Wastewater Quality and the Risk of Intestinal Nematode Infection in Sewage Farming Families in Hyderabad, India

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Abstract. Use of sewage or wastewater in agriculture is becoming increasingly common as a result of a global water scarcity. Intestinal nematode infections have been identified as the main health risk associated with this practice. To protect consumer and farmer health, the World Health Organization (WHO) has established an intestinal nematode water quality standard. However, because of a lack of well-designed studies, the validity of this guideline is questioned. This report presents the findings of a study on the risk of intestinal nematode infections in farming families occupationally exposed to untreated and partially treated wastewater in Hyderabad, India. The study found an increased risk of hookworm (odds ratio [OR] 3.5, 95% confidence interval [CI] = 2.2–5.5), Ascaris lumbricoides (OR = 5.3, 95% CI = 2.0–14), and Trichuris trichiura (OR = 5.6, 95% CI = 1.8–18) infection when untreated wastewater (150 intestinal nematode ova/liter) was used for crop production. Use of partially treated wastewater (28 intestinal nematode ova/liter) was only associated with an increased risk (OR = 3.2, 95% CI = 1.2–8.6) of A. lumbricoides infection. The findings of the study suggest that the current WHO intestinal nematode guideline of 1 ova/liter is sufficient to protect farmer health.

INTRODUCTION

In the next 25 years, the world population is expected to grow by almost two billion persons, a growth that will predominantly take place in urban centers in developing countries. As a result of this unprecedented growth, fresh water will become increasingly scarce and is expected to be the primary constraint for increased food production. The use of (treated) wastewater for crop irrigation has been suggested as one of the possible ways out of a looming water crisis. The construction, operation, and maintenance costs of wastewater treatment plants are high and, the reality is that in many developing countries wastewater is used without any form of treatment.

The use of wastewater poses a number of health risks. Predominant among these is the risk of intestinal helminth infection. A review by the World Health Organization (WHO) identified several epidemiologic limitations in past studies on wastewater use in agriculture, which means that credible evidence to guide policy is lacking. These limitations include one or more of the following: no inclusion of a control group of unexposed farmers; no control for confounding variables; absence of test results and helminth ova concentrations in the water used for irrigation were not assessed. Moreover, few studies quantified intensity of infection, a key determinant of transmission intensity and morbidity risk. Finally, most studies focused almost exclusively on Ascaris lumbricoides and/or Trichuris trichiura, whereas hookworm often exhibits the strongest association with (irrigated) agriculture.

This report presents the findings of a cross-sectional study conducted in three wastewater quality zones in and downstream of the city of Hyderabad, India. The primary objective was to investigate the risk of hookworm infection as a result of occupational exposure to wastewater with varying hookworm concentrations.

METHODS

Study area. The city of Hyderabad (78.47°E, 17.45°N) is located on the Deccan plateau in southern India and has an estimated population of 6.8 million. The city's wastewater is disposed of untreated into the Musi River from where it is used for irrigation. In the city, an estimated 250 households use wastewater from the river to irrigate approximately 500 hectares of land. Downstream of Hyderabad, with the help of weirs (small overflow-type dams), water from the Musi River is used to irrigate 3,100 hectares of agricultural land.

Water quality. A water quality survey was undertaken on the Musi River, which showed typical properties of domestic sewage with high Escherichia coli and helminth concentrations. Water quality improves dramatically with increasing distance from Hyderabad. This finding led to the formulation of the hypothesis that with increased distance from the city lower hookworm prevalence could be expected in farmers occupationally exposed to Musi River water.

