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PERGAMON

OCCURRENCE OF *GIARDIA* AND *CRYPTOSPORIDIUM* IN THE KAU-PING RIVER AND ITS WATERSHED IN SOUTHERN TAIWAN

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Abstract—*Giardia* and *Cryptosporidium* are important waterborne parasites. Thirteen water samples and 32 fecal specimens were collected from the Kau-Ping River and its watershed to test for *Giardia* cysts and *Cryptosporidium* oocysts. The detection methods are immunofluorescence assay and enzyme-linked immunosorbent assay for water samples and fecal specimens, respectively. Seven out of eight samples collected from raw water samples showed the presence of cysts, while six out of eight raw water samples contained oocysts. *Cryptosporidium* was present in 40% of the treated water, while *Giardia* occurred in all of them. Four out of 32 fecal specimens, collected from the hog farming region, tested positive for *Giardia*, and seven specimens tested positive for *Cryptosporidium*. *Giardia* was related only to *Cryptosporidium* but not to the others. © 1999 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Parasites *Giardia* and *Cryptosporidium* are common pathogenic protozoa of the gastrointestinal tract (Cook, 1995). Members of the genus *Giardia* infect the proximal small intestine in humans and other mammals, causing giardiasis with symptoms include diarrhea, stomach cramps, nausea, and fatigue. Many outbreaks of giardiasis have been reported in the last few decades (Smith *et al.*, 1995). Members of the genus *Cryptosporidium* also cause gastroenteritis in humans and animals and are often responsible for waterborne outbreaks. For instance, a significant outbreak in Milwaukee, WI, during 1993 was caused by *Cryptosporidium*, infecting 400,000 people (MacKenzie *et al.*, 1994). 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 and Cross, 1975). Another survey showed that over 50% of the avian species in Taiwan were infected with *Cryptosporidium* spp. (Wang and Liew, 1990). However, information pertaining to *Giardia* and *Cryptosporidium* in the drinking water supply systems in Taiwan is very limited.

Majorities of *Giardia* cysts are oval in shape, ranging from 8 to 14 μm in diameter. *Cryptosporidium* oocysts, which range from 4 to 6 μm in diameter, are characteristically spherical. These thick-walled cysts and oocysts are extremely resistant to commonly used disinfectants such as chlorine (Korich *et al.*, 1990). They can remain viable for several months in water between 4 and 10°C (Medema *et al.*, 1997). The most commonly used laboratory protocols for identifying cysts and oocysts in stool specimens or environmental water samples are immunofluorescent microscopic examination and enzyme-linked immunosorbent assay (EIA) (Leng *et al.*, 1996).

The Kau-Ping River is the major raw water source for the great Kaohsiung area of 2.5 million people, approximately 12% of Taiwan's total population. Another water source in this region is groundwater. A survey on the Kau-Ping River shows that the watershed in the upper region is used for recreation. The middle and lower regions are heavily polluted, due to inputs of domestic sewage, industrial and farm wastewaters. Although conventional prechlorination, coagulation, sedimentation, and filtration processes are employed by

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local water treatment plants, the removal of parasites has never been evaluated.

In this report we surveyed the prevalence of the parasites and the microbes traditionally used as water quality parameters from various locations in the Kau-Ping River and their correlation to water quality parameters. The risk analysis for human infection of *Giardia* and *Cryptosporidium* from drinking water supplies is also included in this study.

MATERIALS AND METHODS

Sampling sites and sampling procedures

Samplings were carried out in three phases. In the first phase, water samples were collected from upstream and downstream of the river running through the hog farming region. The second phase included the collection of ten samples (five raw water samples and five treated water samples) from five water treatment plants using the Kau-Ping River as a water source (Chiya-Shien, Pying-Ding, Cheng-Ching, Ueng-Gungyuan and Kau-Tan). The third phase was carried out to investigate the stool specimens of pasturing animals in the watershed for the presence of parasites. The hog farming region encompasses the middle region of the Kau-Ping River, where approximately 1.04 million pigs and other animals of less quantity are raised. These animals are potential reservoir hosts for the protozoa. Thirty two fecal specimens were randomly collected from farms located in the hog farming region.

