Evaluation of a sanitation programme using eggs of *Ascaris lumbricoides* in household yard soils as indicators

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**Summary**

Soil samples were analysed for the presence of *Ascaris lumbricoides* eggs as indicators of environmental pollution in household yards in Maputo, Mozambique, with the objective of evaluating the impact of a programme for the promotion of improved latrine construction. The locations for soil sample collection were defined by a random grid on which household activities were mapped. In addition, parasitological examinations were carried out amongst household residents. No significant difference was found between the type of latrine in use and the presence of *Ascaris* eggs in the soil or human *Ascaris* infection. Households with at least one infected person appeared more likely to have *Ascaris* eggs in the yard. It was notable that egg counts around the latrines were only slightly greater than in other areas of the yard and less than those immediately in front of the dwelling. This is taken to indicate that faecal pollution of the household environment is due more to promiscuous defecation than to poor construction or maintenance of the latrines. The findings highlight the need to complement sanitation 'hardware' with the necessary health education 'software'. *Ascaris* eggs are useful indicators but robust standardized methods are needed for their extraction from household soils.

**Introduction**

The role of sanitation in improving public health is widely recognized although its exact contribution is still disputed (Churchill 1986). Many Third World countries have given increasing attention to building sanitation infrastructures, particularly in those poorer communities where there is often no hygienically adequate means by which to dispose of human excreta. This process has, since 1980, been reinforced by the International Drinking Water Supply and Sanitation Decade. A number of new technologies such as the VIP (ventilated, improved pit) Latrine have been developed, aimed at meeting the needs of the majority of this population in an effective and affordable way. These technologies normally rely on the on-site disposal of excreta although many are planned to permit future upgrading to waterborne sewage removal (Kalbermatten et al. 1980).

The introduction of programmes to promote improved sanitation using these technologies carries with it the obligation to demonstrate that the interventions do in fact improve the health of the community—insofar as that is one of their objectives, for there are others, such as improved economy, convenience, comfort and even status.

The evaluation of such schemes is widely recognized to be difficult (WHO 1983). It is not easy to obtain detailed and reliable accounts of a community's excretion habits and direct observation is usually unacceptable. Assessment of direct health benefits is handicapped by the host of variables that intervene between excretion and infection, leading Blum and Feachem (1983) to call for more attention to be given to the intervening processes.

An important immediate objective of any sanitation technology is the containment and eventual destruction of pathogens in the environment. It is assumed that the most widely used methods—septic tank systems—will result in the containment of pathogens in the case of on-site sanitation. Feachem et al. (1983) have taken the measure of the presence of *Ascaris* eggs in soil as an indicator of the transmission, as discussed. The study described here is an attempt to ascertain the usefulness of *Ascaris* eggs in soil as indicators of the health status of households in the area.

**Materials and Methods**

**STUDY HOUSEHOLDS**

Ninety-seven households were selected from fifteen neighbourhoods in the Polana Canico market area. The households were grouped into three: those which were promoted by the programme and more rudimentary programme.
Evaluation of sanitation using eggs of Ascaris

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programmes to promote these technologies ion to demonstrate in fact improve the asfar as that is one are others, such as sience, comfort and schemes is widely 3H 1983). It is not eliable accounts of a its and direct obser-
bles. Assessment of diped by the host between excretion um and Feachem tion to be given to e objective of any e containment and eventual destruction of faecal pathogens (which may help to achieve the broader objective of reducing the prevalence of faecally transmitted diseases). The presence or absence of these pathogens in the environment should thus be a useful indicator of its efficacy. The organisms most widely suggested for this purpose in the case of on-site sanitation, due to its persistence in the environment, is Ascaris lumbricoides. Feachem et al. (1983) suggest that it be used as a measure of the pathogenicity of ‘non effluent’ wastes such as night soil, pit latrine contents, septic tank sludges etc. The detection of Ascaris eggs in soil has been used in epidemiological investigations of soil helminths and their transmission, as well as in veterinary studies (Thein-Hlaing et al. 1984; Kazacos 1983). This procedure has also been used to assess the factors predisposing to human helminth infection (Otto et al. 1931; Winfield 1937) and in a few cases specifically to evaluate the impact of health education and sanitation interventions (Ismid & Rukmono 1980).

