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An assessment of water quality and microbial risk in Rio Grande basin in the United States–Mexican border region

Hodon Ryu, Absar Alum, Maria Alvarez, Jose Mendoza and Morteza Abbaszadegan

ABSTRACT

Increased reliance of urban populations on Rio Grande water has necessitated an expanded microbial surveillance of the river to help identify and evaluate sources of human pathogens, which could pose a public health risk. The objectives of this study were to investigate microbial and chemical water quality in Rio Grande water and to perform risk assessment analyses for *Cryptosporidium*. No oocysts in any of the ten-litre samples were detected. However, the limit of detection in the water samples ranged between 20 and 200 oocysts/100 L. The limits of detection obtained in this study would result in one to two orders of magnitude higher risk of infection for *Cryptosporidium* than the U.S.EPA annual acceptable risk level of 10^{-4}. The bacterial data showed the significance of animal farming and raw sewage as sources of fecal pollution. Male specific and somatic coliphages were detected in 52% (11/21) and 62% (24/39) of the samples, respectively. Somatic coliphages were greater by one order of magnitude, and were better correlated with total ($r^2 = 0.6801$; $p \leq 0.05$) and fecal coliform bacteria ($r^2 = 0.7366$; $p \leq 0.05$) than male specific coliphages. The dissolved organic carbon (DOC) and specific ultraviolet absorbance (SUVA) values ranged 2.58–5.59 mg/L and 1.23–2.29 m^{-1} $(mg/l)^{-1}$, respectively. Low SUVA values of raw water condition make it difficult to remove DOC during physical and chemical treatment processes. The microbial and chemical data provided from this study can help drinking water utilities to maintain balance between greater microbial inactivation and reduced disinfection by-products (DBPs) formation.

Key words | *Cryptosporidium*, microbial indicators, organic contaminants, Rio Grande basin, risk assessment

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INTRODUCTION

The Rio Grande River is one of the longest rivers in the United States. It begins in Colorado (CO), passes through New Mexico (NM), Texas (TX) and part of Mexico and continues on to the Gulf of Mexico, travelling a length of 3,200 km (1,960 miles). While irrigation is the largest use of Rio Grande water, municipal water demand in the southern Rio Grande basin has steadily increased over the last decade. The city of El Paso, TX, is already converting some irrigation water to municipal use; 43% of the city's annual water supply comes from the Rio Grande River. The city of Las Cruces, NM, is also planning to use the river as a source of drinking water. *Cryptosporidium* is an important microbial contaminant in surface water in the United States (Fayer 1997, pp. 1–42). Domestic and wild animals such as ruminants, cervids, swine, cats, dogs and other mammals are major sources of *Cryptosporidium* oocysts in the environment (Fayer 1997, pp. 93–110). Expanded populations of these host animals in an ecosystem result in widespread occurrence of *Cryptosporidium* oocysts in environmental waters (rivers and lakes). Cattle farming and ranching is the most prevalent activity in

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the Rio Grande watershed, which can contribute to the microbial burden of Rio Grande water. Moreover, large cattle farms are located in the vicinity of Las Cruces and El Paso.

LeChevallier *et al.* (1991*a*, *b*) found oocysts in 87% of surface water samples and 27% of drinking water systems in the United States, yet few studies examined the fate of oocysts in the environment (Fayer 1994; Fayer & Nerad 1996; Yang *et al.* 1996). Breakdowns or overloading in public water utilities with microbial pathogens have occasionally resulted in community outbreaks of gastroenteritis. There has been a limited microbial surveillance of the Rio Grande River, and no characterization of *Cryptosporidium* oocysts has been reported in this study area. When water supply sources are being investigated for the first time or when new sources are being developed, it is prudent to undertake a wide range of analyses in order to establish the overall safety and wholesomeness of the water. The objectives of this study were to investigate microbial and chemical water quality in Rio Grande water along the US–Mexican border region, and to perform risk assessment analyses for *Cryptosporidium* to determine the public health significance of using Rio Grande water as a source of drinking water.

MATERIALS AND METHODS

Sampling sites

Six sampling sites were selected along the Rio Grande River around the cities of Las Cruces, NM, and El Paso, TX (Figure 1). The sampling sites cover approximately 110 km (70 miles) of the river, south of Las Cruces. Sampling site selection criteria were based on probable sources of pollution. Sampling site 1 is in the vicinity of the city of Las Cruces, NM. Site 2 is downstream of cattle dairies that are approximately 25 km (15 miles) south of Las Cruces. Site 3 was selected due to its location, downstream of a horse race track. The Rio Grande River divides into two branches, the US canal and the Mexican branch of the river, as it reaches the city of El Paso. Approximately 70% of water flows in the US canal, which is not affected by Mexico. Sites 4 and 5 were on the US canal, and the samples were collected from the intake points of the two drinking water treatment plants in the city of El Paso. The last sampling site is downstream of the Alamo gate, approximately 64 km (40 miles) south of El Paso, where both the US and Mexico branches of the river merge.

