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Occurrence and quantitative microbial risk assessment of Cryptosporidium and Giardia in soil and air samples

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SUMMARY

Background: Cryptosporidium oocysts and Giardia cysts can be transmitted by the fecal–oral route and may cause gastrointestinal parasitic zoonoses. These zoonoses are common in rural zones due to the parasites being harbored in fecally contaminated soil. This study assessed the risk of illness (giardiasis and cryptosporidiosis) from inhaling and/or ingesting soil and/or airborne dust in Potam, Mexico.
Methods: To assess the risk of infection, Quantitative Microbial Risk Assessment (QMRA) was employed, with the following steps: (1) hazard identification, (2) hazard exposure, (3) dose–response, and (4) risk characterization.
Results: Cryptosporidium oocysts and Giardia cysts were observed in 52% and 57%, respectively, of total soil samples ($n = 21$), and in 60% and 80%, respectively, of air samples ($n = 12$). The calculated annual risks were higher than 9.9×10^{-1} for both parasites in both types of sample.
Conclusions: Soil and air inhalation and/or ingestion are important vehicles for these parasites. To our knowledge, the results obtained in the present study represent the first QMRAs for cryptosporidiosis and giardiasis due to soil and air inhalation/ingestion in Mexico. In addition, this is the first evidence of the microbial air quality around these parasites in rural zones.
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1. Introduction

Cryptosporidium and Giardia are pathogens transmitted by the fecal–oral route causing gastrointestinal infections in both humans and animals.[1] The public health importance of both parasites results from the very low infectious dose required to cause illness, their resistance to chemical disinfection, and the long period of viability in the environment.[2] The infective stages of Cryptosporidium and Giardia, termed oocysts and cysts, respectively, can be disseminated successfully across several environmental matrices including water, food, and soil.[3,4] Among these environmental matrices, soil represents an important vehicle through which Cryptosporidium and Giardia infect humans. Soil is contaminated by effluents (water runoff, rain and floods, and wastewater) carrying human and non-human fecal material.[3,5]

Parasite exposure caused by soil inhalation/ingestion is a serious health risk to children, who often play outdoors and deliberately place their hands in their mouths. The estimated average soil ingestion by children can reach between 5 and 8 g per day.[6] In adults, small soil particles are inadvertently ingested at a value around 0.01 g per day.[7]

The soil in rural areas is highly predisposed to direct contamination with fecal material because of the lack of sanitary infrastructure (lack of proper water and sewage services, street pavements, and others), resulting in a greater dispersion of soil via airborne dust during the dry season, particularly in those places where people are exposed to large amounts of outdoor dust.[8]

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The process for determining health risk is termed Quantitative Microbial Risk Assessment (QMRA),[9] which involves four steps: (1) hazard identification, consisting of the estimation of Cryptosporidium and Giardia (oo)cysts in soil and air using the Information Collection Requirement Rule (ICR) method, (2) hazard exposure for the Potam population, (3) dose–response modeling, and (4) risk characterization using an exponential model.[10]

Rural areas in Mexico are extensive, and inhabitants of these areas are more in contact with soil and air presumably contaminated with fecal pathogens.[8] The objective of this study was to assess the human health risk of illness by Cryptosporidium oocysts and Giardia cysts from exposure to soil and air in Potam, Sonora, Mexico.

2. Materials and methods

2.1. Study area

Potam is located in the municipality of Guaymas, Sonora, Mexico. It is one of eight rural villages of the Yaqui tribe, which has a population of 6417,[11] and it is situated 10 m above sea level at global position 27°37′35″ N, 110°24′52″ W.

2.2. Identification and characterization of oocysts and cysts in soil and airborne dust samples

Soil samples were collected from five different areas inside the community, once every 2 months for 8 months (July 2010 to February 2011). Soil samples (2–3 kg) were obtained from a 0.9-m^2 area and between 0 and 5 cm of depth. Air samples were collected every 2 weeks for 8 months (August 2010 to April 2011), using a portable air sampler (Graseby GMW) located in the town center, at an elevation of 1.70 m from the ground. The air sampler flow rate was operated at between 1200 and 1800 l/min. Glass microfiber filters (934-AH RTU 90-mm; Whatman; Kent, UK) were used to retain airborne dust as total suspended particulates (TSP) or particulate matter (PM).

2.2.1. Sample characterization

The soil composition was obtained according to NOM-021-SEMARNAT (2000), which includes the following parameters: soil moisture, texture (by Bouyoucos technique), density, and organic matter (by Walkley–Black method). The TSP in the air was determined as defined in NOM-CCAM-002-ECOL (1993).