The study described in this paper had as its objective the evaluation of the Mozambican Improved Latrine Programme using eggs of Ascaris lumbricoides in the soil of household yards as indicators. The distribution of the indicator organism in the yard was used to improve understanding of the mechanisms involved in their dispersion. Faeces of all members of the study households were also examined to provide further information in this regard. A secondary objective of the study was to ascertain the usefulness of Ascaris eggs in soil as indicators of the efficacy of on-site sanitation programmes.

Materials and methods

STUDY HOUSEHOLDS
Ninety-seven households for the study were selected from four blocks (quartierões) in the neighbourhoods (bairros) of Maxaquene and Polana Caniço in the periurban areas of Maputo. The households surveyed were divided into two groups, those with the improved latrines promoted by the programme and those with other more rudimentary traditional latrines. The two types of latrine were found in both bairros and the selection of households was made on a random basis. Socio-economic information for each household was available from a previous survey (Muller 1988). Households where pigs were kept were excluded from the study to avoid false positives due to eggs of Ascaris suum.

SOIL SAMPLES
The soil sampling was carried out during the second fortnight of February 1987. Each household yard was mapped and a two-metre square grid established whose intersections were used as sampling points as illustrated in Figure 1. The number of points per household varied between nine and 37 and depended on the size of the household yard and distribution of buildings within it. One point was always taken immediately in front of the latrine entrance. Grid points at entrances from the road and to the house were specifically referenced as such, and so were areas used for cooking, water storage and washing, rubbish disposal and chicken coops.

Approximately 30 ml of soil were taken at each point using a core sampler 2 cm in diameter. At each point, two core samples were taken. The samples were placed in 200 ml polystyrene containers, covered, and taken to the laboratory of the National Institute of Health in Maputo where they were kept at room temperature in the laboratory (25–30°C) until they could be analysed. To preserve the eggs of A. lumbricoides in their original state at time of sampling, 100 mg of sodium azide was added to each soil sample on the day it was collected and mixed thoroughly (Bundy et al. 1985).

ANALYSIS OF THE SOIL SAMPLES
Separation of eggs from the soil samples was obtained by a sequence of screening, flotation and filtration. Twenty-five millilitres of each soil sample were run through a one-litre sedimentation flask, and washed through with a detergent solution (Tween-40 0.25%). This was continued until the flask was full.

After allowing the solids to settle for 30 min, a 10 ml syringe was used to remove all the sediment from the base of the flask. This was then
transferred to two 25 ml universal tubes which were topped up using the solution from the washings. This solution was allowed to settle for 10 min. The supernatant was then decanted using a fine pipette, leaving the sediment at the bottom of the tubes.

A supersaturated magnesium sulphate solution with 5% potassium iodide was added to the tubes which were then centrifuged at 2000 r.p.m. for 5 min. The sediment was then agitated and the tubes again centrifuged to ensure the flotation of as large a proportion of eggs as possible.

To recover the eggs, water was added to the universal tubes using a No. 21 hypodermic needle bent at 90°C to wash the walls of the tubes. Once the surface of the solution had been covered with water, the entire interface zone plus an extra millilitre or two was aspirated into the syringe. This suspension was then filtered through a 25 mm diameter Nucleopore membrane filter of 12 micron pore size.

The supernatants from both universal tubes in which the filtrate from the sample had been divided were filtered through the same membrane so that the count of eggs on the membrane represented the total count from a single soil sampling point.

After filtration, the membrane filter was transferred to a glass slide, wetted, and covered with a cover slip to prevent it from drying. The slides were examined microscopically and any eggs identified and classified as new or old. Control samples were examined at regular intervals to maintain the quality of the observations.