Sampling method and procedure were adopted from the handout provided in the American Water Works Association training course (AWWA, 1994). They are the same method as specified by the Information Collection Rule (ICR), using indirect fluorescent antibody (IFA) procedures. 15–50 l of the raw water samples and 50–80 l of treated water samples were concentrated through a 254 cm long and 1.0 μm nominal-pore-size polypropylene yarn-wound cartridge filter. The filter fibers were eluted by 1–1.5 l of eluting solution (phosphate-buffered saline, 1% Tween 80, 1% SDS) in a mechanical stomacher, and then the eluate was centrifuged at $1050 \times g$ for 10 min in a 50-ml centrifuge tube in swinging-bucket rotor. Meanwhile, we recorded the packed pellet volume. After the supernatant was aspirated, the pellet was resuspended with an equal volume of 10% formalin and vortexed up to a total volume of 20-ml with an eluting solution in a 50-ml centrifuge tube. The mixture was underlaid with 30 ml of a percoll–sucrose gradient (sp. gravity = 1.10) followed by centrifuging. The sample in the centrifuge tube was collected from the top 20 and 5 ml below interface, and then diluted with eluting solution to 50 ml. This 50 ml suspension was then centrifuged again and the top 45 ml was then aspirated. Finally, the last 5 ml suspension was harvested for manifold filtering.

Immunolabeling of water and fecal samples

Indirect immunofluorescence assay (IFA) was used to detect *Giardia* cysts and *Cryptosporidium* oocysts in water samples. The concentrated water samples were labeled with monoclonal antiserum (Hydrofluor® Combo *Giardia/ Cryptosporidium*; Ensys, Inc., NC, U.S.A.) and examined with epifluorescent microscope at 200×, 400× or 1000× magnification (Olympus, Japan). Cysts and oocysts were identified using following parameters: size, shape, surface feature, and staining reaction. The candidates exhibiting the right size and shape were further identified by epifluor-

Table 1. The concentration of *Giardia* and *Cryptosporidium* and water quality parameters of eight sampling sites in the Kau-Ping River

