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Full Length Research Paper

Co-infection of HIV and parasites in China: *Cryptosporidium* prevalence in environmental water in HIV/AIDS high endemic areas

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The purpose of this study is to explore relationship between water contamination of *Cryptosporidium* parasite and co-infection of HIV with parasites in HIV/AIDS highly endemic areas in China, in order to understand the waterborne transmission routes of *Cryptosporidium* parasite in local environmental settings. The prevalence and contamination degree of *Cryptosporidium* oocysts in surface water and drinking water samples was detected in HIV/AIDS highly endemic areas in Anhui province, China. The surface water was collected from rivers and ponds, and drinking water was obtained from well water, bottled water and tap water. All water samples were detected by using Filta-Max Xpress method employed with immunomagnetic separation (IMS) and fluorescence staining techniques. The main results showed that a total of 32 water samples were collected, of which 9 were collected from the surface water, including 6 of pond water and 3 of the river water; 23 were from the drinking water, including 19 of well water, 3 of bottled water and 1 of tap water. The positive rate of *Cryptosporidium* oocysts was 30.43% in the drinking water, and 55.56% in surface water. The positive rate of *Cryptosporidium* in pond water was significantly higher than in the river water, and that in bottled water was higher than in well water. The highest density of *Cryptosporidium* oocysts in the surface water samples was 8000/100 L, much higher than that in well water (59/100 L) and bottled water (39/100 L). In conclusion, *Cryptosporidium* contamination in local surface water is serious and is a high risk to people lived with HIV-positive (PLWH) to be infected with *Cryptosporidium* spp. And water contamination with *Cryptosporidium* oocysts is one of the major causes that lead to *Cryptosporidium* infection for HIV infected cases. It is necessary to carry out surveillance on *Cryptosporidium* infection in people living with HIV positive.

Key words: HIV/AIDS: Cryptosporidium: Environmental water: China.

INTRODUCTION

Cryptosporidium spp. is a coccidian protozoan parasite that has gained much attention in the last 20 years as a clinically important human pathogen (Casemore, 2009; O'Donoghue, 1995). The protozoa is considered to be an important cause of diarrhea in humans and other animals (Savioli et al., 2006). It was firstly found by Tyzzer from mice in 1907 and the first crypotosporidiosis case of human infection was reported by Nime in 1976 (Nime et al., 1976; Tyzzer, 1907). Cryptosporidiosis has gradually being attentioned worldwide since the emergence of AIDS in 1980, due to its higher prevalence of co-infection between cryptosporidiosis and HIV/AIDS (Petersen, 1992; Tzipori and Widmer, 2008). The important infection stage of *Cryptosporidium* protozoa is its oocyst, which is a

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ubiquitous stage presenting in the environment (Angus, 1983; Fayer et al., 2000; O'Donoghue, 1995). Humans can acquire infections with *Cryptosporidium* oocysts through several routs. Waterborne transmission of *Cryptosporidium* infection was an important mode of transmission, since several severe outbreaks of cryptosporidiosis occurred contributed by the water contaminated with *Cryptosporidium* oocysts (Fayer, 2004; Juranek, 1995). More than 160 waterborne outbreaks of cryptosporidiosis have been reported worldwide, and the most cases reported in the US and UK (Craun et al., 2002; Slifko et al., 2000). The outbreaks of waterborne cryptosporidiosis pose a significant threat to public health (Casemore, 2009; Ongerth and Stibbs, 1987).

The first case of cryptosporidiosis which occurred in People's Republic of China (P. R. C) was discovered by Han Fan in Nanjing in 1987(Han, 1987). Up to now, outbreak of cryptosporidiosis has not been reported in China. Although several increasing investigations on human infections with *Cryptosporidium* parasite were reported during last decades (Huang and Dai, 2002; Shen and Wei, 2005; Song et al., 2004; Xu et al., 2005; Zhou et al., 2005), individual cryptosporidiosis infected through water transmission route were less relatively, so that few investigations on *Cryptosporidium* infection to focus on drinking water transmission route have been reported in China due to the following two reasons. First, infection with *Cryptosporidium* parasite in immunocompetent humans can cause only light symptom although sometime can cause acute diarrhea; Second, most native residents used to drinking boiled water, and *Cryptosporidium* oocysts can be killed in one minute in boiling water (Juranek, 1995; Yang and Wang, 2005).

