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Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia* spp. in environmental samples in Hanam province, Vietnam

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ABSTRACT

Cryptosporidium and *Giardia* are protozoan parasites that cause human diarrheal disease worldwide. This study was done to evaluate the prevalence and concentrations of these protozoa in environmental samples in Hanam, Vietnam and to assess potential contamination sources using molecular epidemiological tools. A total of 134 environmental samples were collected between February 2009 and July 2009, including 24 river water, 24 sewage, 32 fishpond water, 23 canal water, 26 vegetable and five composted waste samples. Samples were analyzed microscopically using an immunofluorescence method. Overall *Giardia* and *Cryptosporidium* were detected in 25.4% and 35.0% of samples analyzed, respectively. In water, a higher percentage of *Cryptosporidium* spp. (41.7%; 43/103) contamination was observed compared to that of *Giardia* spp. 28.2% (29/103). Both *Giardia* spp. and *Cryptosporidium* spp. were found contaminating vegetables at the same level, at 15.4% (4/26) each. Concentrations of *Cryptosporidium* in samples ranged from 10 to 1900 oocysts per 100 ml water or 100 g vegetable/composted waste sample with a median number of 100 oocysts per 100 ml/g. The concentration of *Giardia* cysts ranged from 10 to 1836 per 100 ml/g with a median of 60 cysts per 100 ml/g. Microscopy positive samples were subjected to PCR targeting the SSUrDNA gene for both *Giardia* and *Cryptosporidium* and the β-giardin gene for *Giardia*. PCR amplification and subsequent genetic characterization was successfully performed with 23/34 (67.6%) *Giardia*-positive samples and 15/47 (31.9%) *Cryptosporidium*-positive samples. Molecular characterization indicated the presence of *Giardia duodenalis* assemblages A and B and a high prevalence of *Cryptosporidium suis*. Although the latter being rare in humans, *G. duodenalis* assemblages A and B are potentially zoonotic. In conclusion, this study allowed sources of contamination and human health risks posed by *Cryptosporidium* and *Giardia* in environmental samples in Vietnam to be ascertained. More detailed studies are needed on the host range of different *Giardia* and *Cryptosporidium* species/subspecies, the potential for cross-species transmission, and risk and environmental factors involved in the exposure of the pathogen with the advent of molecular typing tools.
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1. Introduction

The protozoans *Giardia* and *Cryptosporidum* are recognized as major causes of diarrhea and nutritional disorders in institutional and community settings (Savioli et al., 2006). Among the waterborne pathogens, *Giardia* and *Cryptosporidium* are the most common causes of major diarrheal outbreaks globally (Karanis et al., 2007). A FAO/WHO Expert Commitee ranked *Cryptosporidium* and *Giardia* as the 5th and 11th most important global food-borne zoonoses, respectively, using a multicriteria ranking approach (WHO/FAO, 2014). The role of water and food, particularly fresh produce as a source for these protozoan agents is now well-recognized as documented in traceback-outbreak investigations, which is carried out through the detection of oocysts and cysts in vegetables and water samples, e.g. when irrigated in fields (Amoros et al., 2010; Armon et al., 2002; Robertson and Gjerde, 2001; Smith et al., 2006; Smith and Nichols, 2010; Vuong et al., 2007). Forage and fresh produce becomes contaminated when improperly treated with reclaimed low quality water, for example, wastewater is used to irrigate agricultural land, leading to human exposure and disease. Alternatively, direct contamination of fresh produce through handling by farm workers may also occur. The contamination of fruit and vegetables with oocysts and cysts is an important source of human infection because these products are frequently eaten raw or lightly cooked (Cook et al., 2007).