| Sampling site	Sample type	Sampling volume (l)	Giardia (cysts/100 l)	Cryptosporidium (oocysts/100 l)	Heterotrophic bacteria (CFU/1 ml)	Total coliforms (CFU/100 ml)	Fecal coliforms (CFU/100 ml)	pH	Temperature (°C)	Conductivity (μs)	Turbidity (NTU)
Chiya-Shien	raw	25	651	<643	<5	<1	<1	7.05	20.5	437	80
Chiya-Shien	treated	50	57	<56	<5	<1	<1	7.10	19.7	436	0.40
Pying-Ding	raw	50	397	4047	8900	280	31	7.30	25.7	845	20
Pying-Ding	treated	80	40	159	<5	<1	<1	6.60	25.2	595	0.20
Cheng-Ching	raw	50	1367	8972	5300	1200	10	7.70	25.4	526	17
Cheng-Ching	treated	80	14	<41	<5	2.0	<1	7.10	25.5	634	1.4
Ueng-Gungyuan	raw	50	<93	<237	5800	880	337	7.08	25.0	890	5.2
Ueng-Gungyuan	treated	80	12	<35	<5	<1	<1	7.20	22.6	770	0.50
Kau-Tan	raw	50	1107	2540	1.2×10^5	1.0×10^5	3.4×10^4	7.24	22.1	644	11
Kau-Tan	treated	80	49	72	<5	1.0	<1	7.15	24.1	546	0.30
Fwoguang-Shan	raw	40	1398	2378	1.6×10^4	2.4×10^4	100	7.35	23.8	966	7.0
Guei-Yuan	raw	15	3754	12,516	6.0×10^6	5.0×10^7	2.4×10^6	7.35	22.8	881	50
Shyi-Jichang	raw	20	6978	7754	4.2×10^5	7.5×10^4	100	7.28	22.8	630	40
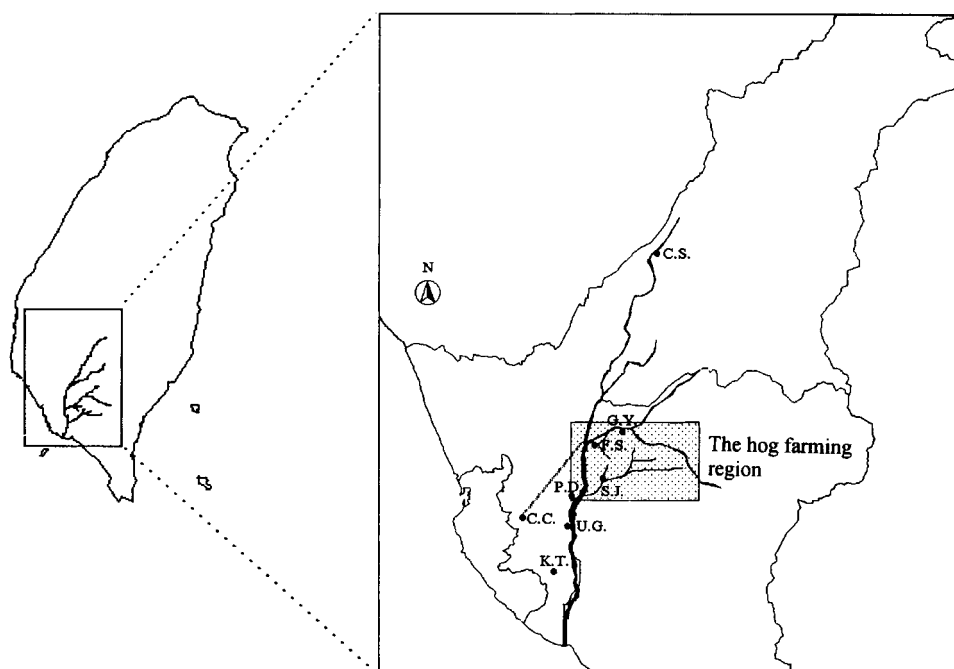
Fig. 1. The watershed of the Kau-Ping River and sampling sites in this study. Abbreviations: C.S.: Chiya-Shien water plant; G.Y.: Guei-Yuan; F.S.: Fwoguang-Shan; S.J.: Shyi-Jichang; P.D.: Pying-Ding water plant; C.C.: Cheng-Ching water plant; U.G.: Ueng-Gungyuan water plant; K.T.: Kau-Tan water plant.

escent microscopy under a bright field according to their internal morphological features. EIA was used to detect the presence of the organisms in fecal samples. ProSpecT *Giardia* EZ Microplate Assay and ProSpecT *Cryptosporidium* Microplate Assay were purchased from Alexon Inc. (Sunnyvale, CA, U.S.A.). Sample preparation and immuno-reaction procedures followed supplier's protocols. Readings of $A_{450} = 0.05$ were interpreted as positive reactions and $A_{450} < 0.05$ as negative. The primary antibodies used in the EIA test are specific to *Giardia* and *Cryptosporidium*. Scheffler and Van Etta (1994) have proved that these antibodies would not cross-react with any other enteric parasites. To assess the sensitivity of the EIA, purified oocysts were serially diluted 10-fold in PBS, and 200 μl aliquots from each dilution were placed in test wells.

Detection of water quality parameters

Water temperature and its pH were measured *in situ* using a portable pH meter (Radiometer Analytical SA, France). Turbidity was measured using a ratio turbidimeter (HACH, Co., U.S.A.). Water samples were collected in sterilized 500 ml Schott bottles, which were kept in a cooler during transportation to the lab. The screening for microbiological parameters must be finished in eight hours after sampling. Heterotrophic bacteria were measured by the spread method. Total coliforms and fecal coliforms were measured by membrane filtration procedures described by the Standard Method for the Examination of Water and Wastewater (Methods 9222 B and D) (APHA, 1995).

Recovery efficiency test

To determine the recovery efficiency, cysts and oocysts were prepared and then calculated with a calibrated hemacytometer. The tests were started with 9.37×10^5 cysts and 8.14×10^4 oocysts and the numbers

detected in 50 l of raw water and 80 l of treated water were calculated using IFA.

