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Giardia duodenalis infection and wastewater irrigation in Pakistan

Jeroen H.J. Ensink [a,b,*], Wim van der Hoek [c], Felix P. Amerasinghe [c]

[a] *International Water Management Institute (IWMI), Pakistan, Iran and Central Asia Regional Office, 12 km Multan Road, Chowk Thokar Niaz Baig, 53700 Lahore, Pakistan*
[b] *Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK*
[c] *IWMI, P.O. Box 2075, Colombo, Sri Lanka*

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Summary The risk of *Giardia duodenalis* (*Giardia*) infection in farmers using untreated wastewater in agriculture was investigated in the city of Faisalabad, Pakistan, through a cross-sectional study. The study found a significantly increased risk of (asymptomatic) *Giardia* infection in wastewater farming households when compared with farming households using regular (non-wastewater) irrigation water (OR 3.3, 95% CI 2.5—4.4). Textile labourers who were employed in the city of Faisalabad but who lived in the same village as the wastewater farmers showed a risk of *Giardia* infection in between that of wastewater and non-wastewater farming households (OR 2.4, 95% CI 1.9—3.1). This study suggests that exposure to wastewater with high *Giardia* concentrations carries an increased risk for (asymptomatic) *Giardia* infection.

1. Introduction

There is sufficient epidemiological evidence that the agricultural use of untreated urban wastewater leads to an increased risk of infection with intestinal helminths among exposed farm workers and their families and consumers of crops that are normally eaten uncooked (Shuval et al., 1986). To a lesser extent the same applies to intestinal bacterial and viral infections (Blumenthal and Peasey, 2002). By contrast, there is no clear evidence that infection with *Giardia duodenalis* (*Giardia*) or other protozoan parasites is associated with wastewater use in agriculture. A large study in Mexico among farming families using wastewater with varying concentrations of *Giardia* cysts found no increased risk as a result of the use of untreated or partially treated wastewater (Cifuentes et al., 2000). This finding was confirmed by smaller studies in India (Sehgal and Mahajan, 1991) and the Southern Punjab, Pakistan (Feenstra et al., 2000a).

The present study was part of a larger research project in Faisalabad, Pakistan, which aimed to quantify both risks and benefits of wastewater irrigation. We previously reported

* Corresponding author. Present address: Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.
Fax: +44 20 7580 9075.
E-mail address: jeroen.ensink@lshtm.ac.uk (J.H.J. Ensink).

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an increased risk of hookworm infection among wastewater farmers in Faisalabad (Ensink et al., 2005). We now present the results of a cross-sectional study on the risk of *Giardia* infection among farming families using untreated wastewater for irrigation.

2. Materials and methods

2.1. Study area and population

Faisalabad city had a population of over 2 million people at the time of study. An estimated two-thirds of the city's untreated wastewater was used for irrigation at more than nine sites in and around the city (Ensink et al., 2004). One of the largest wastewater irrigated sites was situated next to the city's only wastewater treatment plant. The three villages situated in the middle of this site were selected for this cross-sectional study. In addition, a village situated 2 km away was selected where farmers did not have access to wastewater but used water diverted from one of the rivers, sometimes supplemented by groundwater, for irrigation. The main environmental and demographic characteristics of the study site and study population in Faisalabad, Pakistan have been described (Ensink et al., 2005).

The study had a cross-sectional design. Following an initial census, three major exposure groups could be identified: farmer households occupationally exposed to untreated wastewater; textile labourers living in the same villages as wastewater farmers; and the households of farmers not using wastewater. The mean *Giardia* spp. concentration in untreated wastewater was found to be 23 436 cysts l^{-1}, while no cysts were detected in the regular irrigation water (IWMI, unpublished data). As a result of the *purdah* system of female seclusion, only adult males were selected, while children (male and female, between 2 and 12 years of age) of selected adults were also included.

Sample size calculations were based on estimated hookworm prevalence in the three exposure groups (Ensink et al., 2005).

2.2. Data collection

Fresh stool samples were collected in standard 50-ml plastic containers labelled with individual ID numbers in the period August 2002—July 2003. The formalin—ether sedimentation method was used in Parasep® (DiaSys Europe Ltd, Wokingham, UK) faecal parasite concentrator tubes to determine protozoal infection. The tubes were centrifuged at 1000 rpm for 10 min, after which fat and formal water were drained off. Three slides of sediment were analysed in iodine under a 10×40 magnification for the presence of *Giardia* and other protozoa. In order to be able to distinguish clinical cases from asymptomatic cases, all participants were asked at the time of sample collection whether they had experienced an episode of diarrhoea during the last 7 d. Diarrhoea was defined as three unformed stools in one 24-h period (WHO, 1993). The main caregiver, in general the mother or grandmother, was asked about diarrhoea episodes in children.

