Risk Assessment of *Cryptosporidium* and *Giardia* Infection. Case study: fresh vegetable consumption in an indigenous community in Mexico

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Abstract

Consumption of raw vegetables is one of the major sources for pathogen transmission, particularly parasites, such as *Cryptosporidium* and *Giardia*. This research is a complement of quantitative microbial risk assessment in well water, air and soil with the goal of evaluating risk infection by giardiasis and cryptosporidiosis due to ingestion of raw vegetables washed with water from a well in Pótam, Sonora, México. Quantitative Microbial Risk Assessment was the methodology used to predict infection probability of giardiasis and cryptosporidiosis related to consumption of (oo)cysts in pepper and tomato samples. In tomato, *Cryptosporidium* was detected at a concentration of 0.58-1.43 oocysts g^{-1} and a range of 0.58-2.86 cysts g^{-1} for *Giardia*; while a range of 1.9-4.5 cysts g^{-1} were recorded for *Giardia* in pepper, no *Cryptosporidium* oocysts were detected. The lowest risk for cryptosporidiosis per person in the community of Pótam, Sonora was by tomato consumption of 4×10^{-1} per day and one per year. For giardiasis, the lowest risk per day was 9.3×10^{-1} and one per year. The risk of giardiasis by raw pepper consumption was 6.5×10^{-1} and one per year. The results showed that raw pepper and tomato consumption contributed to parasite infection.

Keywords: *Giardia, Cryptosporidium*, Risk Assessment, Yaquis, groundwater, pepper, tomato.

Introduction

The protozoa *Cryptosporidium* and *Giardia* are ubiquitous in the world. Giardiasis and cryptosporidiosis are illnesses that produce symptoms, such as diarrhoea, weight lost, abdominal pain, nausea, vomit and fever. The latent stages of these parasites are oocyst for *Cryptosporidium* and cysts for *Giardia*. These (oo)cysts are important to public health because they transmit the infection to the hosts (human or animal) by the faecal-oral route and principally transported in water and food where they can be viable from weeks to months [1, 2, 3]. The necessary intake of (oo)cysts to initiate the illness ranges from 1 to 100 [4, 5].

In developing countries, consuming raw or unwashed vegetables is the major route of transmission for bacterial or parasite contamination [6]. Both parasites survive well in refrigerated conditions [7]. In the decade of the 2000s, more than 20% of *Cryptosporidium* outbreaks worldwide were correlated to food-borne transmissions [8]. *Cryptosporidium* oocysts have been isolated from food, such as chicken, salads, apples, citron, green onions, unpasteurized milk, lettuce, carrots, tomatoes and cucumbers [9]. Moreover, *Giardia* cysts have been found in tomato, cucumber, lettuce, watercress, cabbage and strawberry, among others [1, 10].

Gastrointestinal infections are more frequent in developing countries, especially in places where populations have risk factors, such as poverty, poor education levels of sanitation, untreated water and low infrastructure for residual water [11]. Native populations are more prone to these types of infections [12]. This

study is a complement to the quantitative microbial risk assessment (QMRA) for *Giardia* and *Cryptosporidium* (oo)cysts in water, air, and soil performed in Pótam community in Valle del Yaqui, Sonora, Mexico (13, 14). The aim of this research was to assess the infection risk of cryptosporidiosis and giardiasis due to fresh vegetable consumption.

Methods

Study site

Pótam is one of eight towns of the Yaqui tribe with a population of approximately 6417 inhabitants [15], located in the municipality of Guaymas, Sonora, México. To select the most consumed raw vegetables in the locality, a cross-sectional study was performed. The following equation was used to calculate the size of the population for survey application:

$$n = \frac{N^{*}Z_a^2\, p^{*}q}{d^2{*}(N-1) + Z_a^2\, p^{*}q} \qquad (1)$$

Where n is the total of the population; Z_a^2 = 1.96 (if safety is 95%); p is the expected proportion (5%); q is equal to 1-p; and d is the precision (5%).