Statistical evaluation

The numbers of cysts and oocysts observed under the microscope were recorded and later normalized to number of counts per 100 l. These numbers were divided by the recovery efficiency to obtain the final counts. When the protozoa were not observed via epifluorescent microscope, we express the protozoa concentration in water samples as less than ($<$) their detection limits. The calculations of correlation coefficients among turbidity, heterotrophic bacteria densities, total coliforms densities, fecal coliforms densities, *Giardia* levels, and *Cryptosporidium* levels were conducted using the STATISTICA software (StatSoft, Inc., U.S.A.). In the statistical evaluation, data under the detectable levels were treated as zero. The mean and standard deviation of the parasites and the parameters of water quality were calculated.

Risk assessment

The exponential risk assessment model (Rose *et al.*, 1991; Crabtree *et al.*, 1996) was used to estimate the number of cases of illness resulting from the measured levels of organisms. The model gives a potential daily risk (P), assuming that a person drinks two liters of water per day. It is calculated using the following equation:

$$P = 1 - \exp(-rN), \qquad (1)$$

where r is the coefficient (0.0198 for *Giardia* and 0.004 for *Cryptosporidium*) and N is the number of cysts or oocysts per two liters of water. The model assumed that all cysts and oocysts were viable and infectious. The overestimate and the underestimate of the risks were canceled by each other.

RESULTS AND DISCUSSION

Distribution of cysts and oocysts in water samples

Thirteen water samples were collected from the water treatment plants and hog farming region of Kau-Ping River watershed. Sampling sites, sample volumes, parasite concentrations and parameters of water quality are listed in Table 1. During the period of sampling, water temperatures of the river ranged from 19.7 to 25.7°C (mean ± SD = 23.5 ± 1.94°C), the pH values ranged from 6.60 to 7.70 (7.19 ± 0.26), and the conductivities ranged from 436 to 966 µs (677? 177 µs). The location of sampling sites and the distribution of protozoan parasites in this research are presented in Fig. 1 and Table 1. It is evident that both parasites are widely distributed in the Kau-Ping River watershed. Twelve out of thirteen water samples showed the presence of cysts, while eight out of thirteen water samples contained oocysts. All the water samples taking from the watershed near the hog farms contained both *Giardia* and *Cryptosporidium*. The percentages for *Giardia*- and *Cryptosporidium*-positive were 80% and 60% in raw water samples of water treatment plants, and 100% and 40% in treated water samples, respectively. Some literatures such as Ahmad *et al.* (1997) have indicated that the lower rate of *Giardia*-positive in raw water samples than in treated water may be due to the interference of detection limit by turbidity.

Relationship between protozoan parasites and water quality parameters

Jennifer *et al.* (1994) conducted a blind survey of sixteen commercial laboratories to evaluate the recovery efficiency for protozoa. The recovery efficiencies of *Giardia* and *Cryptosporidium* ranged from 0.8 to 22.3% and from 1.3 to 5.5%, respect-
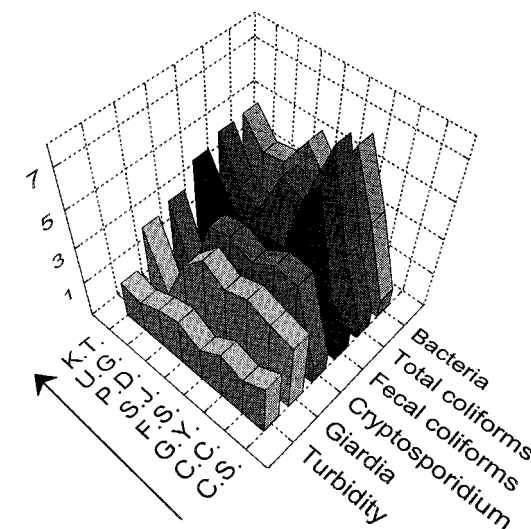

Fig. 2. The trends of protozoan parasites and water quality parameters in raw water samples collected along the Kau-Ping River watershed.

ively, and their average was 9.1% for *Giardia* and 2.8% for *Cryptosporidium*. In our research, the recovery efficiency of cysts ranged from 22.8 to 26.5%, and 8.93 to 8.94% for oocysts. The results of recovery efficiencies were higher than those of Jennifer *et al.* LeChevallier *et al.* (1995) suggested that careful handling in concentration and clarification may result in improvement in the recovery efficiency.