2.2.2. Detection of oocysts and cysts

Only 20 g of each soil sample was processed, whereas in the case of air samples, each filter was processed. The 20-g soil samples and each air filter were eluted by adding 0.2 l of buffered phosphate detergent solution, in accordance with the guidelines of the United States Environmental Protection Agency (USEPA).[12] The preparation obtained from each sample was concentrated by centrifugation at 1050 × *g* for 10 min. The pellets were purified by flotation using a Percoll–sucrose solution with a specific gravity of 1.1, stained with a specific direct fluorescent antibody (Aqua-Glo G/C Kit; Waterborne Inc., New Orleans, LA, USA), and examined under an epifluorescence microscope (Axiolab; Zeiss, Heidenheim, Germany). The results were reported as the concentration (*C*) in terms of oocysts (Cryptosporidium) or cysts (Giardia) per liter of air filtered in the case of air samples, or per gram in the case of soil samples. For negative samples, the reported concentration was the detection limit.

As quality control, the Aqua-Glo G/C Kit was used to evaluate the recovery efficiency (*R*). Soil (20 g) and filters used to collect air samples were intentionally inoculated with a known concentration of oocysts or cysts and were then processed as described above. The *R* value, reported as a percentage, was calculated as follows:

$$R = \frac{(C_o - C)}{C_o} \times 100$$

where *Co* is the known initial concentration of (oo)cysts in the matrix (soil or filters to collect air samples) and *C* is the estimated concentration of (oo)cysts recovered once the ICR protocol was developed. The reported *R* values are the arithmetic mean of triplicate results.

2.3. Exposure assessment

Exposure (*N*) was evaluated considering the following factors: (oo)cyst concentration (*C*) per gram for soil samples or per liter for air samples; amount of matrix (soil or air) ingested or inhaled per day (*M*); the recovery efficiency of the method (*R*), which is considered to avoid underestimation of (oo)cyst concentrations and therefore miscalculation of exposure; and finally the fraction of detected (oo)cysts capable of causing infection (*I*).[13,14] The following equation was applied to determine exposure assessment:

$$N = CR^{-1}IM$$

2.4. Dose–response modeling and risk characterization

An exponential dose–response model was used for risk characterization.[10] The exponential model is given by the following equation:

$$P_{id} = 1 - e^{-rN}$$

where P_{id} is the probability of daily risk of infection, *N* is the exposure as estimated above, and *r* is the probability that the organism survives to initiate an infectious focus. The *r*-values are 0.00419 and 0.0199 for Cryptosporidium and Giardia, respectively.[10] Although current research has reported a new *r*-value for Cryptosporidium ($r = 0.09$),[15] which increases the likelihood of risks because the infectious dose is lower, the *r*-value for Cryptosporidium in the present study was 0.00419.

The estimated daily risk could be extrapolated to calculate the risk of illness over extended periods according to the following equation:

$$P_{iy} = 1 - (1 - P_{id})^{(n)}$$

where P_{iy} is the probability of yearly risk of infection, *n* is the number of days that an individual is exposed to the amount of protozoa, and P_{id} is the daily risk.

Assuming, the risk of illness for both parasites is independent but accumulative, the total risk (soil + airborne dust) can be estimated as follows:

$$P_t = 1 - e^{-rNt}$$

where P_t is the probability of total risk and N_t is the total exposure to pathogens in both samples.

3. Results

3.1. Hazard identification and characterization

The soil characteristics are given in Table 1. The TSP value in Potam was 846.0 ± 523.5 μg/m^3, which is higher than Mexican guidelines (210.0 μg/m^3, NOM-024-SSA1-1993). Cryptosporidium oocysts and Giardia cysts were observed in 52% and 57%, respectively,

Table 1
Soil sample characteristics

Potam district	Texture	Moisture (%)	Bulk density (g/cm^3)	Organic matter (%)
Pozo	Clay loam	3.04	1.14	1.98
Tinaco	Clay loam	2.41	1.16	1.47
Centro	Clay loam	2.83	1.17	0.62
Merida	Clayey	3.01	1.12	1.23
Santa Emea	Clay loam	1.33	1.10	0.47

of total soil samples (n = 21), and in 60% and 80%, respectively, of air samples (n = 12) (Table 2). R-values in soil and air are shown in Table 3.

3.2. Exposure assessment

Assuming a soil consumption (M_s) of 0.02 g/day in adults[16] and 0.5 g/day in children under 7 years old,[17] and the exposure parameters (N) given in Table 3, the estimated N in adults and children by soil ingestion is 2.89 and 72 Cryptosporidium oocysts, respectively, and 2.19 and 54 Giardia cysts, respectively.

