-washing practices.

Table 1 | Occurrence of *Giardia* spp. and *Cryptosporidium* spp. (oo)cysts in water ($N = 200$) used to moisten vegetables at markets in eight districts in Hanoi, Vietnam

	No. of positive samples (%)	Median ^a (oocysts/cysts or cfu/ml)	Range (oocysts/cysts or cfu/ml)
<i>Cryptosporidium</i> spp.	9 (4.5)	10	5–35
<i>Giardia</i> spp.	17 (8.5)	20	5–250
<i>E. coli</i>	95 (47.5)	636	91–23,909

^aThe median values were calculated based on numbers in positive samples.

Table 2 | Sources and handling practices of water used to moisten vegetables by traders at markets ($N = 200$) in Hanoi, Vietnam

Water characteristics and practices of market traders	Proportion of market traders (%)
Water source used to splash vegetables	
Tap water at market	27.0
Tap water from private home	68.5
Location of tap at market	
Public toilet	42.5
Other location	44.0
Water collected and stored in bucket	74.0
Purposes of using water in bucket	
Moisten vegetables	90.5
Hand washing	9.5
Type of practice used to moisten vegetables	
Submerged vegetables in bucket and sprinkle on other vegetables	30.5
Submerged vegetables in bucket only	66.0
Frequency of cleaning bucket with water	
Monthly	70.5
Weekly	7.0
Never	18.5

Traders washed the plastic buckets typically once a month without the use of soap (70.5%) and some traders never cleaned them (18.5%). The markets were divided into sections selling different food items, for example, fish, other meat and live chickens/ducks, and vegetables. The concrete floor was typically wet and open sewage canals were often seen in and around the market areas. Vegetables were stored overnight at the market floor placed on wood or bamboo trays covered with plastic. It is likely that the wet conditions, presence of live chicken, ducks, and the open sewage canals would contribute to fecal contamination of the vegetables.

Splashing water has previously been shown to be an important source of *E. coli* at vegetable markets in Ho Chi Minh city, Vietnam (Ha *et al.* 2008), *Salmonella* in India (Singh *et al.* 2006), and *Cyclospora* spp. at markets in Hanoi (Tram *et al.* 2008). However, contamination of produce can occur along the chain from field to consumer; for example, Vietnamese farmers were found to clean vegetables after harvest in fecally contaminated

surface (pond) water (Vuong 2008). Also, *C. cayetanensis* has been found in surface water in Hanoi (Miegeville *et al.* 2003).

Correlation analysis showed a significant negative correlation between *E. coli* and *Giardia* ($p = 0.833$; $r_s = -0.015$) and *E. coli* and *Cryptosporidium* ($p = 0.245$; $r_s = -0.083$) corroborating previous findings that *E. coli* is a poor indicator of protozoan parasites (Hargy *et al.* 2000; Hayes *et al.* 2003). No significant differences in the median concentrations of protozoan parasites were found with respect to districts ($p > 0.05$).

CONCLUSION

Splashing water used to moisten vegetables at markets in Hanoi was contaminated with *Cryptosporidium* (4.5%) and *Giardia* (8.5%) with median concentrations of 10 oocysts and 20 cysts ml⁻¹, respectively. Water storage and handling practices at markets most likely contribute to high levels of fecal pollution. Although molecular analyses are needed to determine the human pathogenicity of the (oo)cysts, our findings of protozoan parasites in splashing water are likely to represent real food safety hazards. Urgent action is needed to educate traders and the responsible authorities to improve sanitary conditions at markets in Hanoi and elsewhere in less developed countries, to improve food safety and protect public health.