The mean concentration of *Giardia* cysts in raw water samples was 1956 cysts/100 l (SD = 2320), ranging from less than 93 to 6978 cysts; and was 4776 oocysts/100 l for *Cryptosporidium* (SD = 4520), ranging from less than 237 to 12,515
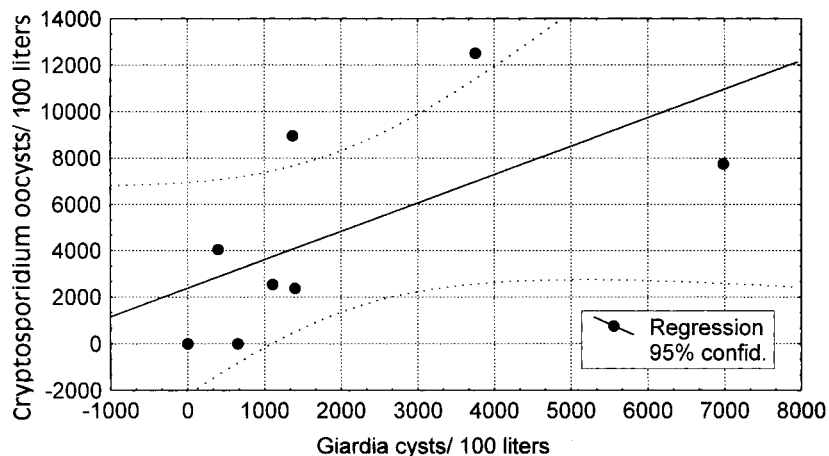

Fig. 3. Relationship between densities of *Giardia* cysts and *Cryptosporidium* oocysts in raw water samples of the Kau-Ping river. Regression line: $y = 2376.3 + 1.23x$; $r = 0.630$, $p = 0.094$.

oocysts (Table 1). Figure 2 is a 3D-diagram showing the distribution of parasites and water quality parameters in raw water samples collected from upstream to downstream of the river watershed. All of the data in the diagram were derived from log transformation. The concentrations of *Giardia* and *Cryptosporidium* along the river exhibited a similar trend, while those of the heterotrophic bacteria, total coliforms and fecal coliforms exhibited a different pattern. Figure 2 also shows that the sampling sites located downstream and near the hog farming region contain more *Giardia* cysts and *Cryptosporidium* oocysts than the upstream ones or those using groundwater affected by the surface water.

In samples collected from the Kau-Ping River watershed, the mean concentration of *Cryptosporidium* oocysts was 1.23 times that of *Giardia* cysts with a correlation coefficient of 0.630 ($p = 0.094$) (Fig. 3). This result was similar to the findings of other investigators. For example, LeChevallier *et al.* (1991) found higher oocyst concentrations in their samples and significant correlations between the concentrations of cysts and oocysts ($r = 0.59$, $p < 0.01$). Rose *et al.* (1988) also found a higher concentration of *Cryptosporidium* oocysts and a significant correlation between the concentrations in a single watershed ($r = 0.778$, $p < 0.01$, $n = 39$). The mean quality of the eight raw water samples was 28.5 NTU (SD = 26) for turbidity, 8.2×10^5 CFU/ml (SD = 2.1×10^6) for heterotrophic bacteria, 6.3×10^6 CFU/100 ml (SD = 1.8×10^7) for total coliforms, and 3.0×10^5 CFU/100 ml (SD = 8.5×10^5) for fecal coliforms. The correlation between protozoan parasites and water quality parameters in these samples was analyzed. The results indicated that neither cysts nor oocysts were significantly correlated with turbidity levels (i.e., $r = 0.300$ at $p = 0.471$ and $r = 0.154$ at $p = 0.715$) (Table 2). Highly positive correlations were observed between *Cryptosporidium* concentrations and the levels of heterotrophic bacteria ($r = 0.713$, $p = 0.047$), total coliforms ($r = 0.692$, $p = 0.057$), and fecal coliforms ($r = 0.690$, $p = 0.058$). It is also noted that no significant relationships were found between *Giardia* and heterotrophic bacteria, total coliforms or fecal coliforms.