Study population. To test the hypothesis, a yearlong water quality survey was conducted and with the help of this survey three water quality zones were identified along the river (Figure 1). Zone A was situated in the center of Hyderabad. Water quality was here classified as untreated wastewater with a mean hookworm ova concentration of 76 ova/L. Zone B was situated in the peri-urban zone of Hyderabad. Farmers used water that was diverted from the first weir on the Musi River; with a mean hookworm ova concentration of 15 ova/L, water was defined as partially treated wastewater. Zone C is a rural zone where farmers used irrigation water free of hookworm ova; water quality was defined as river water. Each zone was separated from the downstream zone by a buffer zone in which no villages were selected. In each zone, three villages, or for Zone A neighborhoods, were randomly selected from a list of villages/neighborhoods within that zone. A population census was completed in each village/neighborhood during December 2003–April 2004.

Sample size for each of the three exposure groups was calculated for hookworm prevalence following cluster simulations for a study power of at least 80%, a 95% confidence interval (CI), and a compliance of 80%. An equal number of households per cluster (i.e., village or neighborhoods) were
selected to maximize the power of between-cluster comparison. This resulted in a required sample of 580 (adult) persons. On the basis of the census, it was estimated that approximately 30 households per cluster were required to meet the calculated sample size.

Households, defined as persons sharing food from one kitchen, were randomly selected from the census list. To ensure that the selected household was involved in agriculture and exposed to the water quality in the selected zone, a brief checklist was used. The aim of this checklist was to exclude farmers working as casual laborers in other water-quality zones and to exclude landowners who had agricultural laborers carrying out all agricultural activities for them. All members of the household greater than two years of age were assigned an identification number.

Data collection. Baseline data regarding age, sex, and education of the selected persons were collected by means of a pre-tested questionnaire. A household questionnaire was designed to collect socioeconomic (type of house construction, ownership of key household items), hygiene (type of water supply and household water use), sanitation (presence or absence of a latrine), and agricultural variables (crops cultivated, type and number of cattle, land ownership). On the day of stool sample collection, an individual questionnaire was administered that dealt with personal hygiene (hand washing before eating and after defecation) and sanitation (preferred place of defecation), agricultural activities involved in, and the use of footwear.

Fresh stool samples were collected during August 2004–May 2005 and analyzed using the formalin-ether concentration technique. Stool sample containers were distributed in the morning and collected within 24 hours after distribution. Approximately 1 gram of stool was fixed in a pre-weighted 10-mL tube containing 6 mL of formalin (30% formaldehyde). The tube containing the stool sample was weighed. The sample was then transferred to a Parasep® (DiaSys Europe Ltd., Wokingham, United Kingdom) fecal concentrator tube, after which 2 mL of ethyl acetate was added. Sediment was analyzed for all nematode ova. A sample was declared negative if five slides yielded no ova. All sediment in the tube was analyzed in case a slide was found to be positive. The intensity of infection was expressed as eggs per gram (epg) of feces.

Data analysis. The association between water quality and intestinal nematode infection was explored using a pre-formulated plan of analysis. Household characteristics per zone were compared using analysis of variance analysis for continuous and chi-square test categorical variables. Two main outcome variables were identified: prevalence of hookworm and heavy hookworm infection and two secondary outcome variables: A. lumbricoides and T. trichiura infection. The WHO has suggested a threshold for what is considered a heavy infection, although this might vary with nutritional status, age, and the species of hookworm. Therefore, the upper 95th percentile of egg counts in the population was chosen as a cut-off point: defined as > 160 epg.

Univariate logistic regression analysis was carried out for all four outcome variables to identify other risk factors and confounders. Variables that showed an association of $P < 0.20$ in univariate analysis were included into a multivariate logistic regression model. Variables that in the logistic regression model showed a non-significant association ($P > 0.05$) were in a backward stepwise mode removed from the model. Risk was reported as odds ratio with corresponding 95% CI. The robustness of the model was tested using the likelihood ratio test. Because helminth infections tend to cluster within households, household was included into the multivariate logistic regression model as a random effect and standard errors were calculated using sandwich estimation. All statistical analysis was conducted in STATA version 7.0 (Stata Corporation, College Station, TX).
Ethical considerations. Ethical clearance for this study was obtained in November 2003 from the ethics committees of the London School of Hygiene and Tropical Medicine and in June 2004 from Osmania Medical College (Hyderabad, India). Written informed consent was given by all households during household selection. All persons with a positive stool sample were provided with a single dose of albendazole (Zentel®; GlaxoSmithKline, Brentford, United Kingdom) and at the end of the study a general health camp was organized for all participating households.

