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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

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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 soil was taken from the container, deposited on a 212 micron screen above 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

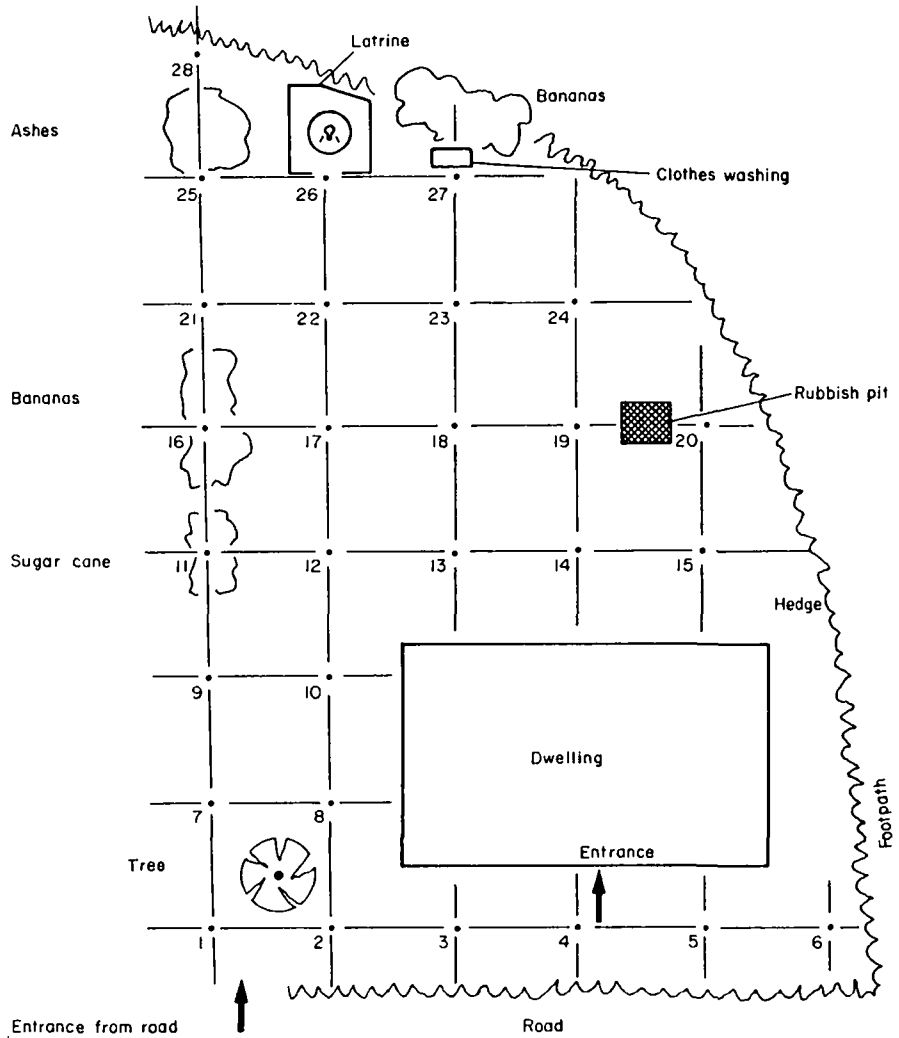

Figure 1. Bairro: Maxaquene C; quartierão: 26; house no.: 22; date: 24 February 1987.

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

Table 1. *Ascaris* eggs found according to site classification

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

*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.

with a cover slip, taking care to exclude air bubbles. The slide was then examined microscopically and any eggs present counted and classified as new, old or embryonated forms. Control samples were introduced blind at irregular intervals to maintain a check on the quality of the observations.

FAECAL SAMPLES

Faecal samples were collected using 200 ml plastic containers with screw thread tops. These were handed to the residents in each household with a label indicating the name of each person in the household. These details had been confirmed during a visit on the previous day. The samples were collected in the morning and taken to the laboratory for immediate analysis.

FAECAL ANALYSIS

The faecal samples were analysed using Ritchie's method (formol ether) which is the standard method used by the Faeces and Urine Laboratory at the National Institute of Health.

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.

The developmental classification of the eggs showed that the majority (79%) were old and probably unviable. Twelve per cent were new and not yet embryonated and 8% were embryonated.

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

Age group	Improved latrine			Traditional latrine			Total		
	No.	Infected	%	No.	Infected	%	No.	Infected	%
0–4	26	4	15.4	16	1	6.3	42	5	11.9
5–15	61	20	32.8	27	11	40.7	88	31	35.2
Adult	63	11	17.5	51	9	17.6	114	20	17.5
All	150	35	23.3	94	21	22.3	244	56	23.0

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

Household infection	Households where eggs were found in soil	%
Persons infected in household		
None	7/17	(41)
One or more	20/28	(71)
Children (0–15) infected		
None	8/19	(42)
One or more	13/18	(72)

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.

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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.

This lack of impact is hardly surprising given the fact that there were more children in improved latrine households than in the others and, more important, that most children were reported to start using the latrine only after the age of five. Since this information was obtained in a survey of household sanitary provision, it is likely to be an underestimate since the respondents would tend to bias their answers to please the interviewer. If at least 20% of the household population do not use the latrine, it is obviously unlikely that the type of latrine installed will make any difference to the faecal contamination observed.

This finding is hardly new. Otto *et al.* (1931) reached similar conclusions about the Scots-Irish descended rural dwellers of Tennessee in the USA who, despite having latrines presented heavy *Ascaris* infections. 'In such families ... the young children have the heaviest infestations and their habits are chiefly responsible for the spread of the parasite'.

The lack of impact in no way calls into question the value of the improved latrine programme. One principle of the programme, which is given tentative support by the socio-economic surveys, is that its products are easier for children to use. The sale of the latrine components also offers a valuable channel for the transmission of health education messages. This health education 'software' (*pace* Walsh and Warren (1979) and other proponents of quick technical fixes for Third World health problems) can only be effective if the corresponding 'hardware' is installed, although it may be fully implemented only after a generation.

ASCARIS EGGS AS INDICATORS

The use of *Ascaris* eggs as indicators of the effectiveness of a sanitation technology depends on the reliability of the results that can be obtained and the extent to which they can provide coherent information about faecal pollution of the environment. The failure in this study to demonstrate a beneficial impact is not unexpected and is therefore no reflection on the method itself. The current study suggests that the method may indeed be valuable, if its limitations are duly recognized. As shown above, and despite the loss of more than half the

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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