Parasitol Res (2001) 87: 163–168

ORIGINAL PAPER

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Analysis for *Giardia* cysts and *Cryptosporidium* oocysts in water samples from small water systems in Taiwan

Received: 6 September 2000 / Accepted: 10 September 2000

Abstract *Giardia* and *Cryptosporidium* have emerged as waterborne pathogens of concern. Twenty-six water samples were collected from small water systems in Taiwan and checked for the occurrence of both parasites. Water quality parameters and characteristics of the sampling sites were also recorded. The frequencies of occurrence for *Giardia* and *Cryptosporidium* were 46.2% for each; and their mean concentrations were 79.5 cysts/100 l and 22.1 oocysts/100 l, respectively. The concentrations of oocysts and heterotrophic bacteria exhibited the highest correlation and followed the concentrations of the two protozoa. The water samples from sites with filtration devices had a lower oocyst concentration than those from sites without filtration devices, while no significant difference was found for cysts. The level of each parasite had no direct relationship with altitude. The cyst concentrations increased proportionally with the consumer population using the water systems. Risk assessment of the parasitic infections suggests that setting up disinfection devices in the small water systems would be needed.

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Introduction

The protozoan parasites, *Giardia* and *Cryptosporidium*, have been recognized as common pathogenic protozoa of the gastrointestinal tract. Many outbreaks of giardiasis and cryptosporidiosis have been reported during the past 25 years (Moore et al. 1994; Lisle and Rose 1995; Frost et al. 1996); human infection with these pathogens was usually through ingestion of contaminated food and/or water (Donnelly and Stentiford 1997; Pell 1997). Outbreaks were associated with filtered and unfiltered surface water sources, groundwater sources, and contamination of the distribution system. In most outbreaks, sources of contamination and deficiencies in treatment and operation were identified (Teunis et al. 1997; Eisenberg et al. 1998; Perz et al. 1998). Experiences with waterborne outbreaks of *G. lamblia* led to the promulgation of the Surface Water Treatment Rule (SWTR) by the United States EPA. From 1986 to 1995, 21 waterborne outbreaks of cryptosporidiosis were reported in the United Kingdom. Therefore, the United Kingdom regulated *Cryptosporidium* in drinking water by setting a numerical standard and requiring tests to meet that standard (Clancy and Hansen 1999).

The first case of giardiasis in Taiwan was discovered on an offshore island in 1975. Of the children residing in the island, 32% were diagnosed with *Giardia* in their stool specimens (Chung and Cross 1975). Another survey also showed that over 50% of the bird species in Taiwan were infected with *Cryptosporidium* spp. (Wang and Liew 1990). Regional surveys (Huang and Hsu 2000) have shown that *Giardia* cysts and *Cryptosporidium* oocysts are widespread in drinking water sources and have been detected in insufficiently treated effluent from water treatment plants. The small water systems in Taiwan are mostly located in mountain areas or in secluded communities. The tap water supplied from these simple water systems are mostly untreated, or only treated by a simple filtration procedure, because these

systems generally have fewer technical and financial resources to cope with new requirements.

The aim of this study was to examine the occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in the small water systems in Taiwan, using the indirect fluorescent antibody method. We investigated the relationships between the parasites and water quality parameters, such as pH, turbidity, conductivity, heterotrophic bacteria, and total coliforms. The correlations between the distribution of protozoan parasites and some characteristics of the sampling sites were analyzed (e.g., size of population using the water facilities, water treatment procedures, and altitude at which the system was located). A risk analysis for human infection and suggestions for improving the small water systems to cope with *Giardia* cysts and *Cryptosporidium* oocysts were also included in this study.