Air consumption (M_a) was calculated using the following equation:

$$Ma = WaT\upsilon B$$

where Wa is the average weight (54.2 kg) of people in Potam,[18] Tv is the volume of air inspired with normal breathing (6 ml/kg weight), and B is the breaths per minute (18).[19] Therefore M_a was 1404 liters per 4 h. Based on these calculations and parameters given in Table 3, exposure due to air inhalation is 18.6 Cryptosporidium oocysts and 15.3 Giardia cysts.

3.3. Dose–response modeling and risk characterization

The risks of cryptosporidiosis and giardiasis for soil ingestion in adults and children are given in Table 4; these results show that the risks are considerably higher in children than in adults. The risks of cryptosporidiosis and giardiasis in 4 h of exposure to air are shown in Table 5.

3.4. Total risk

The total daily risk considering soil ingestion of 0.02 g and air inhalation/ingestion of 1404 l was 6.8×10^{-2} for Cryptosporidium and 2.3×10^{-1} for Giardia. The total annual risk of both parasites was 1.0, with the same consideration for soil and air inhaled/ingested.

Table 2
Cryptosporidium oocysts and Giardia cysts in environmental samples[a]

Sample	Air		Soil	
	Cryptosporidium (oocysts/l)	Giardia (cysts/l)	Cryptosporidium (oocysts/l)	Giardia (cysts/l)
1	$<1.0 \times 10^{-3}$	2.3×10^{-3}	<6.5	<6.5
2	$<1.0 \times 10^{-3}$	2.7×10^{-3}	43.5	72.91
3	4.5×10^{-3}	3.0×10^{-3}	37.5	29.16
4	1.1×10^{-2}	2.4×10^{-3}	<6.5	<6.5
5	8.3×10^{-3}	2.6×10^{-3}	<6.5	<6.5
6	4.5×10^{-3}	$<1.0 \times 10^{-3}$	<6.5	29.16
7	5.3×10^{-3}	5.2×10^{-3}	43.5	72.91
8	5.6×10^{-3}	8.7×10^{-3}	<6.25	87.5
9	$<1.0 \times 10^{-3}$	$<1.0 \times 10^{-3}$	43.5	<6.5
10	$<1.0 \times 10^{-3}$	2.2×10^{-3}	<6.5	<6.5
11	4.7×10^{-3}	2.1×10^{-3}	<6.5	<6.5
12	4.3×10^{-3}	2.6×10^{-3}	14.58	58.33
13			43.5	72.91
14			37.5	58.33
15			14.58	72.91
16			<6.5	<6.5
17			<6.5	<6.5
18			37.5	58.33
19			43.5	72.91
20			<6.5	29.16
21			14.58	58.33
GM	0.0032	0.0025	14.75	22.9
Min/max	0.001/0.011	0.001/0.0087	6.5/43.5	6.5/87.5

GM, geometric mean.
[a] The '<' symbol indicates the concentration detection limits.

4. Discussion

4.1. Soil

Cryptosporidium oocysts and Giardia cysts were found in all environmental soil samples. On dairy farms, 17% and 4% of soil samples were found to be positive for Cryptosporidium and Giardia, respectively.[20] Oocyst and cyst numbers in this study are higher than those reported on the dairy farms. However, the oocyst numbers in soil obtained in this study (Table 2) are similar to those reported in crop soils (0 to 640 oocysts/g).[21]

According to Armon et al.,[21] the higher the moisture in soil, the higher the concentration of cysts detected. High concentrations of (oo)cysts obtained in soil can be associated with the presence of clay particles and a high concentration of organic matter, which

Table 3
Parameters used for exposure calculation

	Parameters to consider for exposure ($N = CR^{-1}IM$)	Data
Air	Concentration of Cryptosporidium oocysts/l (C)	Range 0.001–0.011; GM 0.0032[a]
	Concentration of Giardia cysts/l (C)	Range 0.001/0.0087; GM 0.0025[a]
	Recovery efficiency of Cryptosporidium and Giardia (R)	16.19% and 15.38%, respectively[a]
	Cyst and oocyst viability (I)	100%
	Amount of air inhaled (M)	1404 liters[b]
Soil	Concentration of Cryptosporidium oocysts/g (C)	Range 6.5–43.5; GM 14.75[a]
	Concentration of Giardia cysts/g (C)	Range 6.5–87.5; GM 22.9[a]
	Recovery efficiency of Cryptosporidium and Giardia (R)	10.18% and 20.83%, respectively[a]
	Cyst and oocyst viability (I)	100%
	Amount of soil ingested (M)	0.02 g for adults[16] and 0.5 g for children[17]

GM, geometric mean.
[a] All data were calculated in the present study; see section "Detection of oocysts and cysts".
[b] The amount of air inhaled was calculated in the section "Exposure assessment".