FAECAL SAMPLES
Faecal samples were collected in plastic containers, one for each person in the household, and taken to the Laboratory at the completion of the study. The samples were examined by the standard method of Ritchie's modified faecal analysis.

Of the initial 102 faecal samples studied, 35 were positive for Ascaris lumbricoides from at least one soil sampling point. Only three were faecal samples.
Evaluation of sanitation using eggs of *Ascaris*

<table>
<thead>
<tr>
<th>Site samples</th>
<th>Number of sites in positive households*</th>
<th>Positive sites</th>
<th>Percentage of total sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latrine entrance</td>
<td>35</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Dwelling entrance†</td>
<td>44</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Road entrance‡</td>
<td>50</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Cooking area‡</td>
<td>25</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Water use‡</td>
<td>34</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Chicken coop‡</td>
<td>15</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Other sites</td>
<td>577</td>
<td>62</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>780</td>
<td>92</td>
<td>12</td>
</tr>
</tbody>
</table>

*Any household with at least one positive result was considered positive.
†Some households had more than one sampling point adjacent to these locations.
‡Not all households had clearly defined areas for these activities.

Results

Of the initial sequence of 45 households studied, 35 were found to be positive, in the sense that *Ascaris* eggs were found in the soil from at least one point. Of the subsequent 52, only three were found to be positive. The cause of this decline in the number of positive results is unclear. However, to avoid any spurious results which might result from false negatives, the latter group of 52 households was eliminated from the study.

**DISTRIBUTION OF EGGS**

The distribution of the positive sample points according to the classification of sample sites is shown in Table 1. Of all sites classified, eggs were more likely to be found adjacent to the entrance to the dwelling (23% of households, significantly more often than anywhere else ($P<0.05$)). The entrances to the latrines also showed a higher proportion of positive samples (17%) than the other sites of which 11% were positive, although this difference was not statistically significant. The mean number of eggs found at each point classified varied little (from 2.1 to 3.7) so further analysis was limited to the presence or absence of eggs rather than to egg densities.

**HUMAN INFECTION**

*Ascaris* eggs were found in 23% of the faecal samples examined. Infection was most frequent...
Table 2. *Ascaris* infection by age group and latrine type

<table>
<thead>
<tr>
<th>Age group</th>
<th>Improved latrine</th>
<th>Traditional latrine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Infected</td>
<td>%</td>
</tr>
<tr>
<td>0-4</td>
<td>26</td>
<td>4</td>
<td>15.4</td>
</tr>
<tr>
<td>5-15</td>
<td>61</td>
<td>20</td>
<td>32.8</td>
</tr>
<tr>
<td>Adult</td>
<td>63</td>
<td>11</td>
<td>17.5</td>
</tr>
<tr>
<td>All</td>
<td>150</td>
<td>35</td>
<td>23.3</td>
</tr>
</tbody>
</table>

Table 3. *Ascaris* eggs in household soil in relation to infection of household members

<table>
<thead>
<tr>
<th>Household infection</th>
<th>Persons infected in household</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Households where eggs were found in soil</td>
</tr>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>7/17 (41)</td>
</tr>
<tr>
<td>Children (0-15)</td>
<td>8/19 (42)</td>
</tr>
</tbody>
</table>

amongst the 5-15 age group (35%) followed by the adults (17.5%) with only 12% of the infants (0-4) infected (Table 2).

TYPE OF LATRINE, SOIL CONTAMINATION AND HUMAN INFECTION

Seventy-three per cent (19 out of 26) of households with improved latrines were positive (at least one soil sampling point positive) as compared with 68%, (13 out of 19) households with other types of latrines; the difference was not significant.

There was also no significant difference between the prevalence of infection among people in improved latrine households (23%) compared with those in traditional latrine households (22%). This also held when the groups were divided by age as shown in Table 2.