Molecular taxonomic methods have identified *Cryptosporidium hominis* (which infects humans) and *C. parvum* (which infects cattles, humans and other mammals) as the most commonly detected species of *Cryptosporidium* in surface and wastewater (Paziewska et al., 2007; Smith et al., 2006). An additional *Cryptosporidium* species of animal origin, *C. meleagridis* is the third most common species following *C. hominis* and *C. parvum*, capable of infecting immuno-compromised humans (Silverlas et al., 2012). Furthermore, *C. canis*, *C. felis*, *C. suis* and *C. muris* are minor species responsible for human infections (Xiao, 2010) with most cases detected in HIV-positive patients and in children (Cama et al., 2007; Llorente et al., 2007; Thompson et al., 2005; Xiao et al., 2004). *C. andersoni* has been reported worldwide in post-weened beef and dairy cattle (Olson et al., 2004) and has been implicated as a cause of sporadic human cryptosporidiosis in Australia together with *C. fayeri* (Waldron et al., 2011).

Giardiasis in humans and most other mammals is caused by *Giardia duodenalis*. At least eight genotypes of *G. duodenalis* have been identified (assemblages A–H), however among them, not only assemblages A and B infect humans, but also a wide range of mammalian hosts, making them potential zoonoses. A degree of host-related sub-structuring has been identified within assemblage A, i.e. AI appears to mainly infect animals, AII mainly infects humans, and AIII mainly infects wild ruminants. Assemblages C–H appear generally to be restricted to companion animals, livestock, and rodents while assemblage H so far has only been found in seals and a seagull (Feng and Xiao, 2011).

In a previous study, the prevalence of *G. duodenalis* in humans and dogs in a rural village in Cambodia was 18.3% (40/218) and 10.6% (10/94) as shown by PCR, respectively. *Giardia* assemblages AII and BIII of *Giardia*-positive samples were characterized in humans. *G. duodenalis* assemblages BIII, C and mix infection between C and D of positive-samples were among the dogs (Inpankaew et al., 2014). In Vietnam, molecular epidemiological studies of the species and genotypes of *Giardia* and *Cryptosporidium* infecting humans are few. *C. parvum* human genotype was found in three HIV patients in Vietnam (Gatei et al., 2003). Mostly non-zoonotic isolates of *G. duodenalis* (assemblage E) were detected in cattle and pigs at 201 farms located in five provinces around Hanoi in Northern Vietnam (Geurden et al., 2008). Nguyen et al. (2013) found 28/193 pig fecal samples in central Vietnam positive for *Cryposporidium* oocysts with 12 samples characterized as *C. suis* and two samples as *Cryptosporidium* pig genotype II based on 18S rRNA and HSP-70 gene sequence analysis. Fecal samples from cattle in central Vietnam were found positive for *C. parvum* bovine genotype and *C. andersoni* (Nguyen et al., 2007). The two non-zoonotic species *C. ryanae* and *C. bovis* were detected in native beef calves 2–6 months old in Dac Lac province, central Vietnam (Nguyen et al., 2012). Investigating the molecular epidemiology of *Giardia* and *Cryptosporidium* in environmental samples can provide important information with regard to the potential sources of infection and likely routes of transmission to humans and animals. Thus, the specific aim of the present study was to evaluate the prevalence and concentrations of *Cryptosporidium* spp. and *Giardia* spp. in environmental samples including surface water, compost and fresh vegetables in Hanam, Vienam and to assess potential contamination sources using molecular epidemiological tools. Such knowledge would provide data to aid risk assessment and management measures for preventing contamination of food and water with protozoa in Vietnam.

2. Materials and methods

2.1. Study site

The study was carried out in Hoang Tay and Nhat Tan communes in Kim Bang district, Hanam province located about 60 km south of Hanoi. The two communes border the Nhue river. The water used for rice and vegetable production as well as aquaculture activities in these areas are sourced from Nhue river and Nhue-Day river basins through several pump stations located along the river. Water from these areas were previously found to be one of the most heavily polluted waters in Vietnam. The main pollutants come from industry, handicraft villages, urban wastewater, tourism, agriculture, inland waterway transport and hospitals with the waste generally discharged untreated directly into the river system (ADB/MARD/MONRE, 2007). Effluent from nearby residential areas including grey water from kitchens, bathrooms, and septic tanks; cattle or pig pen cleaning activities are discharged into small irrigation canals surrounding the rice, vegetable fields, fishponds and rivers. Fishponds located in Nhat Tan commune receive water from the Nhue River and surrounding households. Children use the fishponds for swimming and other recreational activities. The fields are irrigated with polluted river water and most farmers work barefooted. Several types of green leafy vegetables (see Section 2.2.3 for details) often consumed raw are grown in the fields. In addition, free-roaming dogs, cats and poultry freely access water sources, fields and

household areas, e.g. indoor kitchen areas. Composted human excreta is commonly used as fertilizer either for household gardening or in fields with rice and vegetables.