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REFERENCES

- Amoros, I., Alonso, J. L. & Cuesta, G. 2010 *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. *J. Food Prot.* **73** (6), 1138–1140.
- Baldursson, S. & Karanis, P. 2011 Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. *Water Res.* **45** (20), 6603–6614.
- Chaidez, C., Soto, M., Gortares, P. & Mena, K. 2005 Occurrence of *Cryptosporidium* and *Giardia* in irrigation water and its impact on the fresh produce industry. *Int. J. Environ. Health Res.* **15** (5), 339–345.
- Dillingham, R. A., Lima, A. A. & Guerrant, R. L. 2002 *Cryptosporidiosis: epidemiology and impact.* *Microbes Infect.* **4** (10), 1059–1066.
- Duris, J. W., Reif, A. G., Krouse, D. A. & Isaacs, N. M. 2013 Factors related to occurrence and distribution of selected bacterial and protozoan pathogens in Pennsylvania streams. *Water Res.* **47** (1), 300–314.
- Gallas-Lindemann, C., Sotiriadou, I., Plutzer, J. & Karanis, P. 2013 Prevalence and distribution of *Cryptosporidium* and *Giardia* in wastewater and the surface, drinking and ground waters in the Lower Rhine, Germany. *Epidemiol. Infect.* **141** (1), 9–21.
- Ha, N. T., Kitajima, M., Hang, N. V., Matsubara, K., Takizawa, S., Katayama, H., Oguma, K. & Ohgaki, S. 2008 Bacterial contamination of raw vegetables, vegetable-related water and river water in Ho Chi Minh City, Vietnam. *Water Sci. Technol.* **58** (12), 2403–2411.
- Hargy, T. M., Clancy, R., Bukhari, Z. & Marshall, M. M. 2000 Shedding UV light on the *Cryptosporidium* threat. *J. Environ. Health* **63** (1), 19–23.
- Hayes, S. L., Rice, E. W., Ware, M. W. & Schaefer, F. W. 2003 Low pressure ultraviolet studies for inactivation of *Giardia muris* cysts. *J. Appl. Microbiol.* **94** (1), 54–59.
- Julio, C., Sa, C., Ferreira, I., Martins, S., Oleastro, M., Angelo, H., Guerreiro, J. & Tenreiro, R. 2012 Waterborne transmission of *Giardia* and *Cryptosporidium* at river beaches in Southern Europe (Portugal). *J. Water Health* **10** (3), 484–496.
- Khouja, L. B., Cama, V. & Xiao, L. 2010 Parasitic contamination in wastewater and sludge samples in Tunisia using three different detection techniques. *Parasitol. Res.* **107** (1), 109–116.
- Miegeville, M., Koubi, V., Dan, L. C., Barbier, J. P. & Cam, P. D. 2003 [*Cyclospora cayetanensis* presence in aquatic surroundings in Hanoi (Vietnam). *Environmental study (well water, lakes and rivers)*]. *Bull. Soc. Pathol. Exot.* **96** (3), 149–152.
- Singh, B. R., Singh, P., Verma, A., Agrawal, S., Badu, N., Chandra, M. & Agrwal, R. K. 2006 A study on prevalence of multi-drug resistant (MDR) *Salmonella* in water sprinkled on fresh vegetables in Bareilly, Moradabad and Kanpur (northern India cities). *J. Public Health* **14**, 125–131.

- Thurston-Enriquez, J. A., Watt, P., Dowd, S. E., Enriquez, R., Pepper, I. L. & Gerba, C. P. 2002 Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. *J. Food Prot.* **65** (2), 378–382.
- Tram, N. T., Hoang, L. M., Cam, P. D., Chung, P. T., Fyfe, M. W., Isaac-Renton, J. L. & Ong, C. S. 2008 *Cyclospora* spp. in herbs and water samples collected from markets and farms in Hanoi, Vietnam. *Trop. Med. Int. Health* **13** (11), 1415–1420.
- Vuong, T. A. 2008 Food safety aspects and occupational health risks of wastewater use in peri-urban aquatic food production in Southeast Asia. PhD Thesis, University of Copenhagen, Frederiksberg, Denmark.
- Vuong, T. A., Nguyen, T. T., Klank, L. T., Phung, D. C. & Dalsgaard, A. 2007 Faecal and protozoan parasite contamination of water spinach (*Ipomoea aquatica*) cultivated in urban wastewater in Phnom Penh, Cambodia. *Trop. Med. Int. Health* **12** (Suppl. 2), 73–81.
- Xiao, G., Qiu, Z., Qi, J., Chen, J. A., Liu, F., Liu, W., Luo, J. & Shu, W. 2013 Occurrence and potential health risk of *Cryptosporidium* and *Giardia* in the Three Gorges Reservoir, China. *Water Res.* **47** (7), 2431–2445.

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