Table 4
Daily and annual risks for Cryptosporidium and Giardia in adults and children, with an amount of soil ingested (M) of 0.02 g and 0.5 g per day, respectively[a]

Risk of infection		Concentration in soil					
		Cryptosporidium (oocysts/g)			Giardia (cysts/g)		
		Min	GM	Max	Min	GM	Max
		6.5	14.75	43.5	6.5	22.9	87.5
Adults	Daily	5.3×10^{-3}	1.2×10^{-2}	3.5×10^{-2}	7.3×10^{-3}	2.5×10^{-2}	9.5×10^{-2}
	Annual	8.5×10^{-1}	9.8×10^{-1}	9.9×10^{-1}	9.3×10^{-1}	9.9×10^{-1}	1
Children	Daily	1.2×10^{-1}	2.6×10^{-1}	5.9×10^{-1}	1.6×10^{-1}	4.8×10^{-1}	9.1×10^{-1}
	Annual	1	1	1	1	1	1

GM, geometric mean.
[a] The r-values are 0.00419 and 0.0199 for Cryptosporidium and Giardia, respectively.[10]

Table 5
Daily and annual risks for Cryptosporidium and Giardia due to ingestion of oocysts or cysts in air during 4 h of exposure per day

Risk of infection	Concentrations in air					
	Cryptosporidium (oocysts/l)			Giardia (cysts/l)		
	Min	GM	Max	Min	GM	Max
	0.001	0.003	0.01	0.001	0.002	0.008
Daily	4.5×10^{-2}	1.1×10^{-1}	3.4×10^{-1}	6.1×10^{-1}	2.3×10^{-1}	9.5×10^{-2}
Annual	9.9×10^{-1}	1.0	1.0	1.0	1.0	1

GM, geometric mean.
The r-values are 0.00419 and 0.0199 for Cryptosporidium and Giardia, respectively.[10]

can provide protection for microorganisms.[22] The recovery efficiency of oocysts in soil samples reported by Kuczynska and Shelton ranged between 6% and 18%,[23] which is similar to the values obtained in the present study.

Although risk assessments in soil for Cryptosporidium and Giardia have not been reported, a lot of research exists on the risk assessment for these parasites in different water sources. The risk of illness in water has been shown to range from 1×10^{-6} to 1 as a function of water quality.[24–27]

4.2. Air

People habitually breathe through the nose; when abundant air passes through the nose, the dust is bound by cilia, preventing the particles from reaching the pulmonary bronchi. The agglomerated dust in the cilia passes to the pharynx, and the esophagus transports the dust to the stomach.[24] During mouth breathing, the airborne dust particles pass directly to the throat and into the stomach. Thus, pathogens that are transported in air and transmitted by the fecal–oral route can infect people.

In Potam, the content of (oo)cysts in air may be associated with TSP values. However, a direct relationship between oocyst/cyst concentrations and TSP detected in air samples was not found. In the study by Cummins et al.,[25] TSP results were not statistically significant ($p > 0.05$) for the months sampled, the same finding as that obtained in the present research.

The risk of illness for air inhalation is very high (Table 5), even above the water guidelines (1.0×10^{-4}).[4] The infectious illness estimates detailed in Tables 4 and 5 may be overestimates as a result of assuming that all of the (oo)cysts (detected in soil and air) were viable when swallowed (samples may have included non-infectious (oo)cysts).

Further research is necessary, extended to other rural areas that need an assessment of the risk for protozoan pathogens in air and soil. Regardless of these characteristic limitations in the QMRA method, this study offers microbial data that could be valuable in the design of strategies or guidelines in inhabited towns, particularly in rural zones.

5. Conclusions

This study provides the first QMRAs in soil for Cryptosporidium and Giardia in Mexico and, to the best of our knowledge, the first demonstration of microbial air quality monitoring in a rural zone. Soil and air are important vehicles of infection for these parasites. In Potam, Sonora, high (oo)cyst concentrations were detected with a yearly risk of 1.0 for each parasite. Effective feces and sewage disposal, as well as strategies to increase hygiene habits, are recommended to reduce the infection risk.

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