Table 3. Removal percentages of *Giardia*, *Cryptosporidium*, and turbidity by water treatment plants

Water treatment plant	Removal percentage of *Giardia*	Removal percentage of *Cryptosporidium*	Removal percentage of turbidity
Chiya-Shien	91.28%	–	99.49%
Pying-Ding	89.87%	96.07%	99.00%
Cheng-Ching	98.98%	> 99.54%[a]	91.88%
Ueng-Gungyuan	< 87.3%[a]	–	90.38%
Kau-Tan	95.6%	97.17%	97.20%

[a]When no parasites were detected in samples, the limit of detection method was recorded.

Relationship between the removals of protozoa parasites and turbidity

All raw water samples had turbidity levels greater than 5.0 NTU (with a mean of 28.5 NTU), while that of the treated water samples showed a mean of 0.56 NTU (Table 1). The removals of turbidity, cysts and oocyts in various water treatment plants were presented in Table 3. The mean removal percentages of all test samples were calculated from the data in Table 3. They are 92.61, 97.59, and 95.59% for cysts, oocysts, and turbidity, respectively. Although the removal percentage of turbidity did not correlate well with either parasite, a positive correlation was found between the removal percentages of both parasites ($r = 0.937$ at $p = 0.226$).

Testing of animal fecal specimens

The data for the occurrences of *Giardia* and *Cryptosporidium* in fecal specimens are shown in Table 4. In the 32 collected samples, four were positive for *Giardia* and seven positive for *Cryptosporidium*. Both parasites were detected in pig and cattle fecal specimens. Only one duck and one sheep were tested positive for *Cryptosporidium*. None of the parasites was detected in any of goose or chicken fecal specimens. Figures 4 and 5 are the EIA results of the fecal samples. When the results of the positive controls were converted with IFA and hemacytometer, samples within the detecting range were estimated to contain 376 oocysts/200 μl and 886 cysts/200 μl for *Cryptosporidium*

Table 2. The linear correlation among the concentrations of protozoa, turbidity and three microbiological parameters (*r*: correlation coefficient; *p*: statistical significance level)

Parameters	*Giardia* cysts	*Cryptosporidium* oocysts
Turbidity	$r = 0.300$, $p = 0.471$	$r = 0.154$, $p = 0.715$
Heterotrophic bacteria	$r = 0.373$, $p = 0.362$	$r = 0.713$, $p = 0.047$
Total coliforms	$r = 0.314$, $p = 0.449$	$r = 0.692$, $p = 0.057$
Fecal coliforms	$r = 0.311$, $p = 0.453$	$r = 0.690$, $p = 0.058$

Table 4. Occurrence of *Giardia* and *Cryptosporidium* in the fecal specimens of hog farming region

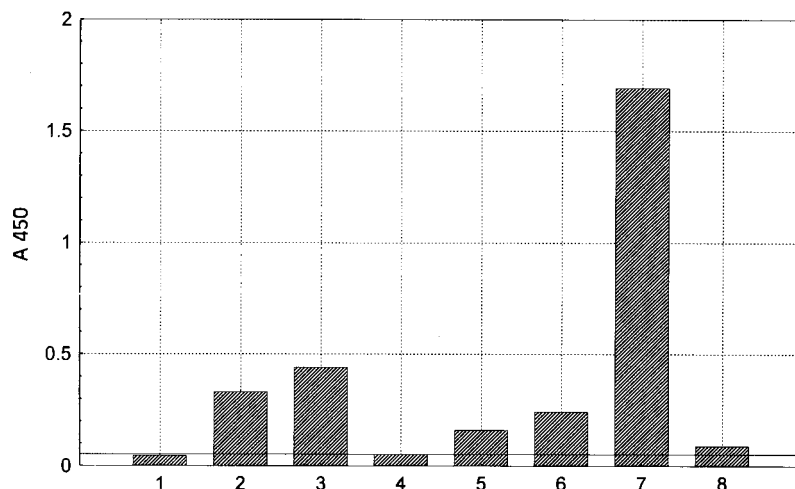| Sample category	Number of samples	Number of positive samples for *Giardia*	Number of positive samples for *Cryptosporidium*
Pigs	17	3	4
Cattle	5	1	1
Goose	1	0	0
Ducks	4	0	1
Sheep	2	0	1
Chickens	3	0	0
Total	32	4	7

