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RISK FACTORS FOR *GIARDIA INTESTINALIS* INFECTION IN AGRICULTURAL VILLAGES PRACTICING WASTEWATER IRRIGATION IN MEXICO

ENRIQUE CIFUENTES, MARIANA GOMEZ, URSULA BLUMENTHAL, MARTHA MARIA TELLEZ-ROJO, ISABELLE ROMIEU, GUILLERMO RUIZ-PALACIOS, AND SILVIA RUIZ-VELAZCO

Instituto Nacional de Salud Publica, Cuernavaca, Mexico; London School of Hygiene and Tropical Medicine, London, United Kingdom; Centers for Disease Control and Prevention, Atlanta, Georgia; National Institute of Nutrition, Tlalpan, Mexico; Instituto de Matematicas Aplicadas a Sistemas, Universidad Nacional Autonoma de México, Copilco, Mexico

Abstract. This study assessed the risk factors for *Giardia intestinalis* infection in an agricultural population in Mexico. Exposure groups included 2,257 individuals from households exposed to untreated wastewater, 2,147 from a group using the effluent from a series of reservoirs, and 2,344 from rain-fed agricultural villages. Stool samples were collected from 6,748 individuals. Wastewater samples were tested for fecal coliforms/100 ml and *Giardia* sp. cysts/L. Untreated wastewater samples contained 10^8 fecal coliforms/100 ml and up to 300 *Giardia* sp. cysts/L. Hydraulic retention (3–7 months) in the reservoirs, however, provided an improved effluent quality (10^1—10^4 fecal coloforms/100 ml and $\leq$ 5 *Giardia* sp. cysts/L). Children 1–14 years of age had the highest prevalence of infection (20%). Data showed marginal associations between storing drinking water in unprotected containers and lack of facilities for feces disposal and the risk of infection (odds ratios [ORs] = 1.76 and 1.19, 95% confidence intervals [CIs] = 0.95–3.23, and 0.97–1.45, respectively). Individuals purchasing vegetables at the city market had higher rates of infection than those buying at the village shop (OR = 2.49, 95% CI = 1.00–6.17). No excess risk was found in individuals exposed to untreated wastewater compared with controls (OR = 1.07, 95% CI = 0.84–1.36); the group using reservoir water was not different from the controls (OR = 1.22, 95% CI = 0.94–1.58). No risk from agricultural activities was detected (OR = 0.83). This pattern of infection may be addressed by primary health care and wastewater treatment.

Wastewater reuse is an ancient practice that has been gradually implemented worldwide. In the United States alone, more than 3,400 water reuse projects have been recorded. In China, more than 1 million farming hectares depend upon wastewater irrigation. In Israel, Egypt, Tunisia, Greece, South Africa, Japan, and a growing number of Latin American countries, wastewater reuse provides a substantial resource for agricultural production. The most compelling reasons for wastewater reuse include job opportunities in rural zones, more and better crops, and less frequent use of chemical fertilizers. Wastewater reuse schemes, when handled safely and efficiently, provide multipurpose rehabilitation opportunities for large extensions of land and, simultaneously, the preservation of fresh water sources for human consumption.[1–3]

Interest in wastewater reuse in agricultural irrigation has been renewed due to recent technologic developments that yield high-quality effluents. Water-stressed countries, however, frequently lack the required financial and technological capabilities for such wastewater treatment systems; crop irrigation with insufficiently treated wastewater may result in health risks. Available evidence shows risks of enteric infections, especially by helminths (i.e., *Ascaris lumbricoides* and *Trichuris trichiura*), in agricultural workers exposed to untreated wastewater irrigation.[4] Additionally, risks for cholera and typhoid fever in consumers of uncooked vegetables have been documented.[5] Based on this evidence, the World Health Organization has published guidelines for the quality of wastewater in agriculture.[6] Basically, These recommend less stringent bacteriologic quality (10^3 fecal coliforms/100 ml) relative to those currently in effect, and for the first time the helminth egg contamination as an indicator of a water quality (< 1 egg/L). The revised guidelines take into account available data on health risk, while emphasizing that high quality may be achieved by treatment involving hydraulic retention in stabilization ponds, a process that consists of sedimentation and natural death of potential pathogens.[7,8] However, the guidelines acknowledged that the actual risk from protozoal infection had not been sufficiently evaluated.[6] The only epidemiologic study available that addressed this problem was carried out in India.[9] It showed no significant difference between the prevalence of *Giardia intestinalis* infection among agricultural workers using untreated wastewater or treated wastewater compared with controls who did not irrigate with wastewater (12.3%, 14.5%, and 11.5%, respectively). Data describing the quality of water used for agricultural production, water treatment technology, and hygiene and sanitation factors were not provided.