Data on possible confounding variables were collected through a questionnaire survey and direct observations. Sanitation- and hygiene-related characteristics were: type of drinking water supply; ownership of a motor pump (reflecting good availability of water for domestic use); and presence or absence of a latrine. Type of house construction was used as a proxy of socio-economic status.

2.3. Data analysis

Analysis was done at the level of the individual. Children were assigned the same exposure as their fathers. Both adults and children were analysed as a single group, as initial data exploration showed no difference in prevalence between the age groups. Statistical analysis was conducted using STATA 7.0 (Stata Corp., College Station, TX, USA). Univariate logistic regression analysis was done with presence or absence of *Giardia* infection as the outcome to explore the impact of wastewater irrigation and other risk factors. Only factors significantly ($P < 0.05$) associated with *Giardia* infection were fitted into a multivariate logistic regression model.

2.4. Ethical considerations

Ethical clearance for the study was obtained in October 2001 from the Institute of Public Health, Lahore, Pakistan. Meetings were held in all four communities to explain the purpose of the planned study. Individual consent was obtained during the census and baseline household survey. Individuals with both a positive stool sample and diarrhoea episode in the week of sample collection were treated with a single dose of tinidazole (Fasigyn, Pfizer, New York, NY, USA), according to age and body weight.

3. Results

3.1. Prevalence of *Giardia* and diarrhoea

The overall prevalence of *Giardia* was high, with 67.2% (1145/1704) of the total study population infected. Only 3.7% (63/1704) of the population reported an episode of diarrhoea in the week before sample collection, indicating that the large majority of detected infections were asymptomatic cases of *Giardia*. Only 2.8% (47/1704) of the total study population was found to be positive for *Giardia* and also to have suffered from diarrhoea in the week before the stool sample collection. The prevalence of *Giardia* and diarrhoea among wastewater farming households and textile labourer households was much higher than among regular farming households (Table 1).

3.2. Risk factors

In univariate analysis, with all three exposure groups combined, the use of publicly recognized defecation sites or the absence of a toilet inside the household was associated with an increased risk of *Giardia* (OR 1.6, 95% CI 1.3—2.1), while the presence of a motor pump and consequently a higher per capita water use was associated with a lower risk of *Giardia* infection (OR 0.6, 95% CI 0.5—0.8). Poor house construction (brick wall, mud plaster and tiled or thatched roof) compared with good house construction (fully concrete)

Table 1 Prevalence of *Giardia* and diarrhoea by exposure group (adult males and their 2—12-year-old children)

	n	*Giardia duodenalis* (%)	Diarrhoea (%)	*Giardia* + diarrhoea (%)
Regular farmers	478	49.2	1.9	1.3
Textile labourers	742	72.1	4.0	2.8
Wastewater farmers	486	77.4	5.2	4.1
Chi-squared test for trend		($\chi^2 = 86$, $P < 0.0001$)	($\chi^2 = 7.1$, $P < 0.008$)	($\chi^2 = 7.3$, $P < 0.007$)

Table 2 Risk of *Giardia* infection, among wastewater and regular farming households and textile labourer households

	n	Odds ratio[a]	(95% CI)	*P*-value
Regular farmers	478	1.0		
Textile labourers	742	2.4	1.9—3.1	<0.001
Wastewater farmers	486	3.3	2.5—4.4	<0.001

[a] Controlled for house construction and age.

was associated with a significantly increased risk of *Giardia* infection (OR 1.7, 95% CI 1.4—2.2).

In a multiple regression model with all variables included, only wastewater exposure group and house construction (reflecting socio-economic status) were significant. The final model showed a better fit when age was included. Table 2 shows the risk of wastewater exposure controlled for house construction and age.

4. Discussion

This study showed an increased risk of (asymptomatic) *Giardia* infection among wastewater farming households and textile labourer households living in the close vicinity of wastewater-irrigated fields. Diarrhoea prevalence was also greater among wastewater farmers and textile labourers, but the study did not establish whether this was due to *Giardia* or any other pathogen.

A study in Brazil identified the visible presence of sewage near the household as a risk factor for *Giardia* infection (Prado et al., 2003). In our study the selected wastewater villages were completely surrounded by agricultural fields, and as a result textile labourers on their way to work or engaged in social activities, such as playing cricket, would be expected to be in daily contact with wastewater-contaminated soil, crops and/or water, which could explain their increased risk of infection. Children of both wastewater farmers and textile labourers were often observed playing in wastewater-irrigated fields, while children of wastewater farmers additionally tended to accompany and help their parents during agricultural activities.