With the development of social economics and changes of living status in China, drinking habits with water supply or bottled water become popular in both urban and rural areas (Lei et al., 2007); the problem of drinking water contamination of *Cryptosporidium* has been paid more attentions recently in China (Cai, 2005). For instance, the item of detecting *Cryptosporidium* has been added into the new National Criteria of Drinking Water (GB5749-2006) published in December, 2006 (China, 2006). Some investigations on prevalence of *Cryptosporidium* in surface water and drinking water had been carried out in Shanghai and Shenzhen cities, showing that contamination of *Cryptosporidium* was quite severe (Bai et al., 2006; Meng et al., 2005; Yu et al., 2003; Zhang et al., 2009). According to the results of a survey on *Cryptosporidium* infection carried out in Fuyang city, the population with HIV positive had a higher infection rate of *Cryptosporidium* with a prevalence rate of 8.28% (Tian et al., 2010). In order to understand the transmission routes of *Cryptosporidium* infection in area with a higher prevalence of HIV/AIDS in residents, we carried out an investigation on *Cryptosporidium* contamination in surface and drinking water in rural areas of Fuyang city, where there are about 3.56% of the HIV/AIDS prevalence in local residents who got the infection mainly through blood transfusion ten to twenty years ago.

MATERIALS AND METHODS

Selection of study area and collection of samples

The study area selected is located in suburb of Fuyang city, Anhui province, People's Republic of China. Fuyang city is located in the northern Anhui province east center part of China, and prevalence of HIV/AIDS was confirmed in over 8% of the population in the study area due to blood transfusion. River networks and surface water abundant are distributed in the study area. Three major rivers branches across the study area (Dongqing, Zhongqing and Xiqing rivers). Well water was the major drinking water for the residents, and a few people drink bottled and tap water.

The samples of surface water, including 6 ponds water and three rivers water, were collected from all villages of the study area. About 20 L sample were collected from the flowing water in rivers, and 10 L from non-flowing water in ponds.

The samples of drinking water were collected from households or surrounding environment. Those households were selected in random from the *Cryptosporidium* infection positives confirmed in previous cross-sectional survey (Tian et al., 2010). About 100 L of daily drinking water were collected from well, tap or bottled drinking water in each study household, respectively. Among all households selected, one household drank tap water and 19 households drank well water and 3 brands bottled water were drunk. So, we collected drinking water samples form 1 tap, 19 wells and 3 bottled water manufactories. The silicified plastic bucket or glass containers were used to collect samples.

Methods of detection

Water samples were pumped through a Filta-Max (IDEXX, Westbrook, ME) filter in a polycarbonate housing, using a car-battery powered pump. Samples ranged from 30 to 100 L, with the exact volume measured by an in-line meter. Oocysts were washed out of the filter according to the manufacturer's instructions (IDEXX 2002). In brief, a bolt is released allowing the compressed filter to expand, and oocysts trapped on a membrane are eluted into 10 ml of fluid. The sample is centrifuged to a pellet of <0.5 ml and then purified by IMS. If the pellet was >0.5 ml the sample was split.

Cryptosporidium oocysts in the samples were isolated from other particulate material by immunomagnetic separation using the commercial kit Dynabeads GC-Combo(Invitrogen Dynal AS, Oslo, Norway), as described in the method 1623 (U.S. Environmental Protection Agency, 1999). Briefly, the procedure involves adding magnetic beads labeled with *Cryptosporidium*-specific monoclonal antibodies to 10 ml of re-suspended pellet and allowing the antibody-antigen reactions to bind the oocysts to the beads. The sample is then magnetized, separating the oocyst-magnetic bead complex from the sample debris, which is then discarded. The beads are then detached and the oocysts are added to a well slide for sample screening, allowed to air-dry completely, and fixed with acetone. (Figure 1).