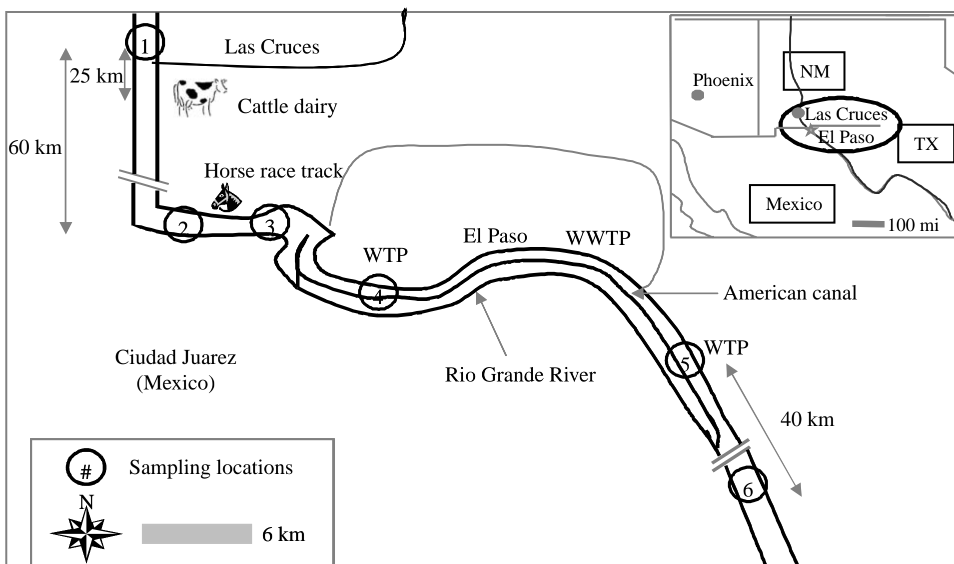

Figure 1 | Sampling locations in the Rio Grande basin.

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Sample processing

Ten-litre grab samples of surface water were collected on a monthly basis from April through December 2001. The water treatment plants are not operated during the winter season; therefore, no samples were collected from site 4 during November and December and from site 5 during December. Ten-litre cubitainers (VWR, Plainfield, New Jersey) were used to collect samples. The samples were placed in ice chests with gel refrigerant ice pack and shipped overnight at 4°C to the Environmental Microbiology Laboratory at Arizona State University, Tempe. The samples for *Cryptosporidium* oocysts were assayed immediately, whereas bacterial and coliphage assays were carried out within 12 h and 36 h, respectively. Samples for natural organic matter were collected in 250 ml of amber glass bottles with Teflon liner (VWR, Plainfield, New Jersey). Turbidity, pH, electric conductivity (EC), dissolved oxygen (DO) and temperature were measured during field sampling. Figure 2 shows the schematic diagram of sample collection and processing.

Detection of *Cryptosporidium* oocysts

Recovery efficiency of oocysts from seeded water samples

Cryptosporidium parvum oocysts were obtained from the Sterling parasitology laboratory at the University of Arizona, Tucson. The oocysts were stored in an antibiotic solution (100 $\mu g\,ml^{-1}$ penicillin and 100 $\mu g\,ml^{-1}$ gentamycin) containing 0.01% Tween 20. The number of oocysts in the stock suspension was determined using a haemocytometer. Recovery efficiency evaluations were performed using tap water, low turbidity raw water from the Chandler water treatment plant, Chandler, Arizona, and high turbidity water from the Rio Grande River. Oocysts were seeded at a level of 100–1,000 per 10 l in the test water. Oocysts were purified by an immunomagnetic separation technique (Dynabeads Anti-*Cryptosporidium*; Dynal AS, Oslo, Norway), labelled using an immunofluorescence assay (Hydroflour Combo; Strategic Diagnostics Inc., Newark, Delaware), and enumerated by epifluorescence microscopy (BX-60, Olympus Optical Co., Ltd, Tokyo, Japan).