One of the largest wastewater reuse systems in the world is located in central Mexico in the Mezquital Valley. Financial constraints, population growth, and water shortages have motivated authorities to develop a wastewater reuse program and adopt water treatment technology different from that of conventional schemes. Currently, cropland irrigation with untreated wastewater is allowed only on fodder and maize, whereas growing vegetables for consumption uncooked is officially forbidden. Previous research indicated a high risk of *A. lumbricoides* infection and diarrhea in families of agricultural workers exposed to untreated wastewater.[4] More importantly, these studies showed that hydraulic retention reduced this risk.[10] No additional data are available on the risk of protozoal infections (e.g., *G. intestinalis*). This paper addresses this issue with respect to infection with *G. intestinalis.*

MATERIALS AND METHODS

The main environmental and demographic characteristics of the Mezquital valley have been previously described.[11] Approximately 45 m^3/sec of untreated wastewater and storm water run off from Mexico City, flow 70 km north through

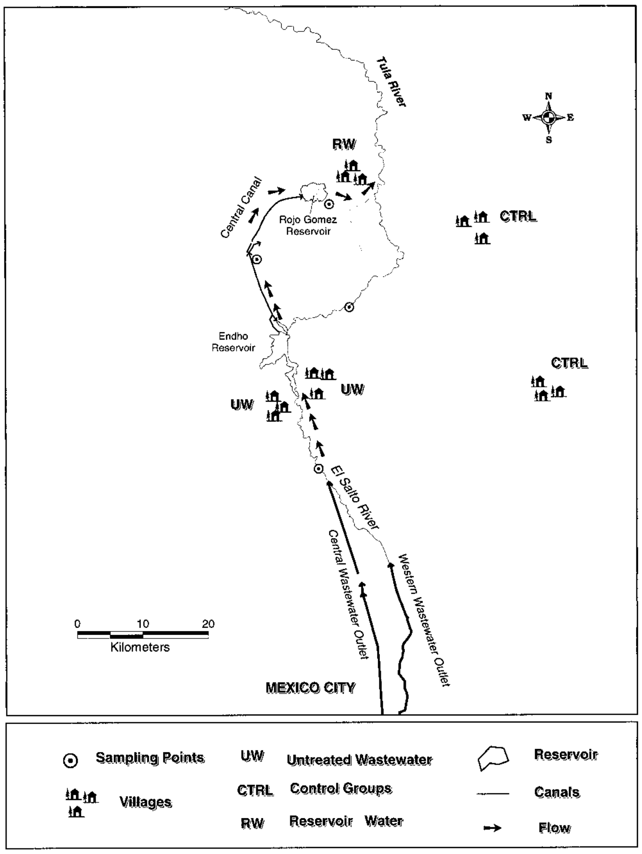

FIGURE 1. Study area in Mexico.

metropolitan outlets, and then irrigate 90,000 hectares of farm land (Figure 1). The first group of villages receive untreated wastewater (UW group), the surplus of which is conveyed further north through a network of canals and retained in interconnected reservoirs. The effluent from these storage reservoirs flows towards another group of villages (RW group). In addition, many communities practicing rain-fed agriculture (CTRL group) are scattered around the Mezquital Valley.

A cross-sectional survey was carried out during the rainy months (July–September 1990). A written explanation (e.g., purpose) of the study was provided to all households and informed consent was obtained from all participants. The study was reviewed and approved by the Institute of Health of Mexico. A total of 11,357 dwellings were visited and numbered. Only households having one or more members actively involved in agricultural production were included in the census. Exclusion criteria included non-agricultural households and those with individuals who had contact with more than one source of irrigation or unknown and unclassified canals. Thus, the sampling units were households and the individual was the unit of analysis. Members of eligible households not directly involved in agricultural work (e.g., infants) were included in the analysis. Every household meeting the eligibility criteria participated in the study. A total of 9,088 individuals were involved. Seventy-five percent completed questionnaires and provided stool samples.

Exposure groups included 2,257 individuals from the UW group, 2,147 from the RW group, and 2,344 from the CTRL group. Information was obtained by interviews using standardized questionnaires and parasitologic tests. Data were gathered that described the agricultural profile, place, and timing of exposure-related activities. Hygienic and sanitation characteristics (e.g., source of drinking water and toilet availability), socioeconomic variables (e.g., land tenure, mother's literacy, dwelling materials) and other potential confounders (age, source of vegetables) were also recorded. At the end of the interview, tagged plastic containers for stool samples were distributed. These were collected the following day. Infection with *G. intestinalis* was assessed by microscopic identification in stool specimens.

