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Journal of Toxicology and Environmental Health, Part A: Current Issues

Publication details, including instructions for authors and subscription information: <http://www.tandfonline.com/loi/uteh20>

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To cite this article: Bing-Mu Hsu, Chihpin Huang, Guo-Ying Jiang, Chih-Li Lilian Hsu (1999) THE PREVALENCE OF GIARDIA AND CRYPTOSPORIDIUM IN TAIWAN WATER SUPPLIES, Journal of Toxicology and Environmental Health, Part A: Current Issues, 57:3, 149-160, DOI: [10.1080/009841099157728](http://www.tandfonline.com/action/showCitFormats?doi=10.1080/009841099157728)

To link to this article: <http://dx.doi.org/10.1080/009841099157728>

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THE PREVALENCE OF *GIARDIA* **AND** *CRYPTOSPORIDIUM* **IN TAIWAN WATER SUPPLIES**

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Giardia *and* Cryptosporidium *have emerged as waterborne pathogens of concern. Thirty-one water samples were collected from nine potable water treatment plants in Taiwan and investigated for the presence of* Giardia *cysts and* Cryptosporidium *oocysts. The immunofluorescence assay was used for the simultaneous detection of cysts and oocysts. The frequency of occurrence of cysts was 77.8% for* Giardia *and 72.2% for* Cryptosporidium *in 18 raw water samples. Ten out of 13 samples collected from treated water samples showed the presence of cysts, while in 5 out of 13 treated water samples oocysts were detected. The risk assessment for adverse human effects arising from the presence of cysts and oocysts indicates the possibility of waterborne transmission of* Giardia *and* Cryptosporidium *infection in Taiwan if water is not adequately treated.*

The protozoan parasites *Giardia* and *Cryptosporidium* have been recognized as common pathogenic protozoa of the gastrointestinal tract (Cook, 1995). Many outbreaks of giardiasis and cryptosporidiosis have been reported in the last few decades (Frost et al., 1996; SoloGabriele & Neumeister, 1996; Kramer et al., 1996). Human infection with these pathogens is usually through either direct dermal contact or ingestion of contaminated food and/or water (Donnelly & Stentiford, 1997; Pell, 1997). Water is perhaps the major route for massive outbreaks of pathogen infection, as a result of contamination of either raw or treated water (Teunis et al., 1997; Eisenberg et al., 1998; Perz et al., 1998). Despite the difficulties in analytical procedures, it has generally been accepted that the number of *Giardia* vary from 0.04 to 66 cysts/L (LeChevallier & Norton, 1995) and for *Cryptosporidium* from 0.005 to 252.7 oocysts/L for surface waters (Rose, 1988; Stetzenbach et al., 1988). Because the assay for pathogen detection does not indicate parasite viability, it is hard to determine if the water sources

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Journal of Toxicology and Environmental Health, Part A, 56:149–160, 1999 Copyright © 1999 Taylor & Francis 0098-4108/99 \$12.00 + .00

Received 14 September 1998; sent for revision 16 October 1998; accepted 20 January 1999.

This work was funded by the Environmental Protection Administration, Republic of China. We are grateful to Dr. Meilin Yu for her assistance in statistical analysis, and to Dr. Jill Ruhsing Pan and Dr. Walter J. Rogan for their editing.

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serve as a source of risk from waterborne pathogens (Teunis et al., 1997; Morris et al., 1998).

Information pertaining to *Giardia* and *Cryptosporidium* in the drinking water supply systems in Taiwan is very limited. The first case of giardiasis in Taiwan was discovered in 1975 on an offshore island. Thirty-two percent of the children residing on the island were diagnosed with *Giardia* in their stool specimens (Chung & Cross, 1975). Another survey showed that over 50% of the avian species in Taiwan were infected with *Cryptosporidium* spp. (Wang & Liew, 1990).

A normal *Giardia* cyst is characteristically oval in shape, ranging from 8 to 14 µm in diameter. *Cryptosporidium* oocysts, which range from 4 to 6 µm in diameter, are approximately spherical. The thick-walled cysts and oocysts are both more resistant to commonly used disinfectants than are other microbes (Haas et al., 1994). Korich et al. (1990) also found that the oocysts are more resistant than cysts to disinfectants. Recent studies have shown that ozone is more efficient than chlorine in the inactivation of *Giardia* cysts and *Cryptosporidium* oocysts (Chiou et al., 1997; Liyanage et al., 1997a, 1997b).