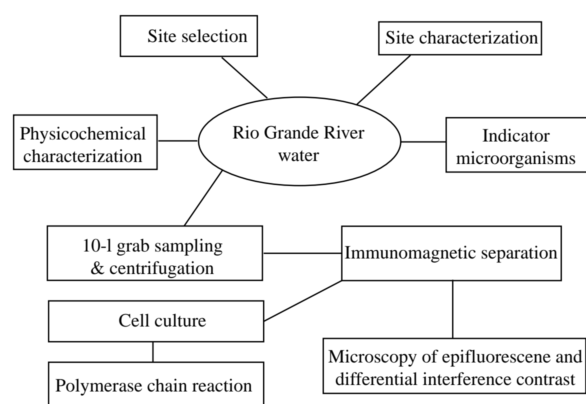

Figure 2 | Schematic diagram of sample collection and processing.

Recovery of oocysts from environmental water samples

Water samples were examined for the presence of *Cryptosporidium* oocysts using immunomagnetic separation–immunofluorescence assay (IMS-IFA) as described in United States Environmental Protection Agency (USEPA) method 1622 (USEPA 2001*a*) and infectious *C. parvum* oocysts were detected using an integrated cell culture-polymerase chain reaction assay (ICC-PCR) as described by Di Giovanni *et al.* (1999). Briefly, ten-litre grab samples of surface water were concentrated by centrifugation at 1,800 × g for 10 minutes using an SLC-4000 rotor (Sorvall, RC. 5C, Newton, Connecticut) with the concentrate purified by IMS. Purified samples (110 μl) were divided into 60 μl and 50 μl aliquots, which were assayed by an IFA microscopy and ICC-PCR, respectively.

Risk assessment of *Cryptosporidium*

The exponential dose-response model developed by Haas (1983) and Haas *et al.* (1996) has been used in various studies for determining the probability of infection from ingestion of various numbers of *Cryptosporidium* oocysts. The following is the model used for estimating the risk of infection for *Cryptosporidium* in the present study.

$$P = 1 - \exp\left(-\frac{N}{K}\right)$$

Where, P is the probability of an infection resulting from ingestion of the number of organisms (N). Regli *et al.* (1991) suggested an annual acceptable risk of 1:10,000 (10^{-4}) from waterborne exposure through potable water. K is the average number of organisms that must be ingested to

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initiate an infection. Using the exponential model with the infectivity data from the study by Dupont *et al.* (1995), the best-fit K value has been determined to be 238.6 (95% confidence limits, 132.0–465.4) (Haas *et al.* 1996).

Detection of indicator microorganisms

Bacteria

Total and fecal coliform, and heterotrophic bacteria (HPC) analyses were performed using a membrane filtration technique on M-Endo LES agar (Difco, Sparks, Maryland), M-FC agar (EM Science, Gibbstown, New Jersey) and nutrient agar (Difco, Sparks, Maryland), respectively. Triplicate samples were filtered using microfunnel II filter cups with a 47-mm diameter, 0.45 μm pore size cellulose acetate membrane (Pall Gelman Laboratory, Ann Arbor, Michigan). The number of total and fecal coliform, and heterotrophic bacteria colonies was counted after 24-hour incubation at 35°C, 44.5°C and 37°C, respectively.

Bacteriophages

Samples were analysed for male specific bacteriophages using two different hosts, *Escherichia coli* F_{amp} (ATCC #700891) and C-3000 (ATCC #15597), and somatic bacteriophages were detected using *E. coli* CN-13 (ATCC #700609) and *Salmonella typhimurium* LT2 (ATCC #19585). Ten ml of samples were assayed using the double agar layer technique (Adams 1959). Briefly, 5 ml of the samples were added to molten top agar with a respective host and plated on bottom agar plates in duplicate. The plates were incubated at 37°C overnight, and plaques were counted after 12 hours. The positive and the negative controls were included in each set of assay for each host group.

Physico-chemical analyses

All glassware for chemical analyses were thoroughly cleaned and rendered free of organic carbon by combustion at 550°C for 4 hours. The samples were filtered through a 0.45 μm Whatman glass-fibre filter (Whatman® International Ltd, Maidstone, UK). To alleviate dissolved carbon leaches, the filters were rinsed with 25 ml of carbon-free deionized (DI) water. The samples were acidified with concentrated hydrochloric acid and stored at 4°C prior to chemical analysis. Dissolved organic carbon (DOC) analysis was performed using a TOC analyser (TOC-5050A, Shimadzu, Columbia, Maryland) with an auto sampler (ASI-5000A, Shimadzu, Columbia, Maryland). Ultraviolet absorbance at 254 nm (UV_{254}) was measured using an UV-spectrophotometer (DR/4000, Hach, Loveland, Colorado).