2.2. Sample collection

A total of 134 samples including 24 Nhue river water, 24 sewage, 32 fishpond water, 23 canal water, 26 vegetable and five composted waste samples were collected from six locations during eight sampling occasions. Diurnal samples were collected every 2 weeks or monthly between February 2009 and July 2009 (Fig. 1).

2.2.1. Collection and preparation of excreta samples

Latrine waste samples were collected and consisted of composted human and animal waste mixed with ashes from open kitchen fires and/or powdered lime. The waste was placed by farmers in a depression in the soil or as composted human waste obtained from the inside of double or single vault latrines just before wastes were applied as fertilizers in the field. For enumeration of parasite oocysts and cysts, a composite sample consisting of five individual 200 g samples was collected and placed in a sterile steel container. All samples were stored in a cooling box and transported to the laboratory at the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi on the day of sampling for further processing. Samples were analyzed according to a previous described method (Vuong et al., 2007). Briefly, 10-g of composite excreta sample was added to 90 ml of distilled water and homogenized for 30 sec in a Pulsifier instrument (Pulsifier®, Filtaflex, Canada). Samples were concentrated by centrifugation followed by a flotation step where 10 ml of the pelleted sample was underlaid with 5 ml of flotation fluid (saturated NaCl solution with 500 g of glucose added per liter; diluted 1:1 with sterile distilled water to a final specific gravity of 1.13, centrifuged for 1 min at $100 \times g$ and the supernatant removed to clean off larger debris). The sample was subsequently washed twice with sterile distilled water to remove remains of the flotation fluid before concentrating the sample volume to 2 ml.

2.2.2. Collection and preparation of water samples

Surface water samples that are used to irrigate rice and vegetables fields, fill up fishponds and for other purposes like swimming, personal hygiene, and vegetables washing were collected from Nhue river, lake, fishponds, field water canals and household sewage effluent (Fig. 1). Composite water samples consisting of five individual 1-l samples (fishpond/river) and two individual 1-l samples