In this study, an immunofluorescence assay (AWWA, 1994) was used for detecting *Giardia* and *Cryptosporidium.* Some parameters of water quality, including pH, turbidity, conductivity, temperature, heterotrophic bacteria, and total coliforms, were also determined. The correlation was analyzed between the concentration of protozoan parasite and these water-quality parameters. The concentrations of cysts and oocysts in the treated water were applied to the risk analysis equation to calculate the risk of human infection from *Giardia* and *Cryptosporidium* in drinking water supplies.

MATERIALS AND METHODS

Water samples were taken from nine potable water treatment plants in Taiwan during a 2-yr period (from July 1996 to January 1998). The distribution of the nine water treatment plants in Taiwan is shown in Figure 1. Taiwan is generally divided into three areas: northern, central, and southern parts. Two water treatment plants are located in northern Taiwan, and another two are in the central part. The remaining five plants are in the southern area. In the first phase, six raw and four treated water samples in total were collected from two water treatment plants in northern Taiwan that used different river water as water sources. In the second phase, six raw and four treated water samples were taken in total from the two water treatment plants in central Taiwan, which drew water from reservoirs. In the third phase, 11 samples (6 raw water samples and 5 treated water samples) were collected from 5 different water treatment plants from the same watershed in southern Taiwan.

The sampling method and detecting procedure were adopted from the guidelines enumerated in the American Water Works Association manual (AWWA, 1994). For the detection and identification of *Giardia* cysts and

FIGURE 1. Sampling water treatment plants in three different parts of Taiwan (north, center, and south). Abbreviations: S. S., Shuang Shi; P. H., Pan Hsing; D. S., Dung Sing; F. Y., Feng Yuan; C. S., Chiya-Shien, P. D., Pying-Ding; C. C., Cheng-Ching; U. G., Ueng Gungyuan; K. T., Kau-Tan.

Cryptosporidium oocysts, an indirect immunofluorescent antibody assay (IFA) specified in the AWWA (1994) manual as an Information Collection Rule (ICR) was used. To determine the recovery efficiency, cysts and oocysts were prepared and then counted with a calibrated hemacytometer. The tests were done by spiking with 9.37 \times 10⁵ cysts and 8.14 \times 10⁴ oocysts into treated water, followed by identical procedures carried out to identify and enumerate the parasites (AWWA, 1994). The concentrated water samples were labeled with monoclonal antiserum (Hydrofluor™ Combo *Giardia/Cryptosporidium*; Ensys, Inc., Research Triangle Park, NC) and examined by epifluorescent microscopy at $200 \times$, $400 \times$, or $1000 \times$ magnification (Olympus, Tokyo, Japan). Cysts and oocysts were identified using the following characteristics: size, shape, surface feature, and staining reaction. The candidates exhibiting the right size and shape were screened and categorized according to their internal morphological features by epifluorescent microscopy under a bright field.

Water temperature and pH were measured on site using a portable pH meter (PHM201, Radiometer Analytical SA, France). Turbidity was measured using a ratio turbidimeter (HACH Co., Loveland, CO). Heterotrophic bacteria were measured by the spread method (APHA, 1995). Total coliforms were measured by membrane filtration procedures described by the Standard Method for the Examination of Water and Wastewater (method 9222 B in APHA, 1995).

The number of cysts and oocysts observed microscopically was recorded and normalized to the number of cysts per 100 L and the number of oocysts per 100 L. The recovery efficiencies were not used in the calculations of parasite concentrations (LeChevallier et al., 1991; Crabtree et al., 1996), and all the values under the detection limit were treated as

zero. Spearman *R* correlation coefficients were calculated between the concentrations of cysts/oocysts and the water-quality parameters such as heterotrophic bacteria, total coliforms, and turbidity, using STATISTICA software (supplied by StatSoft, Inc., Tulsa, OK).