Measurement of UVA_{254} is used to identify the aromatic content (humic and fulvic material) of organic matter in water. Humic substances have been reported to form total trihalomethanes (TTHMs) and five regulated haloacetic acids (HAA5) in drinking water as a result of chlorine disinfection, which has become an emerging health concern (Eggins *et al.* 1997). In addition, they can complex with metals and organic pollutants such as arsenic, cadmium and pesticides. The ratio of UVA_{254} to DOC is termed specific ultraviolet absorbance (SUVA) with units of m^{-1} $(mg/l)^{-1}$. The SUVA values above 4 indicate that the DOC of the water is composed largely of humic material amenable to removal by coagulation, whereas SUVA values below 3 imply less likely removal of DOC (Edzwald 1993). Turbidity was measured in the laboratory using the same spectrophotometer. Dissolved oxygen (DO), conductivity, pH and temperature were measured in the field.

RESULTS

Cryptosporidium oocysts

The recovery efficiency (RE) of oocysts from seeded tap and low turbidity water was 79.2–88.0%, whereas that from high turbidity water averaged 33.1%. The IMS sample concentrate was split and ICC-PCR performed. The results of IMS-IFA and IMS-ICC-PCR were comparable (Table 1).

A total of 51 water samples were analysed for *Cryptosporidium* oocysts using IFA or ICC-PCR. No oocysts were detected in any of the ten-litre grab samples by either assay. The high turbidity of Rio Grande water resulted in a large amount of packed pellet for all samples, which ranged between 0.5 to 5.0 ml. The entire packed pellet for most samples could not be assayed because IMS technique limits the volume of packed pellet up to 0.5 ml per assay. Thus, equivalent sample volumes for IFA assays were 0.5–5.0 l,

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resulting in a maximum detection range of 20–200 oocysts $100\,l^{-1}$ (Figure 3).

A simple exponential equation was used to estimate an annual risk (P_a) for *Cryptosporidium*. The risk assessment model used in this study is based on the following assumptions: 1) a daily consumption of 2 litres of unboiled water for an average consumer (DC); 2) a 33% oocyst recovery efficiency from the Rio Grande River water (RE); and 3) a 3-log removal of oocysts during treatment processes (LR). The daily ingestion of the number of oocysts (N) through potable water is thus: concentration of *Cryptosporidium* (oocysts $100\,l^{-1}$) × DC ($2\,l\ day^{-1}$) × LR × RE^{-1}. The limit of detection of *Cryptosporidium* ranged between 20 and 200 oocysts $100\,l^{-1}$, which was used for the estimation of an annual risk of *Cryptosporidium* infection through potable water. It was concluded that raw water concentrations of 20 and 200 oocysts $100\,l^{-1}$ would result in annual risks of *Cryptosporidium* infection of 1.85×10^{-3} and 1.85×10^{-2}, respectively (Table 2).

Indicator microorganisms

Bacteria

A total of 35 water samples from sites 2, 3, 5 and 6 were assayed for total and fecal coliforms. The results are presented in Figures 4 and 5. Total and fecal coliforms were detected in 97% (34/35) and 94% (33/35) of the samples, respectively. Total coliforms ranged between 2.6×10^2 and 6.8×10^5 with an average of 2.4×10^4 cfu $100\,ml^{-1}$, and fecal coliforms ranged between 3.7×10^1 and 1.4×10^5 with an average of 7.8×10^3 cfu $100\,ml^{-1}$. The greatest numbers of total and fecal coliforms were found in samples from sites 2 and 6.

Table 1 | Oocyst recoveries from seeded water samples and ICC-PCR results

Sample	Packed pellet (ml)	% oocyst recovery	ICC-PCR result
Tap water	<0.1	92.0	Positive
	<0.1	83.9	Positive
Low turbidity*	0.1	87.8	Positive
	0.1	70.6	Positive
High turbidity**	0.5	36.7	Positive
	0.5	29.5	Positive

*Raw water (<5 NTU) from the Chandler water treatment plant, Phoenix, Arizona
**Rio Grande water (>90 NTU)

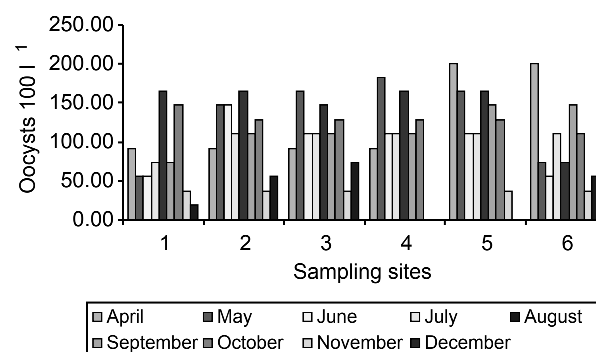

Figure 3 | Spatial and temporal variations of *C. parvum* oocyst detection limit.