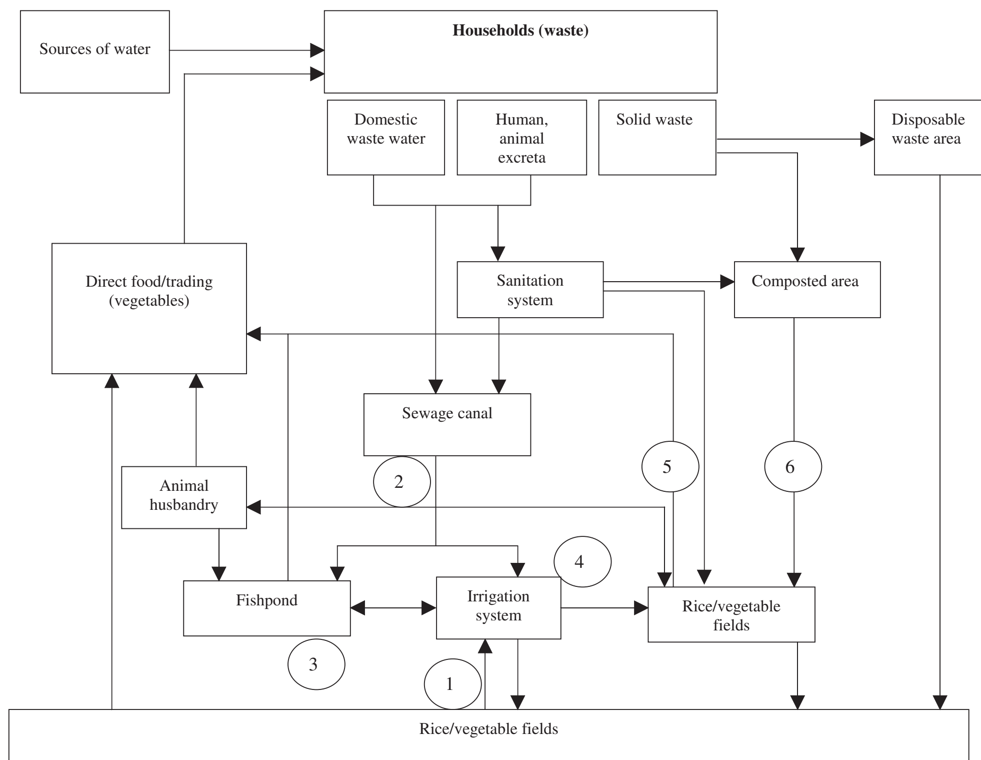

Fig. 1. Contamination routes of *Cryptosporidium* spp. and *Giardia* spp. and sampling locations.

1—Nhue river water samples	2—Sewage effluent samples
3—Fishpond water samples	4—Canal water samples
5—Vegetable samples	6—Composted waste samples

(field water canal/household effluents) were collected in sterile, wide-mouth, screw-capped 1-l bottles. All samples were stored in a cooling box and transported to the NIHE laboratory on the day of sampling for further analysis. Samples were concentrated by centrifugation followed by a flotation step with saturated NaCl–glucose solution to the final volume of 2 ml as described above.

2.2.3. Collection and preparation of vegetable samples

Water spinach grown along the banks of the Nhue river and four types of herbs: Vietnamese mint (*Mentha spicata*), coriander (*Coriandrum sativum*), lettuce (*Lactaca sativa*) and basil (*Ocimum basilicum*) grown in vegetable fields were randomly collected. 200 g of vegetable samples consisting of stems and leaves was picked using a sterile plastic bag. The samples were also stored in a cooling box and transported to the laboratory for the analysis of protozoan parasites. 10 g of vegetables (stems and leaves) was pulsified with 90 ml of 0.01% TWEEN20 (Sigma-Aldrich) in distilled water in a Pulsifier instrument (Pulsifier®, Filtaflex, Canada). Plant washings were also concentrated by centrifugation followed by a flotation step with saturated NaCl–glucose solution to a final volume of 2 ml.

2.3. Quantification of the protozoan pathogens

The final volume of 2 ml sediment was subjected to analysis for protozoan parasites. 200 μl of sediment was incubated at 37 °C for 2–3 h on a Teflon-coated diagnostic slide fixed with acetone which was then stained with a fluorescent monoclonal antibody *Giardia* spp. and *Cryptosporidium* spp. according to the manufacturer's protocol (Crypto/Giardia CEL; Cellabs Pty Ltd, Australia). *Cryptosporidium* oocysts and *Giardia* cysts were identified on the basis of their size, shape, and the intensity of immunofluorescence assay staining (i.e. bright green fluorescence of the cyst wall). The oocysts and cysts were counted at ×400 magnification under green UV light microscopy (500 nm excitation, 630 nm emission).

2.4. Genotyping of the protozoan parasites

2.4.1. DNA isolation

Total DNA was extracted from 250 μl of sediment using a PowerSoil™ DNA Isolation Kit (MO BIO, Laboratories, Inc. California, USA) with minor modifications of the manufacture's protocol by subjecting samples to five cycles of freeze-thaw (freezing in liquid nitrogen for 5 min and thawing at 95 °C in water bath for 5 min) to rupture the *Cryptosporidium* oocysts and *Giardia* cysts. DNA was eluted in 50 μl sterile elution buffer and stored at −20 °C for further processing.

2.4.2. PCR amplification of Giardia and Cryptosporidium

All samples positive by IFA microscopy were screened for the presence of *Cryptosporidium* and *Giardia* by PCR. A nested PCR was performed to amplify a fragment of the 18S rDNA (130 bp) gene of *G. duodenalis* (Hopkins et al., 1997; Read et al., 2002). Samples that were PCR-positive were then subjected to amplification of the *Giardia* β-giardin gene (511 bp) (Caccio et al., 2002; Lalle et al., 2005). For *Cryptosporidium*, amplification of a fragment of the 18S rDNA gene locus (587 bp) was carried out using a two-step nested PCR approach to identify genotypes (Ryan et al., 2003).

