



## 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  $\beta$ -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 *Cryptosporidium* 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 Committee 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-weaned 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 *Cryptosporidium* 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, Vietnam 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

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