A total of 51 water samples were assayed for heterotrophic bacteria. The number of heterotrophic bacteria ranged between 1.0×10^4 and 2.1×10^7 with an average of 1.7×10^6 cfu $100\,ml^{-1}$ (Figure 6). The bacterial numbers were relatively high at site 5 during the months of June, August, September and November.

Bacteriophages

Male specific coliphages were detected in 52% (11/21) and 49% (28/57) of the samples using host *E. coli* F_{amp} and C-3000, respectively. Somatic coliphages and bacteriophages were detected in 62% (24/39) and 48% (10/21) of the samples using *E. coli* CN-13 and *Salmonella typhimurium* LT2, respectively. Figures 7–10 summarize the temporal and spatial variation of bacteriophages in the Rio Grande River.

Relationship between coliforms and bacteriophages

Relationships between the logarithms of total coliforms, fecal coliforms and bacteriophages were investigated. The results are shown in Figure 11. A highly significant correlation ($R^2 = 0.8484$, $p < 0.05$) between total and fecal coliforms was observed, whereas the correlation

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Table 2 | The estimate of an annual risk for *Cryptosporidium* using an exponential model

Parameters for the calculation	Concentration of oocysts (C): oocysts $100\,\mathrm{l}^{-1}$
	Daily consumption of potable water (DC): $2\,\mathrm{l}\,\mathrm{day}^{-1}$
	Reduction of oocysts over treatment (LR): 10^{-3}, 3-log removal
	Recovery efficiency using IMS-IFA (RE): 0.33, 33%
	Daily ingestion of the number of oocysts (N) = (C × DC × LR)/RE
	K*: 238.6 (95% confidence limits, 132.0–465.4)
Annual risk (P_a)	C = 20 oocysts $100\,\mathrm{l}^{-1}$
	$N\,(\text{oocysts per day}) = [(20/100)\times 2\times 10^{-3}]/0.33 = 1.21\times 10^{-3}$
	$K = 238.6\,(132.0 - 465.4)$
	$P = 1 - \exp\left(-\frac{N}{K}\right) = 5.08\times 10^{-6}(9.18\times 10^{-6} - 2.60\times 10^{-6})$
	$P_a^{**} = P\times 365\,(\text{days}) = 1.85\times 10^{-3}(3.35\times 10^{-3} - 9.51\times 10^{-4})$
	C = 200 oocysts $100\,\mathrm{l}^{-1}$
	$N\,(\text{oocysts per day}) = [(200/100)\times 2\times 10^{-3}]/0.33 = 1.21\times 10^{-2}$
	$K = 238.6\,(132.0 - 465.4)$
	$P = 1 - \exp\left(-\frac{N}{K}\right) = 5.08\times 10^{-5}(9.18\times 10^{-5} - 2.60\times 10^{-5})$
	$P_a^{**} = P\times 365(\text{days}) = 1.85\times 10^{-2}(3.35\times 10^{-3} - 9.51\times 10^{-4})$

*Average number of organisms that must be ingested to initiate an infection.
**Assume that daily risks (*P*) are independent and identical.

coefficients between heterotrophic bacteria and both total and fecal coliforms are near zero (data not shown). Total and fecal coliforms are more highly correlated with somatic coliphage ($r^2 = 0.6801$ and 0.7366, $p \le 0.05$) than male specific coliphage ($r^2 = 0.4591$ and 0.5200, $p \le 0.05$).

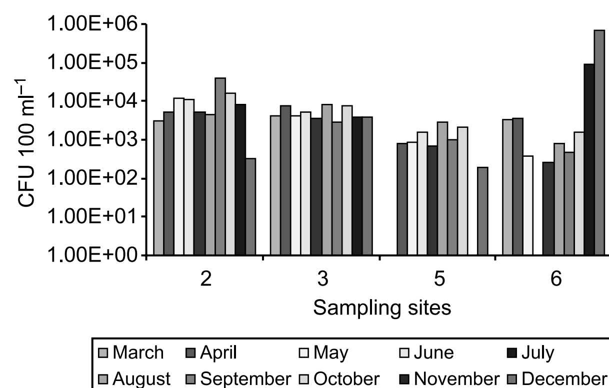

Figure 4 | Total coliform bacteria on M-Endo LES agar media.

