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Detection of *Giardia* cysts and *Cryptosporidium* oocysts in central Taiwan rivers by immunofluorescence assay

Tai-Lee Hu

Department of Environmental Engineering and Science, Feng Chia University, Taichung, Taiwan, ROC

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From November 1998 through 1999, water samples collected from the Faze, Kanwe, and Dajia rivers were analyzed using a combined immunofluorescence antibody method for detecting cysts of *Giardia* spp. and oocysts of *Cryptosporidium* spp. Water samples of the Kanwe River were collected downstream of a piggery; 5 to 469 cysts and 11 to 1102 oocysts per 100 L were found in the water samples. The sampling site of the Faze River was upstream of the same piggery, and there were 110 cysts and 188 oocysts per 100 L of water samples. Water samples from the Dajia River were taken from an inlet of a water purification plant. There were 0 to 14.4 cysts and 14.3 to 50.5 oocysts per 100 L of water samples. Results indicate that the presence of *Giardia* cysts and *Cryptosporidium* oocysts in the surface water is closely related to the discharge of piggery wastes. Cysts and oocysts were found less often in the source water of a water purification plant.

Key words: *Cryptosporidium oocysts*, *Giardia* cysts, immunofluorescence antibody method

Giardia and *Cryptosporidium* are enteric environmentally resistant intestinal parasites that can cause gastroenteritis in humans when they are ingested. These organisms can be transported by water and are documented to cause waterborne outbreaks of giardiasis and cryptosporidiosis [1]. LeChevallier *et al* [2] investigated 66 surface water treatment plants in the United States and Canada, and found that *Giardia* cysts and *Cryptosporidium* oocysts were widely dispersed in the aquatic environment. *Giardia* spp. were detected in 81% of the raw water samples, and *Cryptosporidium* spp. were found in 87% of the raw water locations. Higher cyst and oocyst densities are associated with source waters receiving sewage effluents. *Giardia* and *Cryptosporidium* spp. in drinking water supplies were also studied in the United States [3,4]. Information pertaining to *Giardia* and *Cryptosporidium* spp. in the drinking water supply systems in Taiwan is limited. Hsu *et al* [5] investigated 13 water samples from the Kau-Ping River and its watershed (in southern Taiwan), and found that *Cryptosporidium* spp. were present in 40% of the treated water, whereas *Giardia* occurred in all of the samples.

The Dajia River is the major raw water source of the great Taichung area, which serves approximately 700 000 residents. The Kanwe River is the side stream of the Faze River located in Taichung City. The City Council has planned to develop certain areas in the Faze River for recreational purpose; the water quality of the Faze River, however, declined from slightly to moderately polluted after several small piggeries and duck farms were built along the river.

This study aimed to examine the occurrence of *Giardia* and *Cryptosporidium* spp. in the surface water at the intended development locations of the Faze and Kanwe Rivers, as well as in water samples from source water of a water purification plant.

Materials and Methods

Sampling sites and procedures

Water samples were collected from the Faze, Kanwe, and Dajia Rivers located in central Taiwan. Except for those of the Dajia River, water samples were collected near a piggery located downstream of the Faze River, and upstream of the Kanwe River. Sampling method and procedures were adopted from the Standard Methods for the Water and Wastewater [6]. Raw water samples of 300 to 600 L were concentrated through a 25.4-cm long and 1-μm pore size polypropylene yarn-wound cartridge filter.

Detection of cysts and oocysts

A combined immunofluorescence method was used to detect *Giardia* cysts and *Cryptosporidium* oocysts

Corresponding author: Dr. Tai-Lee Hu, Department of Environmental Engineering and Science, Feng Chia University, 100, Wenhwa Road, Taichung 40724, Taiwan, ROC. E-mail: tlhu@fcu.edu.tw

Table 1. Water quality parameters of the sampling sites

Date mm/dd/yy	Temperature (°C)	Depth (cm)	Turbidity	Sampling site
11/30/98	20	50	++	Kanwe
12/29/98	20	50	+	Faze
03/30/99	25	55	++++	Kanwe
07/20/99	24	65	+++	Kanwe
09/06/99	26	55	+	Dajia
10/12/99	24	44	1.78 NTU	Kanwe
10/26/99	21	40	13.84 NTU	Dajia
11/16/99	22	40	8.19 NTU	Dajia

Abbreviation: NTU = nephelometric turbidity units
Note: + = turbidity measured by visualization

[7]. The HYDROFLOR-Combo (Strategic Diagnostics, NC, US), an *in vitro* procedure for the simultaneous detection of *Giardia* cysts and *Cryptosporidium* oocysts, was used. An epifluorescent microscope (Nikon, Japan) was used to examine the cysts and oocysts.