Materials and methods

Sampling and examination procedures

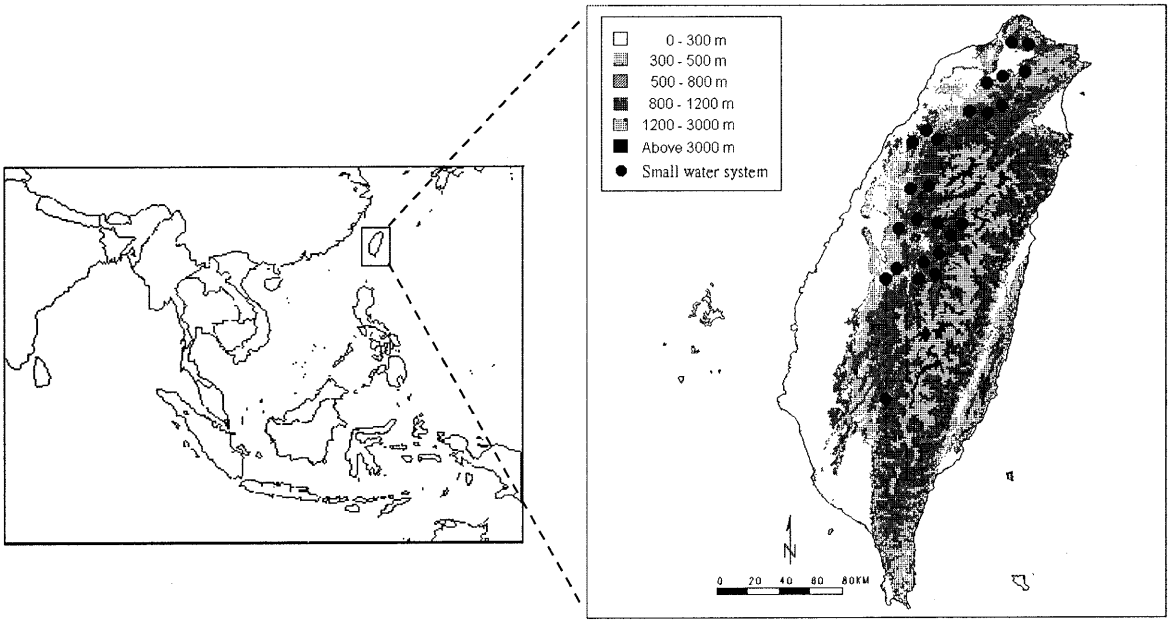
Twenty-six water samples were taken from different small water systems in Taiwan during a 3-year period (From February, 1996, to March, 1999). The distribution of the small water systems and their altitudes are shown in Fig. 1. Water samples were assayed for *Giardia* cysts and *Cryptosporidium* oocysts by the fluorescent antibody procedure as specified by the Information Collection Rule (ICR) method of the United States EPA (1995) for protozoans. This method generally contains three stages: sample concentration and elution, separation of cysts and oocysts from other debris, and the staining and identification of the protozoan parasites. Aliquots of water samples were filtered through 1-μm cartridge filters. The filters were removed from the cartridge, cut off the supporting core, and divided into three parts. The filter fibers were eluted with 0.7–1.2 l of eluting fluid [phosphate-buffered saline (PBS), 1% Tween 80, and 1% sodium dodecyl sulfate (SDS)] in a mechanical stomacher and the eluate was then centrifuged at 1,050 *g* for 10 min in a 50-ml centrifuge tube on a swinging-bucket rotor. The volume of the packed pellet was recorded. After the supernatant was aspirated, the pellet was resuspended in an equal volume of 10% formalin and the eluting fluid was added to a total volume of 20 ml in the same centrifuge tube. The mixture was underlaid with 30 ml of a Percoll-sucrose gradient (sp. gr. = 1.10) followed by centrifuging. The top 20 ml and the 5 ml below the interface were collected, diluted with eluting solution to 50 ml, and centrifuged. The upper-layer liquid was then aspirated until only 5 ml of concentrate was left. After being well mixed, the resuspended sediment samples were applied to individual 25-mm diameter cellulose-acetate membranes, stained with fluorescent-labelled antibodies (Hydrofluor-Combo *Giardia*/*Cryptosporidium*; Ensys, N.C., USA), and examined using an epifluorescent microscope (Olympus, Japan) at 200×, 400×, or 1000× magnification. Cysts and oocysts were identified using the following parameters: size, shape, surface features, and staining reaction. The candidates exhibiting the right size and shape were further identified by their internal morphological features, using an epifluorescent microscope under a bright field.