The survey, applied to the community (N = 72), indicated several data:

personal information (name, date of birth, age and gender), socio-demographic (education, occupation, number of children in the family), clinics and daily consumption (type, quantity and drinks).

The most consumed vegetables were Serrano pepper (97%) and tomato (95%). During the interview, people explained their way of washing food, which was applied in the methodology. Therefore, an exposure of 180 days was calculated (taking into account season, harvest, availability and price) for both vegetables.

Sample collections

For washing vegetables, sufficient water was collected from the only well found in Pótam (8-10 L). Serrano pepper and Saladette tomato were bought in the most common market in the locality (during all the process, the vegetables in this study were handled with gloves). Next, these vegetables were transported to the lab in a freezer. This collection was made monthly (from October 2010 to April 2011) processing 28 samples.

Treatment of samples and application of the modified Information Collection Rule (ICR) method

This study used a modified protozoan version of the ICR method for *Giardia* cyst and *Cryptosporidium* oocysts [16]. All samples were processed in triplicate.

First, the most turgid pieces of pepper and tomato were weighed in a balance; they were washed with Potam well water in a sieve in a revolving way from three to six minutes just like the people surveyed declared they washed their food. After washing, the pieces were immersed into a beaker with 0.2 L of eluent solution, taking care that it covered all the vegetables; the beakers were placed in a rotary shaker for 30 minutes. The eluent solutions resulting were centrifuged 1.050 *xg* for 10 min. The obtained pellet was purified by density gradient with Percoll-sucrose and stained with indirect immunofluorescence (Aqua Glo™ G/C kit, New Orleans, USA). The (oo)cyst count was performed with an epifluorescence microscope (Axiolab Carl Zeiss, Oberkochen, Germany). The slides were examined by completely searching green-apple colour and spherical objects 4-6 µm in size for oocysts and oval-shaped 5-10 µm in size for cysts. Calculated concentrations followed the modified ICR formula:

$$\text{Cyst or oocysts per gram} = \frac{\text{Cysts or oocysts counted}}{\text{FRW}} \qquad (2)$$

Where *F* is 50% of the purified pellet; *R* is the percentage of the floating material examined microscopically; and *W* is the weight of the vegetables. For negative samples the detection limit was calculated where the modified formula was used replacing < 1 in counted structures [16].

Quality control

Aqua Glo™ G/C kit (New Orleans, USA) was used for evaluating the recovery efficiency of the ICR method. Fresh vegetables were inoculated on purpose putting a known quantity of (oo)cysts on their surface. The inoculated vegetables were processed following the steps of the method with the environmental samples. The recovery efficiency (%R) was calculated by the following equation:

$$\% R = \frac{(Co-C)}{Co} * 100 \qquad (3)$$

Where *Co* is oocyst concentration at the beginning in the inoculated food; *C* is the estimated concentration after the method was applied. The R percentage values reported were the average of the triplicate results.

Quantitative Microbial Risk Assessment

For determining the probability of risk infection of *Cryptosporidium* and *Giardia* (oo)cysts, an exponential dose-response was selected. The exponential approach is represented by the following equation:

$$P_i = 1 - e^{-rN} \qquad (4)$$

Where, P_i is the probability of infection; *N* is the number of pathogens; and *r* is the average of the necessary microorganism to initiate the infection; *r*-values were calculated as in the literature for *Cryptosporidium* and *Giardia*, which were 0.00419 and 0.0199, respectively [17].

For evaluating exposure (*N*) to *Cryptosporidium* and/or *Giardia* (oo)cysts by contaminated food, the following equation was used:

$$N = CR^{-1}IM \quad (5)$$

Where *C* is the concentration of *Cryptosporidium* and/or *Giardia* ((oo) cysts/g); *R* is the recovery efficiency of the method; *I* is the fraction of pathogens capable of causing the infection; and *M* is daily consumption of the raw vegetables.

Annual risks were calculated using the following equation:

$$P_i(\text{annual}) = 1 - (1-P_i)^{(n)} \quad (6)$$

Where *Pi (annual)* is the infection risk per year; *n*, is the number of days of exposure; and P_i is the daily risk infection.