RESULTS

A total of 1,078 people in 251 households provided informed consent. In Zone A, insufficient eligible households were identified; therefore, an average of 23 households per cluster were selected instead of the 30 households stated in the study protocol. The reasons for this lower number of selected households were two-fold: 1) only a limited number of households that were exclusively involved in agriculture were identified, and 2) reluctance to participate in the study because of a fear that a negative outcome of the study would result in wastewater use being banned and thus a loss of subsistence. This sampling bias could potentially have affected the association between (waste) water quality and hookworm infection. However, as the results show, hookworm prevalence in farmers using untreated wastewater was high and the lower number of households participating in the study might have affected the strength, but not the direction, of the association. Compliance of those in households that had agreed to participate in the study was 93% (1,007 persons).

Population characteristics. Significant differences in socioeconomic, water, and sanitation characteristics were evident between the three exposure groups (Table 1). Farming families in Zone A had significantly lower socioeconomic status, which was reflected in lower caste, literacy rates, and house and land ownership levels relative to farmers in the other two zones. However, sanitation and hygiene, expressed by the ownership of a latrine, access to water within the household, and the use of footwear were better in Zone A than in the other two zones. Rice was the most commonly grown crop in Zone C, and fodder grass and, to a lesser extent, leafy vegetables were grown in Zone A. Zone B was a transition zone where both rice and fodder grass was cultivated. Children had no clearly defined activities in agriculture, although they did accompany their parents to the fields and were observed playing in the agricultural fields around the village.