The identification of *Cryptosporidium* oocysts was conducted by immunofluorescence assay using the commercial kit Aqua-Glo G/C Direct IF Test (New Orleans, US), according to the manufacturer's instructions. Well slides were washed three times with abundant PBS buffer pH 7.2, and after adding mounting medium, the coverslip was sealed with nail polish. The slides were systematically examined by using epifluorescence microscope (Olympus BX51, Tokyo, Japan) at 400× magnification, searching for bri lliant apple-green fluorescing round to oval objects. Magnification was increased to 1000× for confirmation of presumptive samples and

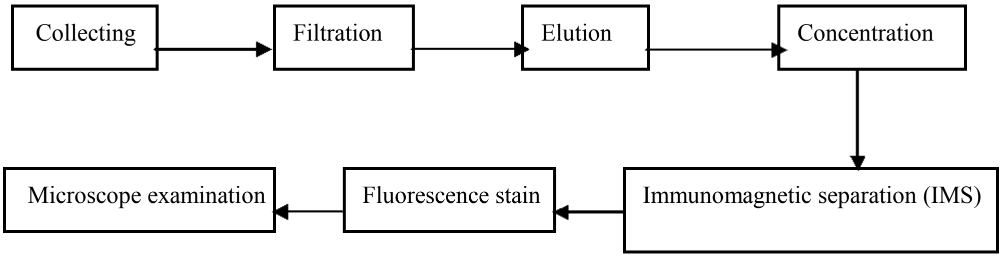

Figure 1. Flow chart of detection methods.

Table 1. Detection of *Cryptosporidium* oocysts from surface water in the village.

Environment of surface water sample	No. sample detected	No. sample positive	Positive rate (%)
Pond	6	4	66.7
River	3	1	33.3
Total	9	5	55.6

Table 2. Detection of *Cryptosporidium* oocysts from drinking water in the households.

Type of drinking water	No. sample detected	No. sample positive	Positive rate (%)
Tap water	1	0	0
Well water	19	5	26.3
Bottled water	3	2	66.7
Total	23	7	30.4

differential interference contrast microscopy was used for identification of internal morphological features. Estimations of the total amount of oocysts were calculated considering the volume of water filtered and the fraction of the pellet analysed. Positive and negative staining controls were routinely included.

RESULTS

Surface water

A total of 9 surface water samples were collected including 6 of pond ater and 3 of river water. The *Cryptosporidium* oocysts positive rate of surface water in the village where human *Cryptosporidium* infection were detected is 55.6%, which was significantly higher in pond water (66.7%) (Table 1).

Drinking water

Among 23 samples of drinking water examined form the households where human *Cryptosporidium* infection detected, 26.3 and 66.7% of samples were detected with *Cryptosporidium* oocysts in well water and bottled water, respectively. No *Cryptosporidium* oocysts was found in tap water (Table 2)

Degree of contamination in water

Among 12 samples of *Cryptosporidium* oocysts positive detected in the study, the degree of surface water contaminated with *Cryptosporidium* oocysts was the highest, varied from 24 to 8000 oocysts/100 L, followed by river water (55/100 L), bottled water (5-55 /100 L), and well water (1-39/100 L). (Table 3)

DISCUSSION AND CONCLUSION

Cryptosporidium was transmitted via the faecal-oral route, with the consumption of contaminated drinking water and foods acquisition of infection in developed countries (Slifko et al., 2000). *Cryptosporidium* are ubiquitous in the aquatic environment and *Cryptosporidium* oocysts may remain viable for several months under a range of environmental conditions (Smith et al., 1995).

Waterborne transmission was a major transmission route of *Cryptosporidium*. According to the reports in China, the regular monitoring of Zhuhai section of the

Table 3. Concentration of *Cryptosporidium* oocysts in positive water samples.

