

Relationships between the occurrence of *Giardia* and *Cryptosporidium* and physicochemical properties of marine waters of the Pacific Coast of Mexico

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ABSTRACT

Untreated sewage has adversely affected the quality of marine recreational waters worldwide. Exposure to marine recreational water with poor microbial quality may pose a threat to bathers. The objectives of this study were to assess the effect of physicochemical parameters on *Cryptosporidium* and *Giardia* presence in marine recreational water of Sinaloa, Mexico, by Logistic Regression Analyses. Thirty-two 10-litre water samples were collected from two tourist beaches, Altata and Mazatlan, between November 2006 and May 2007. Water samples were processed by the EPA 1623 method and pH, temperature, salinity and turbidity were also determined. *Cryptosporidium* and *Giardia* were present in 71 and 57% of the samples collected from Altata, respectively. In Mazatlan, *Cryptosporidium* and *Giardia* were found in 83 and 72% of the samples, respectively. The overall concentration of *Cryptosporidium* ranged from 150 to 2,050 oocysts/10 L with an average of 581 oocysts/10 L and *Giardia* ranged from 10 to 300 cysts/10L with an average of 73 cysts/10L. The occurrence of both parasites increased in water with decreasing temperatures and increasing turbidity of the water.

Key words | *Cryptosporidium*, *Giardia*, marine recreational waters, temperature, turbidity

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INTRODUCTION

Marine recreational waters have become one of the most popular worldwide tourist destinations; therefore, they should be maintained in good physical, chemical and microbiological conditions to avoid risk of transmitting pathogens to bathers (Bartram & Rees 2000). Although the number of gastrointestinal illnesses associated with recreational water use is unknown, the frequency of studies reporting protozoan parasites presence and other waterborne pathogens has recently increased (Johnson *et al.* 1995; Fayer *et al.* 1998; Mathieu *et al.* 2004). Mexican beaches are visited year-round by local and international tourists. Their geographical location and warm temperature make them attractive for swimming, boating and fishing. The city of Mazatlan,

located at the northwestern state of Sinaloa is the eighth most visited year-round beach in Mexico (SECTUR 2006).

Mexican health authorities implemented the 'Clean Beach Program' in 17 coastal states. This program adopts the 'Guidelines for Safe Recreational Water Environments' (WHO 2003; SSA 2004). The program evaluates the microbial water quality based on *Enterococci* levels, excluding enteric pathogen surveillance, even though their presence is commonly associated with non-treated wastewater consistently discharged to the ocean by sewage outfalls and water runoff (Fayer *et al.* 2004). Also, enteric pathogens may originate from bathers themselves (Elmir *et al.* 2007). Gerba (2000) reported that a bather might release up to 10 g

of faecal residue during a 15 min immersion, releasing not only faecal indicators but also potentially waterborne pathogens.

Two of the most common and resistant microorganisms that have been frequently isolated from marine waters are *Cryptosporidium* and *Giardia* (Johnson et al. 1995). These protozoa can cause mild to severe diarrhoea and mortality in certain segments of the population (Marquardt et al. 2000; Thompson et al. 2005). The presence of *Cryptosporidium* and *Giardia* in marine recreational water might originate from river streams contaminated by municipal wastewater, or wild and livestock faecal discharges. Studies conducted by Chaidez et al. (2005) have shown *Cryptosporidium* oocysts and *Giardia* cysts presence in Mexican canals and rivers.

The aims of the present study were to quantify *Cryptosporidium* oocysts and *Giardia* cysts from marine recreational water of the north-western state of Sinaloa, Mexico, and to relate its presence with physicochemical parameters.

MATERIALS AND METHODS

Site selection and sample collection

A total of 32 marine water samples were collected monthly from two and three sampling sites at Altata and Mazatlan beaches, respectively. Altata beach is located between 24°37'53.55"N and 107°55'50.92"W and Mazatlan is between 23°14'35.42"N and 106°25'30.33"W of the Pacific Ocean coastline. Sample collection was conducted between November 2006 and May 2007 according to national and international guidelines for determining the water quality of recreational waters (EPA 2001; WHO 2003; SSA 2004).

Filtration, elution and concentration of *Cryptosporidium* oocysts and *Giardia* cysts from water samples

Spike sample, storage, preparation and quality assurance control were the same as those described at the EPA 1623 method (EPA 2001). A total of 32 10-litre water samples were filtered at flow rate of 2.0 L/min through Envirocheck HV capsule filters (Pall Gelman Sciences), according to EPA 1623 method (EPA 2001). Oocysts and cysts were

eluted from capsule filters with elution buffer and wrist agitation, as specified in method 1623. Eluants were collected in centrifuge tubes, oo/cysts were concentrated by centrifugation at 1,500 × g for 15 min, and the supernatant was carefully aspirated to a final volume of 5 ml above the pellet.