It could be argued that this increased risk was attributable to living in the close vicinity of a wastewater treatment plant. However, studies into the possible dispersion of microorganisms through wastewater aerosols showed no excess risk for enteric diseases (Fattal et al., 1986; Shuval et al., 1989). The waste stabilization pond (WSP) in Faisalabad was a basic stabilization pond, without any form of aeration, and there is no past evidence that living in the close vicinity of a WSP could result in an increased risk of *Giardia* infection. The consumption of food contaminated with *Giardia* cysts could have played a role in the risk for *Giardia* infection, but data on food consumption were not collected in the present study.

4.1. *Giardia* risk and exposure to wastewater

Only a few published studies have investigated the possible association between wastewater irrigation and *Giardia* infection; this is the first study that has shown a significantly increased risk. Three of the previous studies had a small sample size and did not control for confounding variables (Feenstra et al., 2000a; Sehgal and Mahajan, 1991; Srikanth and Naik, 2004), while one study lacked a proper control group (Srikanth and Naik, 2004). The study conducted in Mexico involved 6748 individuals divided over three exposure groups — farmers using untreated wastewater, partially treated wastewater and rain water; it showed a much lower overall prevalence of *Giardia* infection compared with this study and did not report a difference in risk between the exposure groups (Cifuentes et al., 2000).

A possible explanation for the increased risk of *Giardia* infection found in Pakistan but not in Mexico could be the difference in concentration of *Giardia* spp. cysts in the irrigation water. *Giardia* spp. concentrations in untreated wastewater can range from 10 000 to 100 000 cysts l^{-1} (USEPA, 2000), and the *Giardia* spp. concentrations in Faisalabad were within this range, while the mean *Giardia* spp. concentrations in the untreated and partially treated wastewater in Mexico were only 300 cysts l^{-1} and $\leq$5 cysts l^{-1}, respectively.

Although this study was designed as a cross-sectional study with analysis at the individual level, one could criticize the fact that the exposed were living in three villages and the non-exposed in just one separate village. We felt that the chosen design was justifiable, because the communities were located very close to each other and were similar in many aspects.

4.2. *Giardia* prevalence in Pakistan

Prevalence of *Giardia* was high among adults and children in all three exposure groups. Very little information is available about the prevalence of *Giardia* in Pakistan, and therefore the findings of this study are difficult to place in a wider context. Excluding hospital-based studies, only six studies have been conducted on the prevalence and risk factors associated with *Giardia* infection in Pakistan in the last 20 years, and all these studies involved only children. Two studies found a high prevalence similar to the one found in the control village in the present study: the first was conducted in an irrigation system in South Punjab and found a 51.2% prevalence (Feenstra et al., 2000b); the second study was conducted in the peri-urban areas of Karachi and found a prevalence of 50% (Siddiqi et al., 2002). Studies in the northern areas of Pakistan showed a very low prevalence, ranging from 0.1 to 3.3% (Hussain et al., 1997; Khan et al., 1987). Two studies conducted in low-income villages around Islamabad showed a prevalence ranging from 18.3 to 23.7% (Ahmed and Maqbool, 1988; Malik et al., 1993).

4.3. *Giardia* infection and diarrhoea

The present study showed a very high prevalence of *Giardia* and a relatively low prevalence of diarrhoea in the three exposure groups. While it is now generally accepted that *Giardia* can cause diarrhoea, the majority of infections might remain asymptomatic. We used the formalin—ether concentration technique. The ethyl-acetate used in the concentration technique destroys the free-moving *Giardia* flagellates but not the *Giardia* cysts. We could therefore not detect *Giardia* flagellates, which would be present in individuals with symptomatic Giardiasis.

4.4. Conclusions and recommendations

Untreated wastewater irrigation in Faisalabad is associated with an increased risk of hookworm (Ensink et al., 2005) and *Giardia* infection in occupationally and environmentally exposed households. Wastewater treatment to the water quality levels recommended by WHO would reduce the health risks associated with wastewater irrigation. However, in Faisalabad farmers opted to use untreated wastewater because of its reliability and nutrient value, even though irrigation water of better quality was available from the WSP.

Regular treatment with anthelminthic medication has been suggested as a suitable additional method to reduce health risks from wastewater use. However, the regular treatment of at-risk populations with antigiardial drugs cannot be recommended because of the very rapid reinfection in hyper-endemic communities (Gilman et al., 1988). *Giardia* is associated with poor water availability and sanitation, and therefore a suitable risk reduction measure would be to extend the municipal water supply and sewerage system to the studied villages, thereby increasing the general availability of domestic water and thus improving hygiene and sanitation in the villages.

Conflicts of interest

The authors have no conflicts of interest concerning the work reported in this paper.

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