The stock solution containing *Giardia* cysts and *Cryptosporidium* oocysts was produced from Hydrofluor-positive antigen control of Hydrofluor-Combo *Giardia*/*Cryptosporidium* and P101L *G. lamblia* cysts (Waterborne, La., USA). The cysts and oocysts were purified by Percoll-sucrose gradient purification, followed by washing and dilution in 0.1% PBS and were then stored at 4 °C as the stock solution. The numbers of cysts and oocysts in the stock solution were counted before each seeded experiment, using the immunofluorescence assay technique. To do this, samples were mixed thoroughly, pipetted directly from the stock preparation vial onto glass slides (Dynal Spot-On), stained with fluorescent-labelled antibodies (Hydrofluor Combo *Giardia*/*Cryptosporidium*), and counted. The numbers of cysts and oocysts in the solution after clarification were determined according to the ICR protozoan method. Some water quality parameters and environmental factors were recorded simultaneously with protozoan parasite collection.

Data analysis and calculation

To investigate the recovery efficiency of the ICR protozoan method, we collected five water samples from small water systems. The turbidity of water samples ranged over 0.5–1.2 nepholometric turbidity units. The numbers of cysts and oocysts in the stock solution which was seeded into water samples and the numbers in

Fig. 1 Sampling sites of small water systems and their altitudes



solution after clarification were counted, using an epifluorescent microscope. The detection limit of each water sample was calculated as described in Eq. 1 (EPA 1995).

$$X = \frac{100}{V \times F \times R} \quad , \tag{1}$$

where X is the detection limit of cysts/oocysts in 100 l of the water sample, V is the water volume, F is the fraction of the initial eluate packed-pellet volume subjected to clarification, and R is the percentage of suspended sediment examined. The numbers of cysts and oocysts observed under the microscope were recorded, multiplied by the detection limit, and divided by the recovery efficiency to obtain the actual concentration of cysts and oocysts. When no protozoa was observed under the epifluorescent microscope, we expressed the protozoan concentration in water samples as less than (<) their detection limits. In the statistical evaluation, data under the detection limits were treated as zero. Spearman R correlation coefficients were calculated between the concentrations of cysts/oocysts and some water quality parameters, using Statistica software (StatSoft, USA).

Risk evaluation

The risk of infection by protozoan parasites in drinking water depends upon: (1) the actual concentration of cysts or oocysts in the drinking water, (2) the viability of cysts or oocysts, and (3) the daily consumption of unboiled water. For a given drinking water sample, the individual daily dose (N) may be calculated as presented in Eq. 2 (Teunis et al. 1997),

$$N = C \times I \times V, \tag{2}$$

where C is the actual concentration of cysts or oocysts in the treated water, I is the viability of the pathogen, and V is the daily individual consumption of unboiled drinking water.

In order to obtain reliable risk assessment values, the individual dose must be estimated from the parasite concentration in the drinking water and the amount of water consumed over the exposure period (Teunis et al. 1997). The exponential assessment model, Eq. 3, was used to predict the number of cases of illnesses resulting from the measured levels of parasite. This microbiological model of infectivity was proposed by Furumoto and Mickey (Federal-Provincial Subcommittee on Drinking Water 1998) and has been used successfully to assess the risk of infection from *Giardia* and *Cryptosporidium* (Regli et al. 1991; Haas et al. 1994).

$$P = 1 - \exp(-r\,N) \, , \tag{3}$$

where r is a coefficient, P is the potential daily risk, N is the daily dose, and it was assumed that a person drank 2 l water/day. The r value for *Giardia* is 0.0105, as adopted by the Federal-Provincial Subcommittee on Drinking Water (1998). The r value for *Cryptosporidium* adopted in our study was 0.00419, as proposed by Haas et al. (1996).

An annual risk for infection could be calculated as shown in Eq. 4 (Federal-Provincial Subcommittee on Drinking Water 1998):

$$P_A = 1 - (1 - P)^{365}, \tag{4}$$

where P_A is the annual risk and P is the daily risk.