Prevalence and intensity of intestinal nematode infection. Overall, 31.2% of persons were infected by at least one intestinal nematode infection. Of these, hookworm was the most prevalent (29.8%), followed by *A. lumbricoides* and *T. trichiura* (Table 2). The mean intensity of infection ranged from 35 epg for hookworm to 1.7 epg for *T. trichiura*. The highest intensity of infection in a person was found for hookworm (1,789 epg), followed by *A. lumbricoides* (1,333 epg) and *T. trichiura* (336 epg). A significant difference in hookworm, *A. lumbricoides*, and *T. trichiura* prevalence was found between the three zones (Table 3). Farming families in Zone A had a significantly higher prevalence of hookworm, *A. lumbricoides*, and *T. trichiura* infection. In addition, they also exhibited a significantly higher intensity of infection for all three infections relative to farming families in Zones B and C.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Zone A (n = 240)</th>
<th>Zone B (n = 354)</th>
<th>Zone C (n = 413)</th>
<th>Test statistic</th>
<th>P</th>
</tr>
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<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adult</td>
<td>64.6%</td>
<td>61.3%</td>
<td>64.6%</td>
<td>(\chi^2 = 1.1)</td>
<td>0.58</td>
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<tr>
<td>Child</td>
<td>35.4%</td>
<td>38.7%</td>
<td>35.4%</td>
<td></td>
<td></td>
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<tr>
<td>Period worked in irrigated agriculture (years), no. (SD)</td>
<td>11.4 (12.4)</td>
<td>10.3 (12.1)</td>
<td>12.1 (13.7)</td>
<td>(F = 1.9)</td>
<td>0.16</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>63.8%</td>
<td>46.6%</td>
<td>48.7%</td>
<td>(\chi^2 = 19)</td>
<td>&lt;0.001</td>
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<tr>
<td>Male</td>
<td>36.2%</td>
<td>53.3%</td>
<td>51.3%</td>
<td></td>
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<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>59.0%</td>
<td>49.7%</td>
<td>50.6%</td>
<td>(\chi^2 = 7.7)</td>
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<td>&lt; 6 years</td>
<td>14.2%</td>
<td>16.9%</td>
<td>19.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\geq 6) years</td>
<td>26.8%</td>
<td>33.3%</td>
<td>29.5%</td>
<td></td>
<td></td>
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<tr>
<td>Caste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Economically weak</td>
<td>68.3%</td>
<td>52.8%</td>
<td>33.9%</td>
<td>(\chi^2 = 163)</td>
<td>&lt;0.001</td>
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<tr>
<td>Economically strong</td>
<td>31.7%</td>
<td>47.2%</td>
<td>66.1%</td>
<td></td>
<td></td>
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<tr>
<td>Land ownership (acres), no. (SD)</td>
<td>2.6 (1.7)</td>
<td>1.4 (1.1)</td>
<td>1.9 (1.8)</td>
<td>(F = 4.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of latrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13.8%</td>
<td>42.1%</td>
<td>50.4%</td>
<td>(\chi^2 = 88)</td>
<td>&lt;0.001</td>
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<tr>
<td>Yes</td>
<td>86.3%</td>
<td>57.9%</td>
<td>49.6%</td>
<td></td>
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<tr>
<td>Preferred defecation</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Open</td>
<td>22.9%</td>
<td>47.9%</td>
<td>44.6%</td>
<td>(\chi^2 = 42)</td>
<td>&lt;0.001</td>
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<tr>
<td>Latrine</td>
<td>77.1%</td>
<td>52.1%</td>
<td>55.4%</td>
<td></td>
<td></td>
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<tr>
<td>Use of footwear</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>27.8%</td>
<td>62.3%</td>
<td>40.5%</td>
<td>(\chi^2 = 93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sometimes</td>
<td>43.3%</td>
<td>34.6%</td>
<td>48.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>29.9%</td>
<td>3.1%</td>
<td>11.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of water supply</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Outside household</td>
<td>55.0%</td>
<td>67.8%</td>
<td>100%</td>
<td>(\chi^2 = 149)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Household connection</td>
<td>45.0%</td>
<td>32.2%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soap used last time when hands were washed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>99.6%</td>
<td>93.1%</td>
<td>98.3%</td>
<td>(\chi^2 = 24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>0.4%</td>
<td>7.0%</td>
<td>1.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
When compared with farming families in Zone C, farming families in Zone B had a significantly higher prevalence of *A. lumbricoides* infection, although no significant difference in the prevalence of hookworm or *T. trichiura* was found.

**Wastewater quality and intestinal nematode infection.** Among all persons, use of untreated wastewater, when controlled for confounding variables, was associated with an almost four-fold increased risk of hookworm and heavy hookworm infection (Table 3). This result was in contrast to the use of partially treated wastewater, which showed no significant association with hookworm infection when controlled for confounding variables. Use of untreated wastewater further showed a greater than five-fold increased risk of *A. lumbricoides* and *T. trichiura* infection. Use of partially treated wastewater was associated with a greater than three-fold increased risk of *A. lumbricoides* infection. However, no significant association was found for *T. trichiura* infection when controlled for confounding variables.

**DISCUSSION**

This study found a significantly increased risk of *A. lumbricoides*, hookworm, and *T. trichiura* infection in farming communities irrigating with wastewater. The highest risks were associated with use of untreated wastewater, and use of partially treated wastewater was associated only with an increased risk of *A. lumbricoides* infection. Use of untreated wastewater was also associated with a higher intensity of infection, especially for hookworm infection.

With the exception of one study conducted in Vietnam, all past studies on wastewater irrigation have found an increased risk of at least one intestinal nematode infection in farming families occupationally exposed to the wastewater. Studies in Mexico and Morocco have associated wastewater irrigation with an increased risk of *A. lumbricoides* and/or *T. trichiura* infection, and studies on the Indian subcontinent associated wastewater irrigation with an increased risk of hookworm, and to a lesser degree *A. lumbricoides* infection. The current study corroborates findings of all past studies and is the first that found an increased risk for three intestinal nematode infections.