Statistical Analysis

The concentration data was analysed using Stat Graphics Centurion XVI (Madrid, Spain) software obtaining the variance analysis. Daily and annual risks were transformed using a conversion from non-normal distribution to normal obtaining geometric mean (GM) data. Microsoft Excel was used for risk calculations.

Results

Serrano pepper

Cryptosporidium oocysts were not detected in Serrano pepper. On the other hand, *Giardia* cysts were detected in 42.8% of the analysed samples (*n* = 7). The number of *Giardia* cysts in each gram of pepper was found in a range of 1.9-4.5 with a GM of 3.07 (Table 1).

The recovery efficiencies of the method were 16% for *Cryptosporidium* and 13% for *Giardia* oocysts. All the risks were estimated assuming the parameters for exposure in Table 2. Table 3 shows daily infection risks for *Giardia* in Serrano pepper. The risk of daily infection ranged from 4.8×10^{-1} for *Giardia* to the minimum of concentration associated to pepper. Annual risk reached one in all scenarios.

Saladette tomato

The presence of *Cryptosporidium* oocysts in tomato was positive in 14.28%, and that of *Giardia* cysts was of 57% (*n* = 7) from the analysed samples. The numbers of *Cryptosporidium* in tomato were in a range from 0.58-1.43 with GM of 0.9 oocysts g^{-1} while in the case of *Giardia*, they were in a range from 0.58-2.86 with a GM of 1.22 cysts g^{-1} (Table 1).

Table 1 Concentrations of *Cryptosporidium* and *Giardia* oocysts in pepper and tomato washed with well water.

Samples	Pepper (oo)cysts/g		Tomato (oo)cysts/g	
	Cryptosporidium	*Giardia*	*Cryptosporidium*	*Giardia*
1	<1.9	1.9	<1.43	2.86
2	<2.4	<2.4	<0.58	<0.58
3	<2.2	2.2	<0.66	<0.66
4	<4.5	4.5	<1.38	1.38
5	<4.2	<4.2	<0.74	0.74
6	<3.9	<3.9	1.4	2.8
7	<3.5	<3.5	<0.64	<1.29
Median	3.22	3.22	0.95	1.47
GM[a]	3.07	3.07	0.9	1.22
Min/Max	1.09/4.5	1.9/4.5	0.58/1.43	0.58/2.86

Mn: minumun Mx: maximun GM: geometric mean

The parameters considered for the infection risk assessment for *Cryptosporidium* and *Giardia* are shown in Table 2. The calculation for tomato consumption was based on SAGARPA data [18], around 13 Kg per person per year where 56% corresponded to Saladette obtaining 18 g per day per person. The recovery efficiencies of the method were 19% of *Cryptosporidium* oocysts and 15% of *Giardia* cysts for tomatoes.

Table 2 Exposure parameters for the analysis of infection risk by *Cryptosporidium* and *Giardia* oocysts.

Exposure	$N = CR^{-1}IM$	Pepper	Tomato
Concentrations (C)	oocysts/g	Not detected	0.58-1.43; GM= 0.9
	cysts/g	1.9-4.5; GM= 3.07	0.58-2.86; GM=1.22
Recovery efficiency (R)	*Cryptosporidium*	16%	19%
	Giardia	13%	15%
Daily Consumption (M)		3.8* g	18* g
Viability (I)		100%	100%

* [18]

Table 3 shows the daily and annual risks of infection for exposure to *Cryptosporidium* and *Giardia* in tomato. All the risks associated to tomato consumption were assuming the parameters for exposure in Table 2 and taking maximum and minimum concentrations to consider different exposure scenarios. The risks of daily infection ranged from 2.8×10^{-1} for *Cryptosporidium* minimum to 9.9×10^{-1} maximum for *Giardia*. The annual risks reached one for both parasites in all the previous scenarios.

Table 3 Daily infection risks by Cryptosporidium and Giardia oocysts in pepper and tomato.