Results and discussion

Occurrence of cysts and oocysts in small water systems

The sampling sites, sampling volumes, parasite concentrations and parameters of water quality are listed in Table 1. The recovery efficiencies of the ICR protozoan method were 27.3 ± 5.6% ($n = 5$) for cysts and 18.3 ± 6.8% ($n = 5$) for oocysts in this study. The percentage of positives for *Giardia* cysts and *Cryptosporidium* oocysts in the 26 water samples was 46.2% for

Table 1 Sampling sites, sampling volumes, concentration of *Giardia* cysts and *Cryptosporidium* oocysts, and water quality parameters for 26 water samples from small water systems in Taiwan. *CFU* Colony-forming units, *NTU* nepholometric turbidity units

Sampling site	Sampling volume (l)	*Giardia* (cysts/100 l)	*Cryptosporidium* (oocysts/100 l)	Heterotrophic bacteria (CFU/ml)	Total coliforms (CFU/100 ml)	pH	Temperature (°C)	Conductivity (μs)	Turbidity (NTU)
Nan Gang	500	<23.4	105.5	3.4×10^4	1	6.80	–	–	0.15
Fu Guei	500	44.0	98.9	1.3×10^4	3	6.76	–	–	0.07
Song Her	400	<9.5	28.4	2.0×10^3	<1	6.21	–	100	0.02
Gu-Gwan	300	21.6	<32.2	2.7×10^4	24	6.46	–	–	0.12
Dong-Her	300	<20.5	30.6	950	5	6.50	–	–	0.16
Pong-Liai	300	40.7	30.6	450	<1	6.20	–	20	0.24
Fu Shing	400	12.1	36.1	1.1×10^4	56	5.70	–	100	0.17
Jiou-Chiong	400	<8.4	<12.6	3.2×10^4	150	7.03	–	30	0.14
Tsuen-Yang	300	<11.0	<16.4	1.3×10^{44}	34	6.82	–	–	0.10
Wu-Ser	400	59.7	18.0	2.8×10^3	2	6.78	–	–	0.16
Tien Liau	500	2.9	<4.4	4.2×10^3	1	6.64	24.5	221	0.8
Hen San	500	<2.9	4.4	420	79	6.35	26.3	49	1.1
Da Pindi	500	<2.9	<4.4	5.2×10^3	67	6.70	24.0	58	1.2
Chuang Zo	500	<7.3	<10.9	9.0×10^3	28	6.18	26.4	186	1.2
Zue Chu	500	<7.3	<10.9	50	<1	6.70	24.2	835	1.3
Fuon Fuang	500	<7.3	<10.9	327	340	7.16	21.3	222	1.9
Shi Lin	500	<2.9	4.4	40	150	6.70	18.8	200	0.2
Shuang Shi	500	5.9	4.9	4.1×10^3	2.4×10^3	6.70	22.8	250	1.9
Chia Sieng	50	630.0	<313.7	–	–	7.05	20.5	437	80.0
Shuang Shi	500	33.0	<12.6	2.0×10^3	4.6×10^4	7.84	24.4	132	2.4
San Kuang	200	<7.3	<10.9	192	1.7×10^3	7.86	22.0	351	0.3
Luo Fu	250	<24.5	<36.6	550	2.8×10^3	7.96	21.0	343	1.5
Sun Baline	300	<5.1	<7.7	10	12	7.65	14.5	170	0.1
Ping Lin	250	33.3	49.7	2.2×10^4	400	7.68	22.0	60	0.8
Shin Sieng	250	1026	163.9	1.0×10^4	500	7.70	19.5	106	0.3
Chu Xer	250	157.1	<47.0	3.5×10^3	1.3×10^3	7.51	21.7	94	0.4

each. The mean concentration of *Giardia* cysts was 79.5 cysts/100 l, ranging from <2.9 cysts/100 l to 1,025.6 cysts/100 l; and the mean concentration of *Cryptosporidium* oocysts was 22.1 oocysts/100 l, ranging from <4.4 oocysts/100 l to 163.9 oocysts/100 l. The mean concentration of *Giardia* cysts was 3.6 times greater than that of *Cryptosporidium* oocysts in small water systems.