2.4.3. Sequencing

PCR products were purified either directly from the PCR reaction or in the case where multiple bands were present, from 1.5% agarose gels after electrophoretic separation using Wizard® SV Gel and PCR Clean-Up System (Promega, Corporation) according to manufacturer's instruction, with the exception that DNA was eluted using 30 μl of Nuclease-Free Water. Sequencing reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instruction using internal PCR primers. Reactions were electrophoresed through an ABI 3730 automatic sequencer. Bidirectional sequencing was performed and chromatograms analyzed with Finch TVv 1.4.0 (Geospiza Inc.) and compared to reference sequences from GenBank using Clustal W (http://www.genome.jp/tools/clustalw/) and nBLAST searches of the nucleotide database.

2.5. Statistical analysis

Data of the prevalence of the parasites in environmental samples with regard to sampling locations were tested using χ^2 test, whereas the concentration in environmental samples from different sampling locations were tested by Kruskal–Wallis test using SPSS software, version 11.5 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at a probability level of $p < 0.05$.

3. Results

The applications of immunofluorescence microscopy on 134 environmental samples revealed 34 samples (25.4%) positive for *Giardia* spp. and 47 samples for *Cryptosporidium* spp. (35.0%) (Table 1). In 25 of these samples (18.7%), co-contamination by both pathogens was found.

The prevalence and concentration of *Giardia* cysts in the different locations are shown in Table 1. Sewage effluent samples showed the highest contamination with cysts (50.0%) followed by water from Nhue river (25.0%), canal water (21.7%), composted waste (20.0%), fishpond water (18.7%) and vegetables (15.3%). Significantly higher numbers of *Giardia* cysts were recovered in sewage effluent compared to the other water sources ($p < 0.05$). No statistical differences in the concentration of *Giardia* were

Table 1
Prevalence and mean concentration of *Giardia* and *Cryptosporidium* per 100 ml/g environmental samples from Hanam, Vietnam.

Location	No. of samples	*Giardia*		*Cryptosporidium*	
		No. (%) of positive samples	Median no. of parasites (range)[a]	No. (%) of positive samples	Median no. of parasites (range)[a]
Nhue river	24	6 (25.0)	20 (10–100)	10 (41.6)	150 (10–840)
Sewage effluent	24	12 (50.0)	200 (20–1836)	16 (66.7)	125 (20–1900)
Fishponds	32	6 (18.7)	40 (20–60)	8 (25.0)	30 (20–440)
Water canals	23	5 (21.7)	40 (20–140)	9 (39.1)	20 (10–200)
Vegetables	26	4 (15.3)[b]	200 (200–200)	4 (15.3)[c]	400 (200–400)
Composted waste	5	1 (20.0)	600 (600–600)	0	0
Overall	134	34 (25.4)	60 (10–1836)	47 (35.0)	100 (10–1900)

[a] The median values were calculated based on number of positive samples.
[b] Water spinach (*Ipomoea aquatica*) and lettuce (*Lactaca sativa*).
[c] Water spinach (*Ipomoea aquatica*), coriander (*Coriandrum sativum*) and lettuce (*Lactaca sativa*).

observed in water from the Nhue river, fishponds and canals ($p > 0.05$). Based on PCR analysis of the 18S rDNA gene, 23/34 samples produced amplicons of the expected size for *Giardia*. Sequence analysis of the 18S rDNA locus was successful for 7/23 *Giardia*-positive isolates; of these, six belonged to assemblage B and one to assemblage E (Table 2). Sub-typing analysis at the β-giardin locus was successfull for 5/7 *Giardia* isolates and these were identifed as assemblage A2 ($n = 2$), assemblage B3 ($n = 2$) and assemblages B1–2 ($n = 1$) (Table 2), representing an overall result of four single contaminations with assemblage B, one single contamination with assemblage E and two mixed contaminations with assemblages A and B.