The risk of infection by protozoan parasites in drinking waters depends upon (1) the concentration of cysts or oocysts in the drinking water, (2) the recovery efficiencies of the detection methods, (3) the viability of cysts or oocysts, and (4) the daily consumption of unboiled tap water. For a given treated water sample, the individual daily dose (*N*) may be calculated as (Teunis et al., 1997)

$$
N = C \times 1/R \times I \times V \tag{1}
$$

where *C* is the concentration of cysts or oocysts in the treated water, *R* is the recovery efficiency of the detection method, *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 based upon parasite concentrations in the drinking water and the amount of water consumed over the exposure period (Teunis et al., 1997). The exponential assessment model of Eq. (2) was used to predict the number of cases of illnesses resulting from the measured levels of parasites. This microbiological model of infectivity was proposed by Furumoto and Mickey (1967) and has been used successfully to assess the risk of infection from *Giardia* and *Cryptosporidium* (Regli et al., 1991; Rose et al., 1991; Haas et al., 1994; DuPont et al., 1995). The equation is

$$
P = 1 - \exp(-rN) \tag{2}
$$

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

An annual risk for infection could be calculated (Federal-Provincial Subcommittee on Drinking Water, 1998):

$$
P_{A} = 1 - (1 - P)^{365} \tag{3}
$$

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

RESULTS

Distribution of Protozoa and Water Quality Parameters in Water Samples

Limited by the budget of this study, it was only possible to collect 31 water samples from 9 water treatment plants. However, the sampling sites were carefully selected to reflect the most polluted water sources and population at risk. The parasite concentrations and water quality data are listed in Table 1. All six raw water samples from northern Taiwan contained *Giardia* and four of these contained *Cryptosporidium*. The counts ranged from 1.6 to 40 cysts/100 L and from less than 2.3 to 40 oocysts/100 L for *Giardia* and *Cryptosporidium*, respectively. In central Taiwan, *Giardia* was detected in three out of six raw water samples. The counts ranged from less than 4 to 26 cysts/100 L. Six raw water samples tested positive for *Cryptosporidium* with counts ranging from less than 4 to 48.9 oocysts/100 L. In southern Taiwan, out of 6 raw water samples, 5 were *Giardia* positive and 4 were *Cryptosporidium* positive, with counts ranging from less than 21.2 to 311.6 cysts/100 L and from less than 21.2 to 801.4 oocysts/100 L, respectively. Overall, the percentage of cyst-positive samples was 77.8% and of oocyst-positive samples 72.2% among the 18 (number from all 3 phases) raw water samples. For the 13 treated water samples (from all 3 phases), the incidence was 76.9% for cysts and 38.5% for oocysts.

Table 2 lists the means and standard deviations of parasite concentrations and the water-quality parameters from different locations. Raw water samples taken from southern Taiwan showed higher concentrations of cysts and oocysts than samples from the other two regions. These water sources are also more polluted, as indicated by heterotrophic bacteria, total coliforms, conductivity, and turbidity.

Relationship between Protozoan Parasites and Water Quality Parameters

Table 3 shows the correlation coefficients between protozoan parasites and some water-quality parameters (heterotrophic bacteria, total coliforms, and turbidity) in raw water samples. For northern Taiwan, significant correlations were shown between both protozoan parasites, and between *Giardia* concentration and the turbidity levels in raw water samples. For central and southern Taiwan, however, neither cysts nor oocysts displayed significant positive correlation with these water-quality parameters. This may be due to differences in the type of water sources and the small number of samples.

For all raw water samples in the three areas, the correlation coefficient between oocysts and cysts was 0.518 (*n* = 18, *p* = .028). The mean concentration of oocysts was 1.38 times more than that of cysts. Positive correlations were found between the *Giardia* concentrations and turbidity levels (*n* = 18, *R* = 0.491, and *p* = .038) and between *Cryptosporidium* and heterotrophic bacteria ($n = 14$, $R = .614$, and $p = .020$).

Removal of Protozoan Parasites and the Risk from Giardiasis and Cryptosporidiosis Infections

By comparing the concentrations of parasites between raw and treated water from water treatment plants, as listed in Table 1, it was estimated that

 $\overline{4}$

TABLE 1. Concentrations of *Giardia* and *Cryptosporidium* and Water-Quality Parameters of 31 Water Samples in Taiwan

the removal efficiency for *Giardia* was 92.5% and for *Cryptosporidium* was 95.6%.