LeChevallier et al. (1991), surveying water treatment plants from the northeastern United States, found that 81.2% and 87.0% of 83 raw water samples contained *Giardia* cysts and *Cryptosporidium* oocysts, respectively. Rose et al. (1993) found that out of the 257 surface water samples studied, 16% contained 2–140 cysts/100 l and 55% contained 0.25–4,400 oocysts/100 l in the United States. In the study of Huang and Hsu (2000) for large water treatment plants in Taiwan, the percentages of the 24 raw water samples positive in *Giardia* cysts and *Cryptosporidium* oocysts were 79.2% and 62.5%, respectively. The average concentration of *Giardia* cysts was 349.2 cysts/100 l and, for *Cryptosporidium* oocysts, it was 685.0 oocysts/100 l. These results indicated that the occurrence of both cysts and oocysts varied widely in different surveys, and the average (oo)cyst concentrations from raw water samples from Taiwan large water treatment plants were higher than those from small water systems.

Relationship between concentration of protozoan parasites and some water quality parameters

Table 2 shows the correlation coefficients between the concentration of protozoan parasites and some water quality parameters (heterotrophic bacteria, total coliforms, pH, conductivity, and turbidity) in the water samples. During the period of water sampling from small water systems, the concentration of heterotrophic bacteria ranged between 4 CFU/ml and 3.4×10^4 CFU/ml (Mean $\pm$ SD $= 7.9 \times 10^3 \pm 1.0 \times 10^4$ CFU/ml, $n = 25$), while the concentration of total coliforms ranged from <1 CFU/ml to 4.6×10^4 CFU/100 ml (Mean $\pm$ SD $= 2.2 \times 10^3 \pm 9.1 \times 10^3$ CFU/100 ml, $n = 25$). In Table 2, positive correlations were found between the concentrations of *Giardia* cysts and *Cryptosporidium* oocysts ($n = 9$, $R = 0.677$, and $P = 0.045$) and between the concentrations of *Cryptosporidium* oocysts and heterotrophic bacteria ($n = 12$, $R = 0.788$, and $P = 0.0024$). No correlation was found between either parasite and any of the physical or chemical water qualities monitored.

Rose et al. (1988) and LeChevallier et al. (1991) found a significant correlation between the concentrations of cysts and oocysts in their samples. In our previous studies (Huang and Hsu 2000), using raw water samples from large water treatment plants in Taiwan, the correlation between *Giardia* cysts and turbidity ($n = 24$, $R = 0.521$, and $P = 0.009$) was most significant, followed by that between *Giardia* cysts and heterotrophic bacteria ($n = 23$, $R = 0.508$, and $P = 0.018$) and that between cysts and oocysts ($n = 24$, $R = 0.479$, and $P = 0.018$). Although the correlations between the occurrences of protozoa and some indicator microorganisms are expected, the results cannot be universally applied in a comparison with other studies. This is because the indicator microorganisms in water mainly come from feces and the soils while the *Giardia* cysts and *Cryptosporidium* oocysts in water are from the feces of infected hosts. Investigation of the relationship between (oo)cysts and the accompanying microorganisms in the host feces may help to find a more reliable indicator microorganisms.

Relationship between parasite concentration, altitude, number of consumers, and filtration devices

The concentration of the (oo)cysts and characteristics of the sampling sites, such as altitude, number of consumers, and filtration devices used, are shown in Table 3. The concentrations of cysts and oocysts are discriminated at four levels: "–" below detection limit, "+" 1–10 (oo)cysts/100 l, "++" 10–100 (oo)cysts/100 l, and "+++" >100 (oo)cysts/100 l. Small water systems equipped with filtration devices are displayed as "+" and those without the filtration devices are displayed as "–".

The (oo)cyst levels in water samples from small water systems with or without filtration devices are compared in Fig. 2. Oocyst levels in samples from sites with filtration devices were lower than those from sites without filtration devices, while no significant discrepancy was found for cysts.

Figure 3 shows the relationship between each parasite and the altitude of the small water systems. The

Table 2 Non-parametric correlation coefficient between density of protozoa and water quality parameters. *n* Sample size, *P* probability level, *R* Spearman *R* correlation coefficient

Parasite	*Cryptosporidium*	Heterotrophic bacteria	Total coliforms	pH	Conductivity	Turbidity
Giardia	$n = 9$ $R = 0.677$ $P = 0.045$	$n = 11$ $R = -0.1727$ $P = 0.6115$	$n = 11$ $R = 0.0455$ $P = 0.8944$	$n = 11$ $R = 0.518$ $P = 0.102$	$n = 8$ $R = -0.619$ $P = 0.102$	$n = 11$ $R = -0.323$ $P = 0.332$
Cryptosporidium	–	$n = 12$ $R = 0.788$ $P = 0.0024$	$n = 12$ $R = -0.086$ $P = 0.7912$	$n = 12$ $R = 0.462$ $P = 0.130$	$n = 8$ $R = -0.133$ $P = 0.754$	$n = 12$ $R = -0.286$ $P = 0.367$

