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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.

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:

$$N = CR^{-1}\mathrm{I}M$$

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,