Although extended exposure to wastewater with high ova concentrations can be expected to result in a higher intensity of infections, only one study reported this finding. Shuval et al. presented the findings of a study conducted in India in 1973, which reported a significantly higher number of medium and heavy hookworm infections in farmers using sewage than in regular farmers. One of the study sites in the 1973 survey corresponded with the urban sites chosen for this survey. The 1973 survey reports a similar hookworm prevalence (59% versus 55%), although a much higher *A. lumbricoides* prevalence (29% versus 10%). However, because no information was provided on water quality and/or hygiene and sanitation facilities, it is impossible to determine whether this lower prevalence was the result of better water quality or because of improvements in hygiene and sanitary conditions.

The results of this survey can help evaluate the current WHO guidelines on the use of wastewater in agriculture. To protect both farmer and consumer health, the WHO in 1989 recommended a maximum nematode concentration of < 1 ova/L for the unrestricted use of wastewater in agriculture. The validity of this water quality standard has previously been questioned, and it has been suggested that the water quality standard was too strict and could be increased to 10 ova/L. However, few studies have quantified the concentration of ova in wastewater used for irrigation, often just referring to irrigation water as either untreated, partially treated, treated, or clean. Other studies that quantified ova concentration failed to differentiate between different helminth species or ova concentrations in wastewater were too high.
suggest that the current WHO guideline value of (> 700 ova/L) to provide additional evidence to the discussion.\textsuperscript{23}

As a result, there is one study that has investigated the association between ova concentration in wastewater and the risk of nematode infections. Research in Mexico showed no increased risk of infection in adult farmers who worked with wastewater with ova concentrations < 1 ova/L of wastewater than in farmers involved in rain-fed agriculture, although the same study showed an increased risk of infection in their children.\textsuperscript{19} Children often accompany their parents to the fields or play in the fields surrounding the villages and the findings of the study in Mexico resulted in a footnote to the recently updated WHO wastewater guidelines, which now recommend an intestinal nematode guideline of < 0.1 ova/L when children less than 15 years of age are exposed.\textsuperscript{3}

The WHO has set a single nematode water quality standard. However as Table 3 shows, \textit{A. lumbricoides} and \textit{T. trichiura} in this study showed a significant association with use of wastewater at concentrations ranging from 3 to 12 ova/L, and hookworm at similar ova concentrations in wastewater showed no increased risk of infection. This finding would suggest that the WHO guidelines should differentiate between hookworm on the one hand and \textit{A. lumbricoides} and \textit{T. trichiura} on the other hand. This would mean that a more lenient water quality standard can be set when hookworm is the only infection associated with wastewater irrigation, for example in Faisalabad, Pakistan.\textsuperscript{23} The findings would further suggest that the current WHO guideline value of ≤ 1 ova/L is of the correct order of magnitude. The fact that no increased risk of \textit{T. trichiura} was detected when wastewater with a concentration of 0.3 ova/L was used would suggest that the proposed nematode guideline of ≤ 0.1 ova/L when children are exposed to wastewater\textsuperscript{3,19} is too strict.

In this study, examination of the associations between use of wastewater of a particular quality and intestinal nematode infections was hampered by several epidemiologic difficulties, many of which are inherent to the practice of wastewater in agriculture. The first problem is the identification of a suitable control group of clean water farmers. The high cost of municipal water, combined with the high water demand by agricultural crops, means that an urban farmer is almost always synonymous with a farmer that uses sewage and that clean water farmers can not be identified within the urban setting. By selecting rural farmers, an urban-rural bias is introduced. Both \textit{A. lumbricoides} and \textit{T. trichiura} infection are associated with crowded environments and tend to be higher in urban populations than in rural populations.\textsuperscript{29} In addition, wastewater farmers are often the urban poor without well established land or water rights. This was clearly shown in this study where urban farmers were of lower socioeconomic status than rural farmers. This finding could indicate that the association between \textit{A. lumbricoides} and \textit{T. trichiura} and wastewater irrigation is not as strong as is suggested in this survey.