Table 3 (Oo)cysts levels and characteristics of sampling sites. (Oo)cyst concentrations were grouped as:– below detection limit, + 1–10 (oo)cysts/100 l, ++ 10–100 (oo)cysts/100 l, +++ >100 (oo)cysts/100 l. Small water systems equipped with filtration devices are given as "+" and those without filtration devices are given as "–"

Sampling site	*Giardia*	*Cryptosporidium*	Altitude (m)	No. of consumers	Filtration device
Nan Gang	–	+++	900	500	+
Fu Guei	++	++	900	500	–
Song Her	–	++	1,000	1,000	+
Gu-Gwan	++	–	900	1,200	–
Dong-Her	–	++	400	300	–
Pong-Liai	++	++	400	300	–
Fu Shing	++	++	1,000	500	+
Jiou-Chiong	–	–	1,200	500	–
Tsuen-Yang	–	–	1,200	2,000	–
Wu-Ser	++	++	1,200	2,000	+
Tien Liau	+	–	300	1,800	+
Hen San	–	+	300	4,000	+
Da Pindi	–	–	300	450	–
Chuang Zo	–	–	500	200	–
Zue Chu	–	–	500	2,000	+
Fuon Fuang	–	–	900	1,000	+
Shi Lin	–	+	400	1,400	+
Shuang Shi	+	+	300	8,000	+
Chia Sieng	+++	–	800	5,000	+
Shuang Shi	++	–	300	8,000	+
San Kuang	–	–	1,200	300	+
Luo Fu	–	–	1,200	1,000	+
Sun Baline	–	–	1,500	4,000	–
Ping Lin	++	++	400	2,000	–
Shin Sieng	+++	+++	300	1,000	–
Chu Xer	+++	–	300	1,600	+

altitudes were divided into four levels: (A) 100–400 m, (B) 400–700 m, (C) 700–1,100 m, and (D) >1,100 m. The results indicate that parasite levels have no direct relationship with altitude, although the concentrations of (oo)cysts were significantly lower in water systems at altitudes >1,100 m.

The numbers of consumers using the small water systems were also divided into four levels: (A) <500 people, (B) 500–1,000 people, (C) 1,000–2,000 people, and (D) >2,000 people. The relationship between (oo)cyst levels and consumer population are depicted in Fig. 4. The results show that the level of cysts increased proportionally with the consumer population of the water systems. However, no similar trend was discovered for oocysts.

The risk of giardiasis and cryptosporidiosis

The annual risk of giardiasis and cryptosporidiosis of water in 25 small water systems is listed in Table 4. The water samples of small water systems were taken from the tap and the water from these facilities was not disinfected. Therefore, the viability of the pathogen (*I*) was assumed to be 1 and the infection risk of the tap water was directly determined by the detection of (oo)cyst concentrations in the water samples. The average annual risk of giardiasis was 0.997, ranging from <0.199 to 1; and it was 0.491 for cryptosporidiosis, ranging from <0.126 to 0.993. The presence of *Giardia* cysts and *Cryptosporidium* oocysts in small water systems in

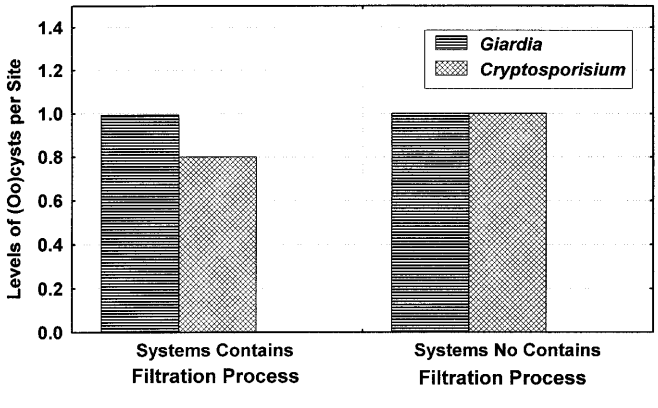


Fig. 2 Relationship between (oo)cyst levels and small water systems with or without filtration devices