Water quality analysis

Additional water quality data, such as turbidity as measured by visualization or nephelometric method (nephelometric turbidity unit, NTU) [6], flow rate, and water level, were provided by the participating utilities. All analyses were conducted according to the Standard Methods for the Water and Wastewater [6].

Results and Discussion

The water quality parameters of the sampling sites are listed in Table 1. A total of 8 samples were collected. Samples of the Dajia River were collected at the inlet of a water treatment plant; the flow rate and depth of water were not determined. Water samples of the Kanwe River, collected downstream of a piggery, have a concentration of *Giardia* cysts ranged from 5 to 469 cysts per 100 L, whereas the concentration of *Cryptosporidium* oocysts ranged from 11 to 1102 oocysts per 100 L. Water samples of the Faze River were collected upstream of a piggery, and the concentration of *Giardia* cysts was 110 cysts per 100 L, whereas the concentration of *Cryptosporidium* oocysts was 188 oocysts per 100 L (Table 2). The occurrence of *Cryptosporidium* oocysts was higher than that of *Giardia* cysts in the Faze and Kanwe rivers, a finding coincided with those described by Rose *et al* [1] and LeChevelier *et al* [2]. The water quality in terms of both turbidity and the presence of *Giardia* cysts and *Cryptosporidium* oocysts in the surface water is related to the discharge of piggery wastes, as similarly observed by LeChevelier *et al* [2] and Bukhari *et al* [8].

The amount of *Giardia* cysts and *Cryptosporidium* oocysts in the Dajia River was between 0 and 14.4 cysts per 100 L and 14.3 and 50.5 oocysts per 100 L, respectively (Table 3). Again, the density of *Cryptosporidium* oocysts is higher than that of *Giardia* cysts. The observation of this phenomenon was concurred by several reports [1-3,5], and may have resulted from the fact that the combined immunofluorescence method is more sensitive to the *Cryptosporidium* oocysts. The occurrence of cysts and oocysts in the surface water correlated well with the water quality parameter of turbidity [2]. The increase in turbidity and pellet volume observed in the sample collected at October 26, 1999 (Table 1) could both have resulted from the powerful earthquake on September 21, 1999, of which the epicenter was in close proximity to the study area and caused a dramatic reshaping of the landscape and stream network. The source water (Dajia River) for the water purification plant was therefore affected. However, 2 months after the earthquake, the turbidity of the same source water decreased from 13.84 to 8.19 NTU (Table 1) and the amount of cysts and oocysts also decreased.

Table 2. Concentration of *Giardia* cysts and *Cryptosporidium* oocysts in raw water samples collected upstream and downstream of a piggery at the Faze River

Site	No. of water samples	*Giardia* cysts/100 L		*Cryptosporidium* oocysts/100 L	
		Range	Mean	Range	Mean
Upstream of piggery	2	0-220	110	2-374	188
Downstream of piggery	4	5-469	137.5	11-1102	313.5

Table 3. Concentration of *Giardia* cysts and *Cryptosporidium* oocysts in the Dajia River

Date mm/dd/yy	*Giardia* cysts/100 L	*Cryptosporidium* oocysts/100 L	Sample volume (L)	Pellet volume (mL)
09/06/99	10	14.3	600	8
10/26/99	14.4	50.5	600	19.5
11/16/99	0	17.2	600	16.5

Although *Giardia* cysts and *Cryptosporidium* oocysts are present in central Taiwan rivers, whether they are pathogenic species needs further investigation. In conclusion, *Giardia* cysts and *Cryptosporidium* oocysts were present in surface water of central Taiwan. The levels of cysts and oocysts in source water were apparently lower than that in surface water. The efficiency of water purification plants in treating cysts and oocysts should be investigated.

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