Dynal IMS procedure for *Cryptosporidium* oocysts and *Giardia* cysts

Immunomagnetic separation (IMS) procedure was performed following the manufacturer instructions. Briefly, 1 ml of test sample was placed in a screw-cap Leighton tube and 1 ml of 10 × solution buffer A, 1 ml of 10 × solution buffer B (Dynal A.S., Oslo, Norway) and 100 µl of *Cryptosporidium* and *Giardia* IMS conjugated beads were also added to each tube and allowed to rotate (18 rpm) for 1 h. Leighton tubes were placed in a magnetic particle concentrator 1 (MPC-1) to separate bead-oocysts/cysts complex from contaminating debris. Beads were resuspended in 1 ml 1 × solution buffer A and 0.5 ml of distilled water, and transferred to a 1-ml Eppendorf tube. Samples were placed in a magnetic particle concentrator S (MPC-S) and rocked for 1 min. Supernatant was aspirated, the magnet removed and 100 µl of 0.1 N HCl was added to each sample for bead-oocysts complex dissociation. The resulting supernatant was placed in the centre of a microscope slide well containing 10 µl of 1 N NaOH.

Staining procedure for recovered oocysts/cysts

Samples were dried, methanol fixed, air dried and labelled with anti-*Cryptosporidium* fluorescein isothiocyanate (FITC) monoclonal antibody (MAb) and anti-*Giardia* FITC MAb (Waterborne Inc., New Orleans, LO). Each sample was examined by epifluorescence microscopy as described in EPA 1623 method (EPA 2001).

Epifluorescence microscopy

A Leica DME fluorescence microscope equipped with a blue filter block was used to detect FITC-conjugated Mab-labelled oo/cysts at a magnification of × 400. *Cryptosporidium* oocysts and *Giardia* cysts were identified according to the EPA 1623 method (EPA 2001).

Physicochemical parameters analysis

Both temperature and pH were measured onsite with thermometer (Hg Taylor 21433) and potentiometer (Oakton wppH) portable devices, respectively. Salinity and turbidity were measured at the laboratory using a refractometer (HANNA Instruments H198203) and turbidimeter (HACH, 2100P), respectively. Results were expressed in °C, OH/H, % (g/L NaCl) and NTU (Nephelometric units), for temperature, pH, salinity and turbidity, respectively.

Statistical analysis

Descriptive statistics were performed to quantify the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts, and sampling times were compared with physicochemical parameters to predict microbial presence. The stepwise binary logistic regression analysis (LRA) was conducted and the odds ratios (OR) were also calculated. Minitab (version 14) was used for statistical analysis with significance level of 5%.

RESULTS

Occurrence of oocysts/cysts

Percentages of marine recreational water samples containing *Cryptosporidium* oocysts and *Giardia* cysts are shown in Table 1. Both protozoan parasites were found at all of the sites selected for sampling with an overall of 78 and 65% for *Cryptosporidium* oocysts and *Giardia* cysts, respectively. At Altata beach, numbers of marine recreational water samples containing *Cryptosporidium* oocysts and *Giardia* cysts were 10 (71%) and 8 (57%), respectively; both protozoa were present in 7 (50%) samples. In Mazatlan, numbers of seawater samples containing *Cryptosporidium* oocysts were 15 (83%), and *Giardia* cysts were detected in 13 (72%); both protozoa were present in 12 (66%) samples.

Table 1 | Percentage of marine recreational water samples containing *Cryptosporidium* oocysts and *Giardia* cysts

Beach	No. of samples	<i>Cryptosporidium</i> oocysts	<i>Giardia</i> cysts	Both
Altata	14	71.42	57.14	50
Mazatlan	18	83.33	72.22	66.66

Table 2 | Concentration ranges and average values for *Cryptosporidium* oocysts and *Giardia* cysts

Protozoan parasites Oo/cysts	Range (oo) cysts/10 L	Mean	SD*
<i>Cryptosporidium</i>	150–2,050	581.25	662.07
<i>Giardia</i>	10–300	73.75	75.89

*Standard deviation.