The prevalence of *Cryptosporidium* in the different locations ranged from 15.3% to 66.7% with the highest prevalence and concentration found in sewage effluent (Table 1). The median concentrations of *Cryptosporidium* in sewage effluent and water from the Nhue river were significantly higher than fishpond and canal water ($p < 0.05$). Nevertheless, no statistically significant difference was found between sewage effluent and Nhue river water samples ($p = 0.08$). Clear and readable sequences were obtained from 9/15 amplicons and identified as *C. suis* (one vegetable sample and eight sewage effluent samples) (Table 2). The DNA sequence of a 587 bp region of the 18S rRNA gene of these samples showed 99.8% similarity to *C. suis* (GU254171).

4. Discussion

Giardia and *Cryptosporidium* are highly infective, environmentally robust pathogens, insensitive to a number of disinfectants, ubiquitious in domestic and wild animals, and capable of zoonotic transmission. These factors combined, make fresh-produce and water in areas lacking adequate wastewater treatment, sanitation and proper food safety standards as primary sources of infection for humans (Slifko et al., 2000). In this study, environmental samples were sourced from a region where urban and rural practices overlap and where water and in turn vegetable quality is determined by the quality of surface water and the use of composted livestock manure and human excreta for irrigation and as agricultural fertilizer. In Vietnam, the habit of ingesting raw vegetables and herbs is common and health risks of contracting protozoan diseases are therefore likely to be associated with transmission of pathogens from contaminated irrigation water to vegetables or when workers handle the produce. High proportions of environmental contamination with both *Cryptosporidium* oocysts (35%) and *Giardia* cysts (25%) in concentrations significantly exceeding estimated infectious dosage for human (i.e. 1–100 cysts/oocysts) were recorded in the communities studied (Razzolini et al., 2011; Teunis et al., 2002). With the increased awareness of foodborne protozoan diseases, more attention should be paid to the management of food safety risks associated with protozoa in the FAO/WHO Codex Alimentarius. During recent years, several waterborne outbreaks by *Giardia* spp. and *Cryptosporidium* spp. have been documented worldwide (Baldursson and Karanis, 2011). No significant waterborne outbreaks of cryptosporidiosis and giardiosis have been reported in humans in Vietnam (Gatei et al., 2003; Ngan et al., 1992; Verle et al., 2003). Due to the highly endemic nature of both protozoa among the general population coupled with strong host immunity maintained by low-level exposure through food, water and environmental sources, the morbidity reportedly associated with these protozoa may be masked and it tends to hinder advocating the control and prevention of *Giardia* and *Cryptosporidium* (Cotruvo et al., 2004),

Table 2
Giardia and *Cryptosporidium* genotypes in environmental samples in Hanam, Vietnam.

Locations	*Giardia* ($n = 23$)		*Cryptosporidium* ($n = 15$)
	Species (n)	Subspecies (n)	Species (n)
Nhue river	NI	NI	NI
Sewage effluent	B (4)	A2 (2) and B3 (1)	*C. suis* (8)
Lake/pond water	B (1) and E (1)	B1–2 (1)	NI
Canal water	NI	NI	NI
Vegetables	B (1)	B3 (1)	*C. suis* (1)

NI = not identified.

Despite the high concentrations of *Giardia* cysts and *Cryptosporidium* oocysts recovered in sewage effluent (Table 1), successful genetic characterization was only possible for 33.3% and 50.0% of microscopy-positive samples with *Giardia* and *Cryptosporidium*, respectively. These findings can be explained by the poor sensitivity of molecular tests due to empty oocysts and cysts lacking DNA (Smith and Nichols, 2010). On the other hand, IFA methods are known to cross-react with nontarget organisms such as algae (Rodgers et al., 1995) thereby providing false positive microscopy results. Alternatively, false negative PCR amplification may arise due to the inhibitory effects of substances such as humic acids, present in sewage/environmental samples (Mayer and Palmer, 1996).