To analyze the risk from *Giardia* and *Cryptosporidium* infections, the concentrations of parasites in the treated water were adjusted by considering the recovery efficiency and viability. In drinking water samples, the average recovery efficiency for cysts and oocysts was 26.5% and 8.9%, respectively. In evaluating parasite viability in Taiwan water supplies, it was assumed that these treatment plants can provide sufficient *C* × *T* values to achieve 3-log and 2-log inactivation for *Giardia* and *Cryptosporidium* under the following conditions: pH 7.0; temperature 25°C; residual chlorine concentration in tap water 0.6 mg/L; and enough contact time (more than 2 d) (Federal-Provincial Subcommittee on Drinking Water, 1998; Casemore, 1995).

By means of Eqs. (1), (2), and (3), the concentrations of parasites from treated water samples in three different areas were converted to an annual risk of giardiasis infection. The values from north to south were 2.31×10^{-4} , 9.85×10^{-4} , and 2.60×10^{-4} , respectively. The average annual risks for cryptosporidiosis infection from north to south were 1.64×10^{-2} , 8.22×10^{-3} , and 1.40 \times 10⁻², respectively.

DISCUSSION

Giardia and *Cryptosporidium* are prevalent in Taiwan surface waters, which are the main sources of drinking water for 22 million people. River water in southern Taiwan, reaching water treatment plants, flows through the hog farming region and is significantly polluted by domestic sewage and wastewater from farm practices. In central Taiwan, water sources for supply come from reservoirs and water quality is somewhat affected by some agricultural activities from the upper region. As for water sources in northern Taiwan, high population density in this area in conjunction with lack of sewer systems results in a high pollution loading of the source water. Because of different degrees of pollution, due to various farming activities, different correlations between protozoan parasites and other water-quality parameters were discovered in these three parts of Taiwan. Madore et al. (1987) indicated that agricultural and farming effluents might be important sources for *Giardia* and *Cryptosporidium*. Data show that the mean concentrations for *Giardia* and *Cryptosporidium* in the raw water samples in southern Taiwan were 9.2 and 23.2 times greater than those in northern Taiwan, and 18.3 and 21.4 times higher than those in central Taiwan, suggesting that farming activities play an important role in the prevalence of the protozoan parasites. Since *Giardia* and *Cryptosporidium* are commonly found in Taiwan surface water supplies, both parasites need to be regulated under stricter laws and treatment guidelines to protect public health.

Gray (1994) suggested that it was possible to remove *Giardia* cysts from a large reservoir if the retention time was longer than 6 wk, provided mixing

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TABLE 2. Mean and Standard Deviation of Protozoan Parasites and Water Quality Parameters Classified by Their Locations

| Location | Parasites | Cryptosporidium | Heterotrophic bacteria | Total coliforms | Turbidity |
|-------------------------------|-----------------|---------------------------------------|---------------------------------------|---------------------------------------|---|
| Northern | Giardia | $n = 6$ $R = 0.794$ $p = .059$ | $n = 4$ $R = -0.80$ $p = .200$ | $n = 4$ $R = -0.20$ $p = .800$ | $n = 6$ $R = 0.794$ $p = .059$ |
| | Cryptosporidium | | $n = 4$ $R = -0.632$ $p = .368$ | $n = 4$ $R = -0.949$ $p = .051$ | $n = 6$ $R = 0.588$ $p = .219$ |
| Central | Giardia | $n = 6$ $R = -0.122$ $p = .686$ | $n = 5$ $R = -0.359$ $p = .552$ | $n = 5$ $R = 0.616$ $p = .269$ | $n = 6$ $R = -0.832$ $p = .040$ |
| | Cryptosporidium | | $n = 5$ $R = 0.800$ $p = .104$ | $n = 5$ $R = 0.700$ $p = .188$ | n ₆ $R = 0.638$ $p = .173$ |
| Southern | Giardia | $n = 6$ $R = 0.616$ $p = .269$ | $n = 5$ $R = -0.100$ $p = .872$ | $n = 5$ $R = 0.500$ $p = .391$ | $n = 6$ $R = 0.200$ $p = .747$ |
| | Cryptosporidium | | $n = 5$ $R = 0.205$ $p = .870$ | $n = 5$ $R = 0.410$ $p = .493$ | $n = 6$ $R = 0.103$ $p = .870$ |
| Total raw water samples | Giardia | $n = 18$ $R = 0.518$ $p = .028$ | $n = 14$ $R = 0.011$ $p = .970$ | $n = 14$ $R = 0.038$ $p = .896$ | $n = 18$ $R = 0.491$ $p = .038$ |
| | Cryptosporidium | | $n = 14$ $R = 0.614$ $p = .020$ | $n = 14$ $R = 0.294$ $p = .307$ | $n = 18$ $R = 0.331$ $p = .179$ |