Samples code	Type of water samples	Concentration of *Cryptosporidium* oocysts (per 100 L)	Degree of contaminated
1	Pond water	8000	++++
2	Pond water	225	+++
3	Pond water	93	++
4	Pond water	24	++
5	Bottled water	55	++
6	Bottled water	5	+
7	River water	75	++
8	Well water	39	++
9	Well water	12	++
10	Well water	5	+
11	Well water	2	+
12	Well water	1	+

Xijiang river showed that that the water concentration of *Cryptosporidium* oocysts was from 0 to 182 /100 L. *Cryptosporidium* oocysts detection rate was 46% in raw water source of Guangzhou city in 2003. The survey results showed that the local surface water *Cryptosporidium* positive rate was 55.56%; surface water contaminated was relatively high. It may be related to local residents living habits and improper excreta disposal. Patients and asymptomatic people infected with *Cryptosporidium* was the major transmission source. There were 400 million *Cryptosporidium* oocysts in once stool excreted from people infected with *Cryptosporidium* (Li, 2004). Most of the toilets in the locality were not water-rush, and it was very widespread that the excretions of human and animals as well as domestic wastewater without any treatment were dropped directly into the ponds or ditch. It was the main reason the surface water was contaminated by *Cryptosporidium* in the locality.

According to criterion of daily drinking water in China (China, 2006), the number of *Cryptosporidium* oocysts per 100 L must be less than 1 in daily drink water. According to present study results that all the well water that were detected *Cryptosporidium* positive were from less than 8 m depth wells instead of more than 20 m depth wells. It suggested that less than 8 m depth wells were more easily contaminated by surface water. In order to protect local residents health and decrease *Cryptosporidium* contaminated surface water and pond water, we should strengthen education and management of feces, reasonable treat domestic wastewater and refuse. On one hand, we should strengthen feces management and health education to reduce water contamination; on the other hand, we should enhance the improvement of water and toilets by encouraging the use of tap water or more than 20 m depth wells water.

The present study showed that, among three bottled water samples, two were positive with *Cryptosporidium* oocysts, and another was suspected positive. Although bottled water was not totally popular in the locality, bottled water contamination had become a severe public health problem. It was common that drinking boiled water in local residents, even if well water was contaminated by *Cryptosporidium* oocysts, most of the *Cryptosporidium* oocysts can be killed after water was boiled. Bottled water was often drunk without boiling, however, especially in summer, it was more popular, so bottled water was more significant as an infection source than well water. *Cryptosporidium* oocysts were very small, diameter was 4 µm and resistant regular chloridize disinfector, is not eliminated by regular filter. According to reports in Japan, *Cryptosporidium* oocysts positive rate was still 35%, even after being filtered; in Canada, it was 3.5% in treated water (Wallis et al., 1996). So, it is suggested that we should enhance management and monitoring of bottled water, and encourage the drinking of boiled water.

The method of Filta-Max xpress, which was admissive by Environmental Protection Bureau of America (EPA), was employed to detect *Cryptosporidium* oocysts in water. This method was in accord with the criterion of EPA, and detection speed was quick and can be used as a substitute of Envirochek HV and Filta-Max (Li and Chen, 2008). Due to time consumption and cost of this method, the limitation of this study is that we were only able to detect the water samples collected from those representative environments in the study villages.

In conclusion, *Cryptosporidium* contamination in local surface water is serious and there is a high risk to HIV infected cases to be infected with *Cryptosporidium* spp. And water contamination with *Cryptosporidium* oocysts is one of the major causes that lead to *Cryptosporidium* infection for HIV infected cases. Therefore, it is suggested that management of feces and daily refuse and education on local residents have to be strengthened in the local settings. Mostly, the behavior on improving personal hygiene and not drinking bottle water directly instead of drinking boiled water are recommend for those people lived with HIV/AIDS. Furthermore, it is imperative to carry out surveillance on *Crptosporidium* infection in people living with HIV positive.

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