An additional difficulty is caused by the characteristics of wastewater and its geographic isolated use. The one-to-one comparison between geographically distinct areas (untreated wastewater versus river water and partially treated wastewater versus river water), which was adopted in this study, and all other wastewater studies is in principle statistically flawed.\textsuperscript{30} However, inclusion of additional random clusters is complicated by the fact that wastewater quality, and especially pathogen concentrations, will vary widely from neighborhood to neighborhood, and as a result wastewater quality used at different sites is not uniform. Moreover, wastewater irrigation can only occur where sewage is disposed of; this finding means that it is geographically constrained to a very limited number of sites and in the case of small cites often only one. In Hyderabad, relatively uniform water quality was found because all wastewater ended up in the Musi River where it mixed and from where it was used for agricultural purposes. This result enabled selection of three independent clusters (villages/neighborhoods) per water quality zone to address the one-to-one comparison problem, although from a statistical point of view, this is only a marginal improvement.

The WHO water quality standards are based on the best available epidemiologic evidence and on the principle that there should be no additional cases of disease as a result of wastewater use in agriculture. This approach has made the WHO guidelines vulnerable to criticism, especially from supporters of the United States Environmental Protection Agency guidelines, which advocate a much stricter water quality standard on the basis of the principle that there should be no additional risk as a result of wastewater use in agriculture.\textsuperscript{31} This discussion seems trivial but for developing countries a water quality standard that is too strict will either mean needless investments in wastewater treatment technology or a situation, like in Hyderabad, where municipalities ignore the use of wastewater, pretending that it does not take place.\textsuperscript{4} To help strengthen the WHO guidelines, more research is needed. A potential improvement on the chosen study design, which would strengthen the association between exposure to wastewater of particular quality and nematode infection would be a re-infection study, whereby exposure to wastewater and wastewater quality will be monitored on regular intervals.

On the basis of WHO standards,\textsuperscript{3} wastewater used in Hyderabad to irrigate was unfit for crop production. The most obvious way to mitigate health risks would be wastewater treatment. However, this would require large investments in wastewater treatment technology and an even larger investment in the sewage system. Currently, all sewage pipes drain into the Musi River, and a complete reconstruction of the current infrastructure in Hyderabad is prohibitively expensive. Thus, the only option would be to ban wastewater use, and consequently the removal of farmers that use wastewater from their land. In addition to the questionable legality of such a move, it also highly undesirable because an already vulnerable group would be deprived of their sole subsistence.\textsuperscript{32} Because no crops are grown that are consumed uncooked, use of wastewater does not pose a risk to the general public, and fodder grass production is key to the dairy industry in Hyderabad,\textsuperscript{32} thus making an important contribution to overall population health in Hyderabad.

Thanks to wide availability of cheap, single-dose anthelmintic drugs, nematode infections pose a relatively easy containable risk.\textsuperscript{13} Farmers using wastewater in Hyderabad have fought several court cases over the last decades to retain access to wastewater and are thus well organized and easily mobilized. This would make twice yearly anthelmintic treatment programs in the wastewater farming communities an effective way to minimize the impact of wastewater irrigation in Hyderabad.

To help formulate national guidelines, the WHO recom-
mends epidemiologic studies with a water quality component, similar to the one presented here.33 This study provided further evidence that use of wastewater was associated with an increased risk of nematode infections. Although this study was unable to provide conclusive evidence about the validity of the current WHO wastewater nematode guideline, the findings suggest that for *A. lumbricoides* and *T. trichiura* infection the current WHO nematode guideline of ≤ 1 ova/L is appropriate but that the nematode guideline of ≤ 0.1 ova/L when children are exposed is too strict. No increased risk of hookworm infection was detected when wastewater with a mean concentration of 15 ova/L was used, which would suggest that, at least for the risk of hookworm infection, the WHO nematode guideline is too strict and that a more lenient guideline can be set if hookworm species are the predominant ova in wastewater. However more studies are needed to confirm this suggestion.

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