TABLE 3. Nonparametric Correlation Coefficient Between the Densities of Protozoa and the Microbiological Parameters

Note. n, Sample number; *R*, Spearman *R*; *p*, *p* levels.

and current were minimal. Gray (1994) also found that the settling velocity of *Giardia* in a reservoir was 11 times greater than that of *Cryptosporidium*. Northern and central Taiwan were found to have similar mean levels of *Cryptosporidium*, while the mean level of *Giardia* in northern Taiwan was two times higher than in central Taiwan, whose source water originated from a reservoir. More *Giardia* might thus have settled in the water during the storage period as a consequence of a more efficient settling velocity.

In this study, smaller volumes of samples were collected from southern water supplies to compensate for the high turbidity. It was noted that the limit of detection was determined by the volume of original water sample, fraction of eluate packed pellet volume, and percentage of floated sediment examined (AWWA, 1994). The combination of these factors result in different detection limits in each water sample. The detection limits for both parasites from the southern part, because of smaller volume, were thus higher than from the northern and central parts.

Table 4 lists the parasite concentrations in Middle Eastern and Asian surface waters recently reported by Ahmad et al. (1997), Zuckerman et al.

| Area | Sample type | Range and mean level for Giardia (cysts/100 L) | Range and Mean level for Cryptosporidium (oocysts/100 L) | Reference |
|-----------|---|---|---|-------------------------|
| Hong Kong | Rivers | 200-46,800 | $30 - 300$ | Ho and Tam (1998) |
| Israel | Surface water in northern part | $0 - 78.3$ 13 ± 22 | $0 - 190$ 69 ± 80 | Zuckerman et al. (1997) |
| Malaysia | Raw water of water treatment plant | $0 - 6000$ $1,206 \pm 1,560$ | ND. | Ahmad et al. (1997) |
| Taiwan | Raw water of water treatment plant | $< 2.3 - 311.6$ 64 ± 96 | $< 0.9 - 801.4$ 88 ± 10 | This study |

TABLE 4. The Range and Mean Level of *Giardia* and *Cryptosporidium* in Different Middle Eastern and Asian Areas

Note. ND, no oocysts were detected.

(1997), and Ho and Tam (1998). Data from our study are also included in this table for comparison. Data in Table 4 were not adjusted for recovery efficiency. Mean levels of *Giardia* cysts in Malaysia were found to be 18.8 times higher than in Taiwan, and the range of cysts found in Hong Kong was also greater. On the other hand, the mean level of cysts in Israel was only one-fifth that in Taiwan. Mean levels of *Cryptosporidium* oocysts from Middle Eastern and other Asian areas were less than that found in Taiwan. Oocysts were not found in the water samples in Malaysia.

The U.S. Environmental Protection Agency (EPA) *Federal Register* (U.S. EPA, 1989) included that 1 case of microbiologically caused illness per year per 10,000 individuals is acceptable as a drinking water standard. To achieve this goal, Rose et al. (1991) suggested that tap water should not contain more than 7×10^{-4} *Giardia* cysts/100 L on the basis of a geometric mean for 1 yr. Haas and Rose (1995) stated that an outbreak would probably occur if tap water contains more than 10 to 30 *Cryptosporidium* oocysts/ 100 L. Because the information about dose-response relationships for both parasites is insufficient in Taiwan, results predicted from this model are variable and uncertain. Despite this, it is recommended that point-of-use equipment be used for the safety of drinking water.

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