Fig. 4. The A_{450} values of purified cysts, EIA controls and positive fecal specimens for detecting *Giardia* cysts by EIA. (No. 1 and 2: EIA negative and positive controls, No. 3–6: positive fecal specimens, No. 7 and 8: 8860 and 886 cysts in 200 μl reaction volume.)

and *Giardia*, respectively. False-positives might have occurred due to the presence of the antigens in stool rather than complete cysts and oocysts. This was not considered while interpreting the raw data.

The risks of giardiasis and cryptosporidiosis infections

The concentrations of cysts and oocysts observed in the effluents of the five water treatment plants ranged from 12 to 57 cysts/100 l (mean $\pm$ SD = 34 $\pm$ 20 cysts/100 l) and from less than 35 to 159 oocysts/100 l (46 $\pm$ 70 oocysts/100 l). The detection of the protozoan parasites in treated water indicates a potential risk for waterborne dis-

eases. The average risk of acquiring *Giardia* and *Cryptosporidium* infections from drinking unboiled tap water from these five plants were estimated to be 132 and 58 people per 10,000 people per day. However, few people in Kaohsiung drink tap water without further treatment. A survey conducted in the Kaohsiung metropolitan area (Chuang, 1996) showed that 64.6% of the entire population consumed bottled waters containing distilled water, R.O. water, or mineral water. The rest of the people chose tap water as their main drinking water, which they either boiled before drinking or treated with in house water purifying device. This explains why there is no outbreak of giardiasis and cryptosporidiosis in this area.
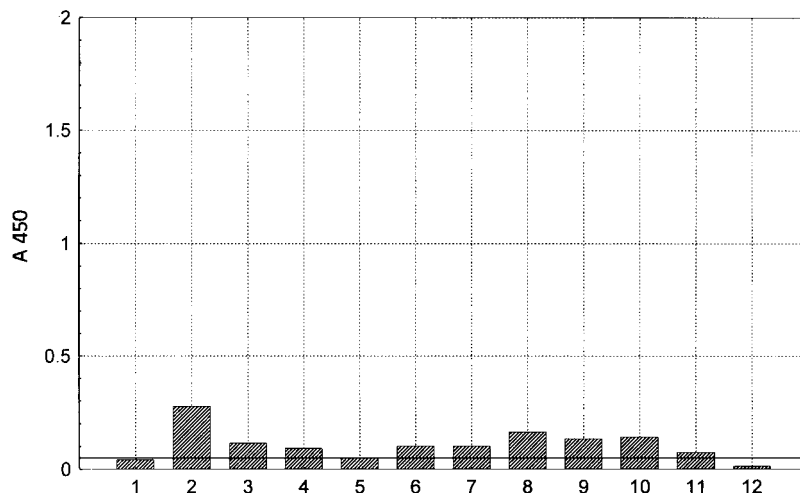

Fig. 5. The A_{450} values of purified oocysts, EIA controls and positive fecal specimens for detecting *Cryptosporidium* oocysts by EIA. (No. 1 and 2: EIA negative and positive controls, No. 3–10: positive fecal specimens, No. 11–12: 376 and 37.6 oocysts in 200 μl reaction volume.)

CONCLUSION

Giardia and *Cryptosporidium* are prevalent in the Kau-Ping River which supplies the drinking water for 2.5 million people. The waters from the hog farming region and its downstream sampling sites contain more parasites than the water from upstream sampling sites. The concentrations of the cysts and oocysts correlated well with each other. The occurrence of *Cryptosporidium* also related to those of heterotrophic bacteria, total coliforms and fecal coliforms, which proves that the upstream farming plays an important role in the contamination of these parasites, which indicates the importance of establishing regulations to prevent domestic sewage, industrial and pastured wastewater from directly reaching the river. Risk assessments of the parasitic infection suggest that the tap water in southern Taiwan is still not suitable for drinking without boiling or point-of-use treatment.

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