The use of molecular diagnostic tools to characterize species and genotypes of protozoa in clinical and environmental samples provides valuable insight into the potential sources of contamination contributing to human infection. Nevertheless, a number of challenges remain. (Monis et al., 2009; Smith and Nichols, 2010; Xiao, 2010). The use of 18S rDNA locus has been recommended as a target for screening *Giardia* and *Cryptosporidium* from stool and environmental samples owing to its high copy number within the genome, therefore enhancing detection sensitivity. For example, *Giardia* has been estimated to have 60 copies of the rDNA repeat (Boothroyd et al., 1987). Compounding the sensitivity of the PCR assays, we were only able to successfully sequence a limited number of samples. Similar results were obtained in other studies conducted in Hungary (Plutzer et al., 2008), Spain (Castro-Hermida et al., 2011) and France (Coupe et al., 2006). Environmental samples are likely to contain multiple mixed assemblages of *Giardia* and species/genotypes of *Cryptosporidium* which makes it difficult to identify assemblages/species. Use of second-generation sequencing technologies may assist in overcoming these limitations (Paparini et al., 2015). Despite of the limited number of successful sequences, future studies might aim at targeted amplification and quantification of the zoonotic species and genotypes. Using specific molecular markers/probes will provide a more informative approach to assessing the risk posed by these pathogens from environmental samples.

One purpose of this study was to examine the distribution of potentially zoonotic *Cryptosporidium* isolates. The predominance of *C. suis* contamination in sewage effluent in this community implicates effluent discharged from cleaning of domestic pigpens as a major source of environmental contamination of *Cryptosporidium*. Currently, *C. suis* is recognized as zoonotic and it has been identified in immunocompetent humans (Xiao et al., 2002), however owing to the low proportion of successfully characterized samples, information pertaining to the risk of human transmission of *Cryptosporidium* remains incomplete. Therefore, effluent used as untreated irrigation water on the open fields can provide a source of potentially zoonotic *Cryptosporidium* to humans.

The majority of water and vegetable samples contaminated with *Giardia* belonged to assemblage B (6/7) and only one sample of fishpond water was contaminated with livestock-specific assemblage E. *Giardia* A2 and B3 subgenotypes present in sewage effluent may be a reason for public health concern. Water contaminated with untreated sewage effluent is commonly used for agricultural irrigation purposes and poses a significant source of infection to humans, through the consumption of raw produce. Moreover, farm workers may be placed at direct risk through contact with wastewater during the process of irrigating fields. In this study, the sewage effluent discharged into the irrigation system and Nhue river was mainly of domestic origin sourced from both humans and animals. These findings highlight that both anthroponotic (human) and zoonotic (community dogs, livestock) sources may be responsible for contibuting to the contamination of the environment and in turn may contribute to infection in humans.

Findings of *Giardia* assemblage B and *C. suis* in vegetables (Table 2) likely represent direct health risks to people consuming raw vegetables irrigated with the water analyzed. For example, in a study that quantifed diarrhea risk related to wastewater, it was found that eating morning glory collected from a wastewater canal represented an infection risk of 100% by *Giardia* (Ferrer et al., 2012). The health risks of other activities such as collecting morning glory, contact with canal, swimming, and fishing in the wastewater canal was also very high for both *Giardia* and *Entamoeba histolytica*, reaching a yearly risk of almost 100%. However, it is still uncertain if these estimated alarming high health risks are real. Indeed, public health is threatened by water pollution through the consumption of microbiologically contaminated food and drinking water. There is therefore a need to classify the major sources of pollution and explain possible modes of disease transmission involving wastewater by identifying and quantifying *Cryptosporidum* and *Giardia* species/genotypes. Results herein can be useful in building an intergrated pathogen management strategy aimed at protecting public health where extensive use of wastewater was common.

In conclusion, the present study represents new knowledge on prevalence and molecular characteristics of *Giardia* and *Cryptosporidium* in environmental sources in Vietnam. Genotyping and subtyping enteric parasites in environment can be an effective supplement to conventional surveillance and epidemiologic tools. Thus, more comprehensive molecularly epidemiological studies in their genetic diversity, transmission route, and zoonotic potential should be conducted in more wide range of hosts in Vietnam.

Conflict of interest

All coauthors declare no conflict of interest.

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