Daily infection risks	Concentration*	*Cryptosporidium*	*Giardia*
For tomato	Mn	$2.8x10^{-1}$	$7.2x10^{-1}$
consumption	Mx	$5.6x10^{-1}$	$9.9x10^{-1}$
	GM	$4.1x10^{-1}$	$9.3x10^{-1}$
For pepper	Mn	N/D	$4.8x10^{-1}$
consumption	Mx	N/D	$7.9x10^{-1}$
	GM	N/D	$6.5x10^{-1}$

*Per gram of fresh produce
N/D: Not detected

Discussion

This research study assessed the infection risk by *Giardia* and *Cryptosporidium* implicating consumption of raw vegetables washed with well water in the Yaqui community in Pótam, Sonora, Mexico using QMRA. No significant differences were observed between the concentrations of oocysts or cysts within sampling months ($p > 0.05$).

The concentrations of *Cryptosporidium* and *Giardia* (oo)cysts detected in Serrano pepper and Saladette tomato are shown in Table 1. Occurrences of

(oo)cysts in vegetables have been frequently reported in other studies as *Cryptosporidium* in tomato of 32% (n = 200) [19], *Giardia* cysts in Philippine cabbage of 1.25% (n = 80) [6], and for *Cryptosporidium* and *Giardia* in raw green vegetables of 29.3 and 6.7% (n = 300), respectively [20]. The results of this study were compared with those of Mota *et al*. [22], who also looked at the potential contamination of fresh produce by water in Mexico but with different concentrations of (oo)cysts in retained irrigated water on vegetables. Hence, our study obtained higher values because it included the contamination with well water and that from fresh food surface involved in their cultivation, growth, crop, marketing, preparation and consumption.

Contamination of Serrano pepper and Saladette tomato with *Cryptosporidium* and *Giardia* oocysts were caused by different elements. Firstly, vegetables were contaminated by soil contact because the ground is fertilized frequently with farm animal manure that might be host of parasites. Secondly, they were contaminated by wastewater irrigation from drainage canals used to freshen the vegetables (common practice of the sampled community) to increase their preservation and prevent dehydration. Thirdly, the samples bought in the small local shops had a high concentration of oocysts; the vegetables could have been touched by infected local customers or food handlers/farm workers while selecting them (direct contamination of fresh produce), which could have introduced the pathogen in the products. Finally, from previous studies made in Potam well water, *Cryptosporidium* and *Giardia* concentrations were calculated obtaining 2.3 oocysts

L^{-1} and 3.07 cysts L^{-1}, respectively [13].

Producers use irrigation water coming from canals to freshen and wash the vegetables before selling or distributing them to commercial businesses. Buyers can also contaminate the vegetables during their selection. Sia Su *et al*. [6] reported that the occurrence of parasites is greater in vegetables bought in big markets than at small markets ($p < 0.05$).

The Yaqui community has a traditional vegetarian diet; consequently, the days of exposure and the amount of vegetable they consume could make the risk greater than that estimated. Therefore, green leafy vegetables, such as crops of greens, *Portulaca oleracea, Chenopodium*, and *Medicago sativa*, all abundant in the region, are often consumed raw, and they were not considered in this study although it has been demonstrated that leafy vegetables favour the adhesion of oocysts in vegetable surfaces [21, 22, 23].

Conclusions

The following sources of contamination of the analysed vegetables were considered: crop field, irrigation water, soil contact, distribution, trading, handling and inappropriate wash. Other related research studies in water and soil support that *Giardia* cysts and Cryptosporidium oocysts are endemic in the Potam community [13, 14]. Therefore, local health and environmental authorities should ensure sanitary conditions are improved where vegetables are grown and

emphasize the importance of washing and disinfecting them correctly before consumption**.**

Limitations

- The risks of infection calculated in this investigation could be overestimated because it is assumed that all the (oo)cysts detected are viable or able to infect.
- The detection method does not identify the species and genotypes.

Abbreviations

(OO)CYSTS: refer to both, cyst of *Giardia* and oocysts of *Cryptosporidium*

QMRA: Quantitative Microbiological Risk Assessment

ICR: collecting information on rules and requirements

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