Concentration ranges for all analysed marine recreational water samples are listed in Table 2. Oocysts/10 L ranged from 150–2,050, and cysts/10 L ranged from 10–300 with a geometric mean value of 581 and 73, respectively. Samples collected at Altata beach showed a concentration ranging from 150 to 2,050 oocysts/10 L and a mean concentration of 532 for *Cryptosporidium*. *Giardia* cysts showed a concentration range from 50 to 150 cysts/10 L, with a mean concentration of 60 cysts/10 L (Table 3). The highest levels of *Cryptosporidium* oocyst concentration detected at Altata beach occurred in January 2006 (2,050 oocyst/10 L) and the highest *Giardia* cyst levels were detected in April 2006 (150 cysts/10 L) when water temperature was warm. Mazatlan results showed a concentration range from 150 to 2,000 oocysts/10 L, and a mean concentration of 619 oocysts/10 L, while *Giardia* cysts showed a concentration range from 10 to 300 cysts/10 L and a mean concentration 83 cysts/10 L (Table 3). The highest concentrations of *Cryptosporidium* oocysts were observed in Mazatlan in November 2006 and February 2007 (2,000–1,850 oocysts/10 L), while the highest *Giardia* cysts concentration was detected in February 2006 (300 cysts/10 L).

The detection limit for protozoan parasites was set to 50 (oo) cysts/10 L. The estimated average recovery efficiency for the detection method was 98 and 55% for *Cryptosporidium* oocysts and *Giardia* cysts, respectively.

Table 3 | Concentration ranges and average values for *Cryptosporidium* oocysts and *Giardia* cysts

Protozoan parasites Oo/cysts	Altata		Mazatlan	
	Mean	Range	Mean	Range
<i>Cryptosporidium</i> *	532.14	150–2,050	619.44	150–2,000
<i>Giardia</i> †	60.71	50–150	83.88	10–300

*Oocyst/10L.

†Cyst/10L.

Table 4 | Physicochemical parameters of marine recreational water samples

Beach	pH Mean (range)	Salinity*	Turbidity†	Temperature (°C)
Altata	8.09 (7.95–8.06)	29.42 (22.5–35)	2.96 (2–4.4)	24.35 (19–33)
Mazatlan	7.73 (4.5–8.18)	28.16 (25–32.5)	3.43 (2–5.1)	25.13 (19–36)

* ppt, part per thousand.

†NTU, nephelometric units.

Physicochemical parameters

Results obtained for physicochemical parameters in marine recreational water samples are shown in Table 4. Water temperature and turbidity varied from 19°C to 36°C and from 2 to 5 NTU, respectively, but variation in occurrence within each sampling season was not significant for *Cryptosporidium* and *Giardia* ($P = 0.323$ and $P = 0.896$), respectively.

Cryptosporidium prediction models

Data analysis to evaluate correlation between *Cryptosporidium* oocysts presence/absence and temperature, turbidity, pH and salinity was conducted as follows. The estimated model was:

$$P(\text{Pres}) = \frac{1}{1 + e^{-0.28T + 1.083Tu}} \quad (1)$$

The goodness-of-fit test, based on Pearson's correlation coefficient, showed $P = 0.097$, indicating that the model was appropriate. Only temperature (T) and turbidity (Tu) parameters were significant, $P = 0.036$, $P = 0.135$, respectively. The estimator coefficients for temperature and turbidity were -0.28 and 1.083 , with *odds ratios* of 0.76 and 2.95, respectively. Results showed that the occurrence of *Cryptosporidium* oocysts were less at higher temperatures and lower turbidities. Fitted risk values from marine recreational water temperature and turbidity data are shown in Table 5.

Thus, the occurrence of *Cryptosporidium* oocysts increases as temperature decreases and turbidity increases. These conditions are commonly present during the rainy season, which may cause organic/inorganic runoff toward coastal zones, conferring protection to the attached oocysts (EPA 1999). Water movement may cause suspension of

organic/inorganic matter for longer periods of time, increasing the probability of detecting *Cryptosporidium* oocysts in marine recreational waters.

Giardia prediction models

The correlation between presence/absence of *Giardia* cysts and temperature, turbidity, pH and salinity was evaluated. The obtained estimated model was:

$$P(\text{Pres}) = \frac{1}{1 + e^{-0.30T + 2.26Tu}} \quad (2)$$

The goodness-of-fit test, based on Pearson's correlation coefficient, showed $P = 0.481$, indicating that the model was appropriate. Only temperature (T) and turbidity (Tu) were significant, $P = 0.021$ and $P = 0.012$, respectively. The estimator coefficients for temperature and turbidity were -0.30 and 2.26 , with *odds ratios* of 0.74 and 9.55, respectively. Logistic regression analysis showed that the occurrence of *Giardia* cysts decreases as temperature increases and turbidity decreases. Fitted risk values are shown in Table 6.