Physico-chemical characteristics

The DOC and SUVA (UVA_{254}/DOC) values of Rio Grande water were in the range 2.58–5.59 $\mathrm{mg\,l^{-1}}$ and 1.23–2.29 $\mathrm{m^{-1}}$ $(\mathrm{mg/l})^{-1}$, respectively. The concentrations of DOC were consistent at all sampling sites except for site 6 (Figure 12), the converging point of US and Mexican branches of the Rio Grande River. Turbidity in

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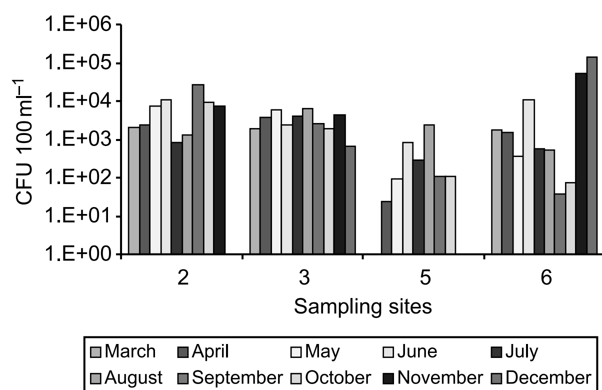

Figure 5 | Fecal coliform bacteria on M-FC agar media.

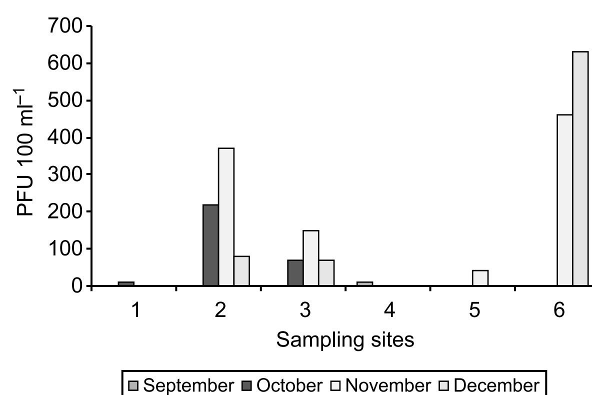

Figure 8 | Male specific coliphages in Rio Grande water using *E. coli* F_{amp} host.

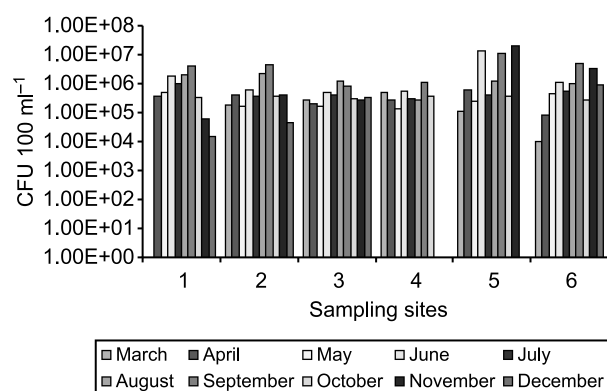

Figure 6 | Heterotrophic (HPC) bacteria in Rio Grande water.

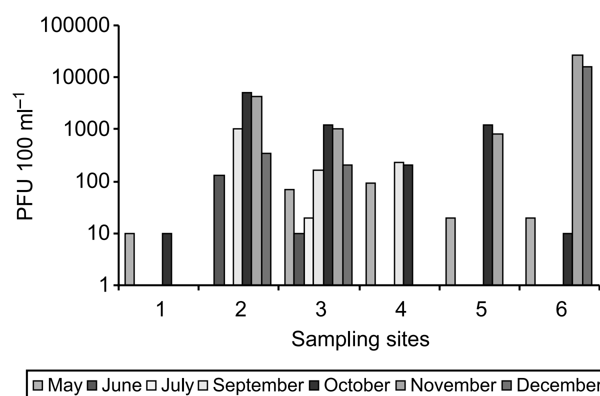

Figure 9 | Somatic coliphages in Rio Grande water using *E. coli* CN-13 host.

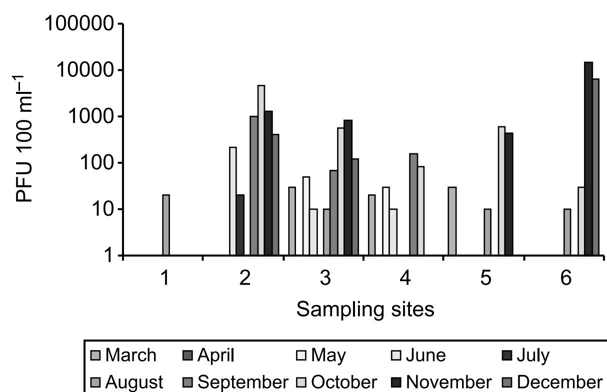

Figure 7 | Male specific coliphages in Rio Grande water using *E. coli* C-3000 host.