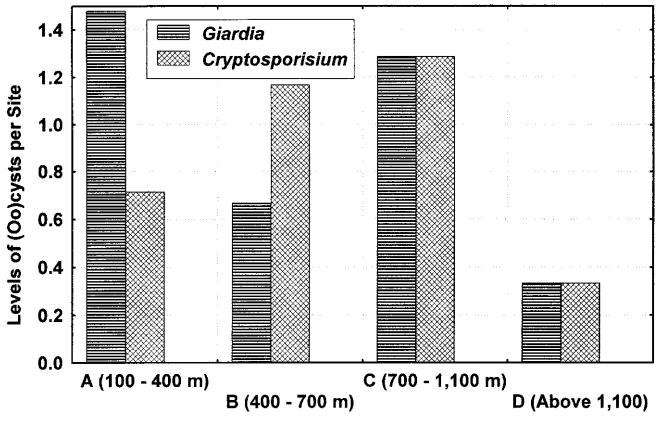


Fig. 3 Relationship between (oo)cyst levels and altitude (m) of small water systems

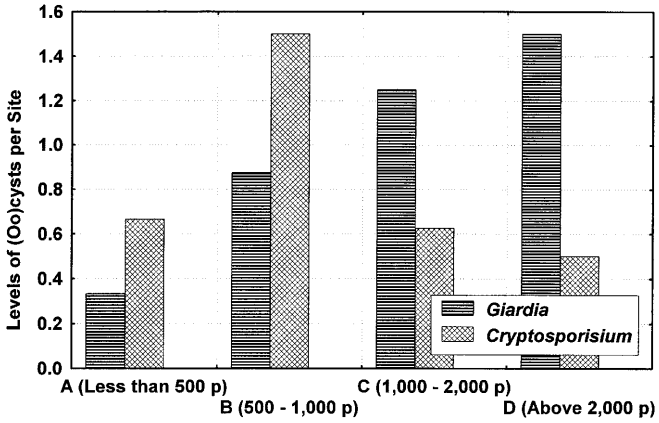


Fig. 4 Relationship between (oo)cyst levels and the consumer population for each small water system. *p* People

Table 4 Annual risk of giardiasis and cryptosporidiosis from water in 25 small water systems

Small water system	Annual risk of giardiasis	Annual risk of cryptosporidiosis
Nan Gang	< 0.833	0.960
Fu Guei	0.965	0.951
Song Her	< 0.517	0.580
Gu-Gwan	0.809	< 0.627
Dong-Her	< 0.792	0.608
Pong-Liai	0.955	0.608
Fu Shing	0.604	0.669
Jiou-Chiong	< 0.474	< 0.320
Tsuen-Yang	< 0.569	< 0.394
Wu-Ser	0.989	0.423
Tien Liau	0.199	< 0.126
Hen San	< 0.199	0.126
Da Pindi	< 0.199	< 0.126
Chuang Zo	< 0.428	< 0.283
Zue Chu	< 0.428	< 0.283
Fuon Fuang	< 0.428	< 0.283
Shi Lin	< 0.199	0.126
Shuang Shi	0.363	0.139
Chia Sieng	1	< 0.999
Shuang Shi	0.920	< 0.320
San Kuang	< 0.428	< 0.283
Luo Fu	< 0.847	< 0.608
Sun Baline	< 0.324	< 0.230
Ping Lin	0.922	0.781
Shin Sieng	1	0.993
Chu Xer	0.999	< 0.763

Taiwan is more prevalent than previously recognized; and the infection risk when consuming water from small water systems is higher than the risk when consuming tap water from large water treatment plants, which use disinfection devices (Huang and Hsu 2000). Therefore, disinfection devices would be needed for the small systems where (oo)cysts were detected.

Acknowledgements This work was supported by the R.O.C. Environmental Protection Agency. We are grateful to Mr. Yeong-Fua Hsu and Mr. Peang-Rong Wang for their experimental work, and to Dr. Jill Ruhsing Pan for her editing.

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