Values employed (temperature and turbidity) were obtained during the sampling period (Nov 2006–May 2007). According to Table 6, the combination of 30°C and 4 NTU results in almost 50% of the probability of detecting

Table 5 | Fitted risk prediction values for *Cryptosporidium* oocysts presence/absence in marine recreational waters

(Temperature*, Turbidity†)	Risk‡
(19, 3)	0.89
(25, 4)	0.94
(27, 4)	0.96
(36, 5)	0.99

*°C.

†NTU, nephelometric units.

‡Risk based on presence/absence.

Table 6 | Risk prediction for *Giardia* cysts presence/absence in marine recreational waters

(Temperature*, Turbidity†)	Risk‡
(19, 3)	0.25
(20, 3)	0.31
(25, 4)	0.17
(36, 5)	0.38

*°C.

†NTU nephelometric units.

‡Risk based on presence/absence.

Giardia. The probability of *Giardia* occurrence diminishes as temperature increases despite an increase in turbidity. This could be attributed to the presence of organic and inorganic matter transported to the coastal zone by water run-off, which may also contain faecal matter that will be reflected in the increase of *Giardia* occurrence.

DISCUSSION

Cryptosporidium oocysts and *Giardia* cysts presence in water has been widely studied due to their importance as waterborne pathogens. Various studies have shown the ample distribution of *Cryptosporidium* and *Giardia* in aquatic environments. LeChevallier et al. (1991) reported the presence of both *Cryptosporidium* and *Giardia* in 87 and 81% of superficial waters, respectively. Data comparison must be made with precautions due to the diversity of employed methodologies as well as environmental factors that may show inconsistencies. The present study showed that *Cryptosporidium* oocysts were present in greater numbers than *Giardia* cysts, and their prevalence was higher at Mazatlan (83%) than at Altata beach (71%). The intense and continuous tourist activities taking place at Mazatlan beach might partially explain this prevalence. According to the official reports by Mexican environmental authorities (SEMARNAT 2006), both beaches (Altata and Mazatlan) met the *Enterococci* safety standard (<500 CFU/mL) when our sampling was taking place and thus both places were recommended for recreational use. The results of this study are similar to others where it was found that *Enterococci* and other bacterial indicators are not reliable tools for predicting *Cryptosporidium* and *Giardia* presence (Harwood et al. 2005).

The presence of protozoa in marine recreational water relies on environmental factors as well as local and tourist populations (Gerba 2000). Water temperature and turbidity showed significant variation during sampling collection. The highest *Cryptosporidium* oocyst concentration was observed in periods of lower water temperatures (November 2006 and February 2007), while the fewest *Cryptosporidium* presence was at warmer temperatures (July 2007). However, when the risk prediction estimation was performed, it was observed that the occurrence of *Cryptosporidium* oocysts increases when turbidity increases. Studies have shown this event when water run-off carries organic/inorganic materials that might be a source for oocysts/cysts protection. Even though turbidity is not a direct indicator for human health risk, various studies have demonstrated that when turbidity is low protozoan parasites are also reduced (EPA 1999).

The highest and lowest concentrations of *Giardia* were observed at cooler and warmer temperatures, respectively; however, risk prediction estimation showed that when temperature increases the risk is diminished (Table 6). Salinity and pH did not show a significant effect in oocysts/cysts presence/absence. Studies have demonstrated that saline environment does not affect its infectivity (Johnson et al. 1995, 1997; Freire-Santos et al. 1999; Fayer et al. 1998, 2004).

CONCLUSIONS

Based on the results of this study, *Cryptosporidium* oocysts and *Giardia* cysts are present in marine recreational water in 78 and 65% of the samples collected, respectively. The results may lead to protozoan viability studies that might help to develop microbial risk assessment based on parasite concentrations. Monitoring for *Giardia* and *Cryptosporidium* is recommended to better determine the risks of illness from these two protozoan parasites.

ACKNOWLEDGEMENTS

This research was supported by The Fondo Sectorial CONAGUA-CONACYT, 2004, (FON-CNA-2004-01-010). The authors thank Celida Martinez, Andres Medrano and Nestor Gallardo at Food and Environmental Microbiology

Laboratory of Center for Research in Food and Development for the aid in collection the samples analysed in this research work.

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First received 27 October 2008; accepted in revised form 18 March 2010. Available online 2 June 2010