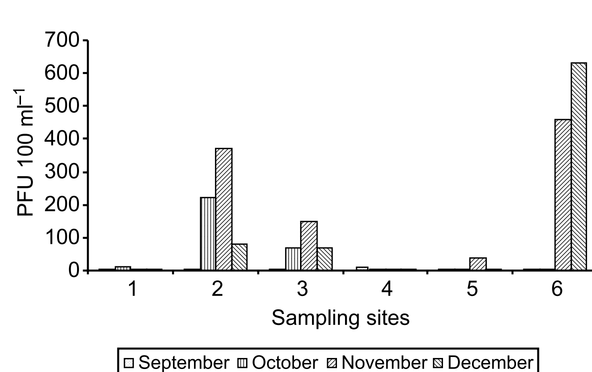

Figure 10 | Somatic bacteriophages in Rio Grande water using *S. typhimurium* LT2 host.

water samples ranged from 3 to 612 NTU, whereas DO, conductivity, temperature and pH range observed were 3.59–11.3 mg l^{-1}, 288–2,709 μmhos cm^{-1}, 5.7–27°C and 7.4–8.9, respectively.

DISCUSSION

The recovery efficiency of oocysts from Rio Grande water using IMS-IFA method averaged 33.1%. The equivalent

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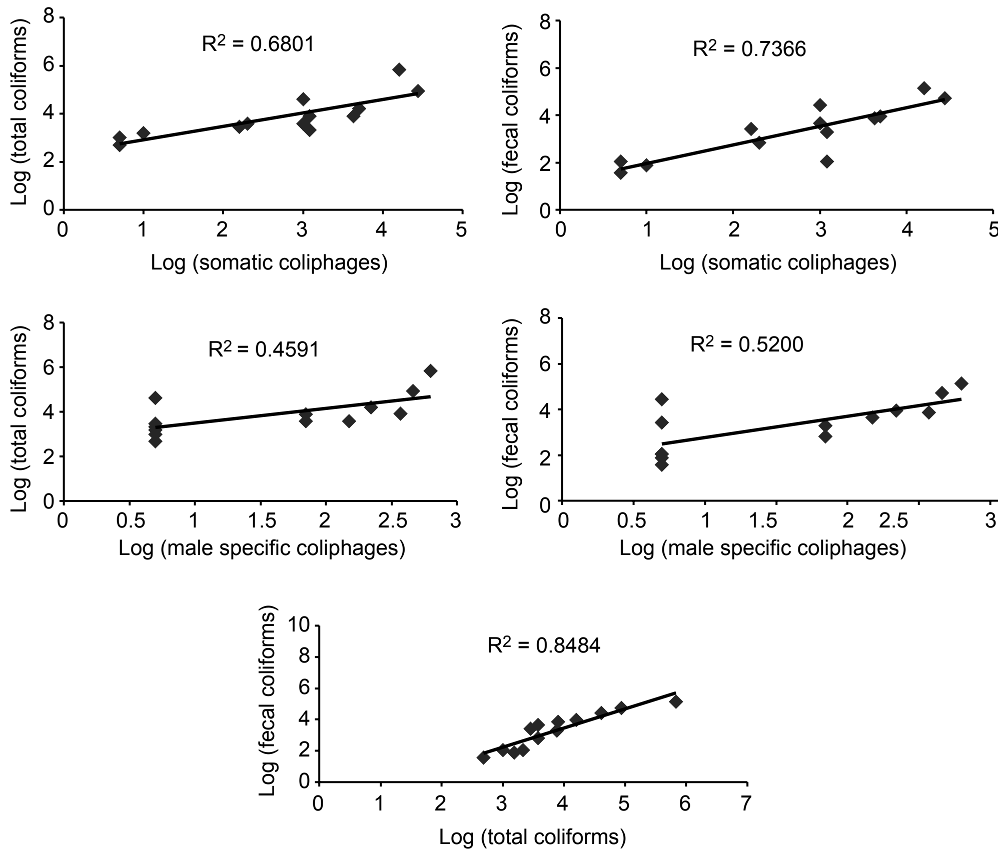

Figure 11 | Correlation between coliforms and coliphages.

sample volume assayed by IMS-IFA ranged between 0.5 and 5 l, because the maximum packed pellet was 0.5 ml. The limit of detection in the samples ranged between 20 and 200 oocysts 100 l^{-1}. The limits of detection obtained in this study would increase the risk of *Cryptosporidium* infection to one or two orders of magnitude above the annual acceptable risk level of 10^{-4} (Table 2).