Wastewater samples were collected monthly from selected sites (Figure 1). The main objective of these tests was to assess the quality of wastewater, particularly after storage in the reservoirs. Water quality indicators were the number of *Giardia* sp. cysts/L and fecal coliforms/100 ml. Hydraulic retention time was calculated using the formula designed by Peasey (Peasey AE, unpublished data). Intestinal infection with *G. intestinalis* was assessed by means of microscopic identification of cysts using the merthiolate and iodine concentration technique.[12]

Logistic regression was used for bivariate analysis. Because person-to-person transmission (household clustering) was possible, an intrafamily correlation structure was assessed and examined as a source of bias. Generalized estimation equations were developed and used in this analysis to account for autocorrelation within the data, while allowing for the use of time-dependent covariates.[13] The interpretation of the regression coefficients followed the usual conventions.

Age was analyzed as a continuous variable and the odds ratio (OR) was interpreted as the likelihood of infection compared with subjects 1 year younger.[14] Statistical analysis was performed using Stata 5.0 (Stata Co., College Station, TX).[15] A socioeconomic index was generated by factor analysis[16] of a set of variables that would indirectly allow characterization of the living conditions of the population. The variables that made up this index included ownership of the dwelling, types of flooring and roofing, available farming commodities (e.g., tractor), crowding, and weekly frequency of meat consumption during the 2 weeks prior to the interview. Data from the population was compared with the information generated by the National Institute of Statistics and Geography (Aguascalientes, Mexico).[17]

Wastewater samples were tested for fecal coliforms and *Giardia* sp. cysts. For fecal coliforms, the technique used was the most probable number. Confirmation was made using fecal coliform fermentation medium at 44.5°C, as recommended by the American Public Health Association.[18] *Giardia* sp. cysts were tested by a membrane filtration and concentration technique. Wastewater samples were obtained with a pump from selected canals (plastic flow controller, 15–35 L/min) previously subjected to chlorination. Samples were collected in polypropylene plastic jars and transported in an ice pack to the laboratory, where they were filtered (1 μ fiber membrane; United Filters, Houston, TX). To enhance the sensitivity of the method, increasing volumes of water were filtered, depending on the source and turbidity of the sample. Debris was removed by flotation procedures; membranes were rinsed and read in a Sedwick-Rafter (Manches-

TABLE 1
Sociocultural characteristics of the Mezquital Valley in Mexico, 1993

	Control group	Reservoir effluent	Untreated wastewater
Individuals	2,344	2,147	2,257
Households	470	441	556
Extreme poverty	17.5	49.5	9.3
Crops cultivated (%)			
Maize	77.5	87.1	64.0
Beans	9.9	4.0	5.0
Chilies	0.0	1.3	7.0
Fodder (alfalfa)	0.0	4.1	11.7
Vegetables	9.0	1.4	1.2
Other (e.g., oats, wheat)	3.6	2.1	10.9
Drinking water supply (%)			
Bottled	3.1	2.6	4.2
Piped inside	7.9	8.9	5.8
Piped outside	82.6	74.6	87.9
Well	2.7	3.3	0.0
Public tap	3.2	8.9	2.1
Other (e.g., trunk)	0.5	1.7	0.0
Feces disposal facilities (%)			
None (e.g., open air)	50.0	53.9	56.1
Flush toilet	29.2	30.2	32.9
Latrine	15.0	9.9	7.5
Septic tank	5.8	6.0	3.5

ter, United Kingdom) chamber using iodine staining for microscopic counting of the cysts (100× and 400×).[19]

RESULTS

A total of 9,088 individuals participated in the study and their general characteristics are shown in Table 1. Seventy-five percent (6,750) provided epidemiologic and stool samples. Extreme poverty affected approximately 50% of the population from the RW group, whereas it only affected 9.3% in the UW group and 17.5% in the CTRL group. During the rainy season, the most frequently cultivated crop was maize (64% in households from the UW group, 87% in the RW group and 77.5% in the CTRL group). Fodder (alfalfa) was detected mostly in irrigated villages (11.7% and 4% in the UW and RW groups, respectively), but not in the CTRL group. Cultivation of chilies was also reported mostly by families from irrigated communities (7.0% and 1.3% in the UW and RW groups, respectively), but not in the CTRL group, in which most (9%) vegetables were found. Most

