Giardia duodenalis infection and wastewater irrigation in Pakistan

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Received 4 July 2005; received in revised form 24 August 2005; accepted 26 August 2005
Available online 5 December 2005

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.

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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...
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untreated wastewater was found to be 23,436 cysts l−1 using wastewater. The mean concentration in untreated wastewater was used for irrigation at more than nine sites in and around the city (Ensink et al., 2005). 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 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 formal 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 responsible for all 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 absence or presence 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)
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 farmers were completely surrounded by agricultural communities, while textile farmers were located very close to each other and were similar in many aspects.

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.

### 4.1. 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 ≤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

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#### Table 1

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

<table>
<thead>
<tr>
<th>Exposure Group</th>
<th><em>Giardia duodenalis</em> (%)</th>
<th>Diarrhoea (%)</th>
<th><em>Giardia</em> + diarrhoea (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular farmers</td>
<td>478</td>
<td>49.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Textile labourers</td>
<td>742</td>
<td>72.1</td>
<td>4.0</td>
</tr>
<tr>
<td>Wastewater farmers</td>
<td>486</td>
<td>77.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>

**Chi-squared test for trend**

- ($\chi^2 = 86.0, P < 0.0001$)
- ($\chi^2 = 7.1, P < 0.008$)
- ($\chi^2 = 7.3, P < 0.007$)

#### Table 2

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

<table>
<thead>
<tr>
<th>Exposure Group</th>
<th>$n$</th>
<th>Odds ratio$^a$</th>
<th>(95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular farmers</td>
<td>478</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Textile labourers</td>
<td>742</td>
<td>2.4</td>
<td>1.9–3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wastewater farmers</td>
<td>486</td>
<td>3.3</td>
<td>2.5–4.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Controlled for house construction and age.
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 anthelmintic medications 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.

Acknowledgements

The first two authors are sad to report the untimely death of friend and co-author, Felix P. Amerasinghe. We gratefully acknowledge the financial support from the German Federal Ministry for Economic Cooperation and Development (BMZ) for IWMI research on the use of wastewater in Pakistan. We thank M. Mukhtar, Nyla Tabassum, Tariq Mahmood, M. Saleem, Aqela Naeem and Farah Iheedd for their dedication to the fieldwork and data management.

References