SOIL CONTAMINATION AND HUMAN INFECTION

There was an apparent association between the presence of at least one person infected with *Ascaris* in the household and the occurrence of a positive soil sample (Table 3). Seventy-one per cent of the households with an infected person were positive compared with 41% of those with no infected person. This result was not however statistically significant. A similar result was found when only the presence of infected children (0-15) was considered. Seventy-two per cent of households with an infected child were soil positive compared with 42% of those households without an infected child, although again the result was not statistically significant.

SOCIO-ECONOMIC DATA

Socio-economic information of relevance to the present study was extracted from the records of the previous survey. It was notable that the mean number of children per household in improved latrine households was considerably greater (3.35) than in the other households (2.26). The mean INDEC (a composite standard of living index with a range from 0 to 7) was 4.0 for improved latrine households and 3.45 for the others.

The mean INDEC of soil-positive households (3.43) was substantially lower than that of soil-negative households (4.33). There was however little difference in the mean INDEC between households with at least one member infected by *Ascaris* (4.03) and those with no person infected (3.88).

Only 28% of children in improved latrine households and 25% in other households were reported to begin using the latrine before the age of five years.

Discussion

There are two distinct concerns addressed by this study: the analysis of the impact of the improved latrine programme, and the more general application of the technique used.
PROGRAMME IMPACT

What impact may be expected from an improved latrine programme? Since the overwhelming majority (97%) of the households in the study area had some form of latrine, any impact detected would not be the difference between households with and without latrines but between households with different types of latrine. If it is to alter beneficially the health status of the household, the improved latrine must therefore be either more effective at containing the faecal pathogens or more attractive to the members of the household who are supposed to use it.

The potential of traditional latrine constructions to be foci for infection has long been a concern and this, together with practical and economic considerations, was at the origin of Mozambique's improved latrine programme as explained elsewhere (Brandberg 1983). The hazards posed by a fouled latrine, particularly in relation to the transmission of hookworm but also as a source of the dispersion of faecal pathogens to the domestic environment, are clear. However, an equally important hazard of a fouled latrine is that it will discourage potential users. Their reluctance to use it will be compounded if it is of insecure construction. Any evaluation of a latrine programme must address both these aspects, as attempted in the present study.

The distribution of Ascaris eggs in the household yards indicated to us that the latrine was not the major source of faecal pollution. Were it to be so, there would be a gradient of pollution, high close to the latrine, dropping away with distance from it. In fact, the dwelling itself appeared to be more frequently associated with Ascaris eggs and the overall level of faecal pollution in the yards was not much less than at the latrine entrance. The number of latrines where Ascaris eggs were found was too small to allow for comparison to be made between the two types in use.

The generalized distribution of Ascaris eggs in the yard points more to a problem of non-usage of the existing latrines. Here, the impact of the improved latrine might be expected to be found in a reduced occurrence of eggs in improved latrine households. However, no such relationship was found.
sample households which considerably reduced the power of the study (in sample size and in the ability to use egg densities as more informative indicators of faecal pollution than simple present/absent observations) coherent results were obtained. The relationship found between the presence of infected persons and soil contamination in their household yards, the two peaks in spatial distribution of eggs in the yards and the relationship found between a lower standard of living and an increased prevalence of soil contamination all suggest that the occurrence of *Ascaris* eggs in the yards is not a random phenomenon but related to the key study variables.

We had previously expressed reservations about the laboratory aspects of the study (Muller 1987) and the difficulties experienced bore these out. If this type of work is to be repeated, some attention must be given to developing and validating simple standard methods.

Separation of eggs from the soil and subsequent microscopic examination is a time-consuming activity and any simplifications which could be introduced would assist in the routine use of the method. Our use of membrane filtration is one such contribution. If *Ascaris* eggs are to be widely used as indicators, it will be in those countries where the new sanitation technologies are being introduced. Laboratory procedures must therefore be appropriate for their limited human and technical resources.

**Acknowledgements**

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**References**