TABLE 3
Giardia intestinalis infection rates (%) by age group, in the Mezquital Valley in Mexico, 1990

Age group (years)	Control group	Reservoir effluent	Untreated wastewater
Individuals	2,344	2,147	2,257
<1	2.1	2.1	2.8
1–4	19.6	20.8	20.8
5–9	13.6	17.1	14.7
10–14	9.0	11.5	10.8
15–19	5.3	8.8	5.1
20–29	2.4	6.9	4.0
30–39	5.6	6.6	2.5
40–49	1.5	4.9	4.2
50–59	2.9	5.0	4.0
≤60	3.6	4.2	1.5

households got drinking water from taps located in the yard of their dwelling (87.9% in the UW group, 74.6% in the RW group, and 82.6% in the CTRL group). The highest proportion of individuals getting water from public taps was in the RW group (8.9%), followed by the CTRL (3.2%) and UW (2.1%) groups. Defecation outdoors was a common practice (50–56%), while flush toilets were used by approximately one-third of the population.

Data on retention time showed that wastewater was stored more than 2 months in each reservoir, and up to 6 months during the winter. Table 2 shows that untreated wastewater contained high concentrations of fecal coliforms (10^8/100 ml) and *Giardia* sp. cysts (125–300 cysts/L). Lower concentrations of these water quality indicators were detected in samples from the effluent of the reservoirs (10^1–10^4 fecal coliforms/100 ml, and ≤ 5 *Giardia* sp. cysts/L).

Table 3 summarizes the age-related prevalence of *G. intestinalis* infection. Children ≤ 1 year of age had a low prevalence of infection (3%) compared with those 1–4 years of age in all three exposure groups (20%). Lower rates of infection were detected in older individuals. The prevalence of infection was higher in individuals in RW group (10.9%), followed by the UW and the CTRL groups (8.1% and 7.8%, respectively). No excess risk of infection with *G. intestinalis* was detected in individuals from the UW group compared with the controls (adjusted OR = 1.07) (Table 4). Similar results were observed when the RW and CTRL group were compared (OR = 1.22). Individuals from older age groups had a lower risk of infection than younger individuals (ad-

TABLE 2
Microbiologic quality of wastewater in the Mezquital Valley in Mexico, 1990

	Central outlet*	Tula River†	Influent reservoir I†	Central canal‡	Effluent reservoir II§
Number of samples	6	6	6	6	7
Fecal coliforms/100 ml					
Mean	1.19×10^8	7.62×10^7	1.23×10^8	9.05×10^5	7.41×10^3
SD	9.45×10^7	9.77×10^7	2.35×10^8	1.28×10^5	1.24×10^4
Maximum	2×10^8	2×10^8	6×10^8	3×10^6	3×10^4
Minimum	6×10^6	9×10^5	3×10^6	4×10^4	2×10^1
Giardia sp. cysts/L, range	125–300	50–95	ND¶	ND¶	≤5

* Untreated wastewater.
† Diluted wastewater.
‡ Effluent, first reservoir.
§ Effluent, second reservoir.
¶ ND = no data.

TABLE 4
Risk factors for *Giardia intestinalis* infection in the Mezquital Valley in Mexico, 1990*

Factor	Infected individuals	(%) positive	No. examined	OR	95% CI	*P*
Exposure group						
Control	183	(7.8)	2,344	1		
Reservoir effluent	234	(10.9)	2,147	1.22	(0.94–1.58)	0.13
Untreated wastewater	184	(8.1)	2,257	1.07	(0.84–1.36)	0.55
Age†	601	(9.0)	6,748	0.96	(0.96–0.97)	0.00
Length of exposure/agriculture						
Less than 1 year	449	(9.0)	4,998	1		
1–4 years	83	(13.8)	602	1.35	(0.99–1.82)	0.05
5 years and more	69	(6.0)	1,148	1.16	(0.81–1.67)	0.40
Activities in the field						
Cattle raising	534	(9.6)	5,575	1		
Planting	45	(4.6)	969	0.58	(0.39–0.88)	0.01
Weeding	22	(10.8)	204	0.83	(0.50–1.38)	0.48
Source of vegetables						
Local shop	594	(8.8)	6,710	1		
Mexico City market	7	(18.4)	38	2.49	(1.00–6.17)	0.04
Store drinking water						
Protected recipients	586	(8.8)	6,637	1		
Unprotected tanks, bucket	15	(13.5)	111	1.76	(0.95–3.23)	0.06
Basic sanitation						
Flush toilet or latrine	236	(7.6)	3,080	1		
No facilities	365	(9.9)	3,668	1.19	(0.97–1.45)	0.08