A gradual decrease in total and fecal coliform numbers was observed downstream of dairy farms (site 2), and thereafter a sudden increase in bacterial numbers was observed at Alamo Gate (site 6). High numbers of coliform bacteria were found near the urban areas (sites 2–5) during the autumn (September and October), whereas site 6 showed the highest level of coliform bacteria during the winter (November and December) (Figures 4 and 5). This indicates that fecal contamination sources in a rural area (site 6) may be different from the urban area. Site 2 is immediately downstream of cattle dairy farms, and site 6 is

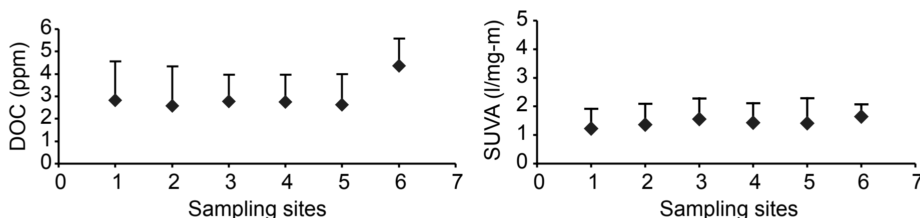

Figure 12 | Variation of dissolved organic carbon and SUVA at each sampling site.

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located downstream from the cities of El Paso, TX and Ciudad Juarez, Mexico. Cattle dairy farms and the Mexican branch of the river are identified as significant sources of fecal contamination. The greatest numbers of heterotrophic bacteria were found in samples from site 5, indicating the impact from the wastewater treatment plant in the city of El Paso (Figure 6). The bacterial numbers at site 1 were similar to the urban area sites, suggesting that run-off from a catchment area may be a major source of these bacteria, representing a non-anthropogenic contamination.

E. coli C-3000 and *Salmonella typhimurium* LT2 have traditionally been used for the detection of male specific and somatic bacteriophages, respectively. However, it has been reported that these hosts are not specific for a respective group of bacteriophages. The United States Environmental Protection Agency (USEPA) method 1601 (USEPA 2001*b*) recommends *E. coli* F_{amp} and CN-13 as hosts to detect male specific and somatic coliphages, respectively. The number of coliphages detected using *E. coli* CN-13 host was very similar to that using *E. coli* C-3000 host (Figures 7 and 9). It appears that *E. coli* C-3000 host not only detects male specific coliphages, but it can also detect some somatic coliphages. In this study, the number of somatic coliphages was higher by one order of magnitude (Figures 8 and 9), and had a better correlation with total (r^2 =0.6801; $p \le 0.05$) and fecal coliform bacteria ($r^2 = 0.7366$; $p \le 0.05$) than male specific coliphages (Figure 11). Araujo *et al.* (1997) reported that somatic coliphages were detected in numbers approximately one order of magnitude higher than male specific coliphages in waters highly contaminated by fecal pollution. Therefore, somatic coliphage seems to be a good indicator of fecal contamination in the Rio Grande River.

The removal of dissolved organic carbon (DOC) and regulated disinfection by-products (DBPs) such as TTHMs and HAA5 is an important issue in drinking water treatment processes. The specific ultraviolet absorbance (SUVA) value, which represents the aromatic contents of DOC, is a critical factor in DOC removal during physical and chemical treatment processes. It is known that greater SUVA values in water are correlated with better DOC removal (Edzwald 1993). The Rio Grande water contains relatively high concentrations of DOC with low SUVA values, which makes it relatively difficult to remove DOC. Improving the removal of DOC as a DBP precursor is important in order to minimize DBP formation. According to the USEPA Disinfectants/Disinfection By-Product (D/DBP) Rule (Stage 1), compliance would be achieved when DOC and SUVA values for source water were $< 2\,mg\,l^{-1}$ and $< 2.0\,m^{-1}\,(mg/l)^{-1}$, respectively (USEPA 1998). In the context of low DOC and low SUVA values of Rio Grande water, the drinking water utilities in the area need to achieve a balance between the tasks of greater microbial inactivation and reduced DBP formation. The data provided from this study can help them to achieve these objectives.

CONCLUSIONS

The following conclusions can be drawn from the present study:

1. No oocysts in any of the ten-litre grab samples were detected; however the limit of detection in the samples ranged between 20 and 200 oocysts $100\,l^{-1}$.
2. The limits of detection obtained in this study would result in one to two orders of magnitude higher risk of infection for *Cryptosporidium* than the annual acceptable risk level of 10^{-4}.
3. Total and fecal coliform bacteria data showed the significance of animal farming (site 2) and raw sewage (site 6) as sources of fecal pollution.
4. Somatic coliphage seems to be a good indicator of bacterial and fecal contamination in the lower Rio Grande basin.
5. High concentrations of DOC with low SUVA values were measured.

The microbial and chemical data provided from this study can help the water utilities to enhance their understanding of source water quality and help them in the process of decision making to produce better quality drinking water.

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