* OR = odds ratio; CI = confidence interval.
† Continuous variable.

justed OR = 0.96). Individuals with the longest time of exposure to agricultural activities (5 years or more) had the lowest prevalence of infection (6%) compared with those who experienced 1–4 years of exposure time (13.8%, OR = 1.35). When agricultural-specific activities were included in the analysis, individuals who were involved in grazing and weeding had a higher prevalence of infection (9.6% and 10.8%, respectively) than those involved in seeding and planting (4.6%, OR = 0.58). In addition, individuals from households who purchased vegetables from the market in Mexico City had a higher prevalence of infection with *G. intestinalis* than those who tend to patronize local shops (OR = 2.49). Individuals from households with unprotected tanks and buckets to store their drinking water had a higher prevalence of infection than those with covered containers (OR = 1.76). The prevalence of infection was higher in individuals from households without basic sanitation than in those with a latrine or flush toilet (9.9% and 7.6%, respectively).

DISCUSSION

This study detected no increased risk of infection with *G. intestinalis* in individuals who used untreated wastewater. Long periods of hydraulic retention and partial improvement of wastewater quality did not reduce the health risk. Infection rates in individuals from the RW group were higher than those in the UW and CTRL groups in most age groups. The highest prevalences of infection with *G. intestinalis* were detected in 1–4-year-old and school age children. The rates of infection decreased in older subjects after adjusting for exposure to irrigation. Individuals with the longest period of exposure time to agricultural duties (≥ 5 years) had a lower prevalence of infection compared with those with shorter exposure. Furthermore, individuals involved in grazing cattle or weeding (drier duties) showed higher infection rates than those performing hand-mud activities (e.g., planting). This may reflect host characteristics (not measured in this study), e.g., passive immunity (breast-feeding) and behavior (i.e., weaning habits, person-to-person transmission) rather than excess risk from wastewater exposure. Individuals from households in which vegetables were purchased at the city's market had a higher prevalence of infection than those who used local shops.

Associations were found with several known risk factors such as individuals from households with lower standards of storing drinking water and without facilities for disposal of feces. This may indicate fecal-oral transmission and contamination of drinking water with *G. intestinalis.* Similar results were reported in Egypt[20] and rural Lesotho.[21] This pattern suggests that socioeconomic and cultural characteristics not identified in this study may contribute to transmission in some villages. The findings are similar to the overall picture observed in this setting reported from India,[9] and do not suggest a waterborne outbreak.

The potential limitations of this study deserve comment. Microscopic examination of *G. intestinalis* cysts in stools may be less sensitive than ELISAs or duodenal aspirates. Thus, some individuals may have been incorrectly classified as negative for infection with *G. intestinalis.* Nevertheless, it is unlikely that our findings reflect a bias in isolation rates since the same laboratory tests were used for all 3 groups.[22,23] In fact, detection rates for infection with *G. intestinalis* in this study were similar to those in previous studies of shanty towns in Mexico City.[24] In addition, data that described wastewater quality must be interpreted with caution since the methods for detecting *Giardia* sp. cysts in water samples are less reliable than those currently available.

Despite widespread practice of wastewater reuse, gaps in knowledge of the risk of giardiasis limit our ability to make definite recommendations. Nevertheless, it should be stressed that setting water quality guidelines without consideration of the epidemiology of intestinal parasitic infections and cultural conditions will contribute to unregulated agricultural practices and risk of disease. Protective measures for children against protozoan infections may consist of providing primary health care and health education, fostering breast-feeding, safe weaning practices, disinfection of vegetables, safe drinking water containers, and domestic sanitation.

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Authors' addresses: Enrique Cifuentes, Mariana Gomez, Martha Maria Tellez-Rojo, Instituto Nacional de Salud Publica, Av. Universidad 655, Sta. María Ahuacatitlan CP 62508, Cuernavaca Mor., Mexico. Ursula Blumenthal, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT, London, United Kingdom. Isabelle Romieu, Centers for Disease Control and Prevention, Mailstop F-46, 4770 Buford Highway, Atlanta, GA 30341-3724. Guillermo Ruiz-Palacios, Department of Infectology, National Institute of Nutrition, Vasco de Quiroga 15, Tlalpan 14000, Mexico. Silvia Ruiz-Velazco, Instituto de Matematicas Aplicadas a Sistemas, Universidad Nacional Autonoma de México, Ciudad Universitaria, Copilco 04320, Mexico.

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