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THE MINIMISATION OF MICROBIOLOGICAL HAZARDS ASSOCIATED WITH LATRINE WASTES

D. Wheeler* and R. F. Carroll**

*Robens Institute, University of Surrey, Guildford, Surrey GU2 5XH, U.K.
**Building Research Establishment, Garston, Watford WD2 7JR, U.K.

ABSTRACT

Investigations of latrine wastes in Botswana were undertaken to verify that sludge stored in a pit latrine chamber for at least one year does not constitute an unacceptable microbiological hazard on handling or reuse. Levels of faecal bacteria, Ascaris, Taenia, Schistosoma and human enteric viruses in sludges which had been stored for periods of more than one year were compared with levels in improperly stored sludges. In all cases, the importance of long term storage was confirmed. However, it is noted that substantial sociological and educational inputs are required if latrine users are to obtain the maximum health and economic benefits of latrine sanitation and sludge reuse, particularly in cultures where there is traditionally little interest in such practices.

KEYWORDS

Sanitation; latrine; faecal waste; sludge storage; health; microbiology; parasites; faecal bacteria; enteric viruses.

INTRODUCTION

It is frequently asserted that the reduction of faecal oral infections in less developed countries depends on a multi-factorial approach to public health protection (Feachem, 1986). And in many cases the successful introduction of new sanitary facilities will necessitate a fundamental change in behaviour of the recipient population requiring substantial educational inputs. However, it has also been suggested that technical solutions may have an inherent relative value with respect to reducing disease transmission and that of all available interventions, the provision of adequate excreta disposal facilities is the most important (Feachem, 1983). Thus, many authorities have devoted particular attention to the examination of options for low cost sanitation in developing countries (Rybczynski et al., 1982).

But there are health risks as well as health benefits associated with the concentration of solid human wastes eg in latrine chambers. Not least of these is the possibility that waste containing high levels of enteric pathogens may be prematurely removed from storage, or else not treated at all before application as agricultural or aquacultural fertiliser (Blum and Feachem, 1985).

The use of human excreta as an economic resource has been reviewed in the context of social behaviour and culture (Cross, 1985). It was concluded that although cultural and religious beliefs are a constraint on the promotion of reuse in some countries, they do not constitute an insurmountable barrier. Thus it is quite possible that the practice may be extended to many more countries in future as economic and ecological arguments for the practice gain wider acceptance. If such developments do occur, it is important that an additional threat to human health is not created.

The use of raw or untreated excreta in agriculture or aquaculture has been associated with the transmission of a variety of parasitic infections, but there is little information relating to the risk of using properly stored or treated sludges (Blum and Feachem, 1985). For this reason, it is suggested that further epidemiological studies be conducted which involve multidisciplinary teams in a variety of social, economic and cultural settings.

Nevertheless, tentative minimum storage times for faecal waste have been suggested with a view to minimising the risk of excreta-related disease (Strauss, 1985). More sophisticated time-temperature relationships of microbial inactivation in sludge have also been drawn from extrapolations of survival experiments involving a variety of micro-organisms (Feachem et al., 1983). These relationships tend to confirm the recommendation that a minimum of one year storage at an ambient temperature in excess of 25°C is required to render faecal waste largely free of pathogenic organisms including the most persistent of potential pathogens such as Ascaris.

It is the purpose of this paper to report microbiological investigations conducted in Botswana which confirm the tentative guidelines for the adequate storage of latrine waste cited above. The study involved bacteriological, parasitological and virological assays of latrine waste with a view to assessing the efficacy of storage and latrine maintenance arrangements practised in an urban environment.

URBAN SANITARY PRACTICE IN BOTSWANA

In Botswana there is no tradition of using human faecal waste in agriculture or aquaculture (unlike societies of the East,eg China, it appears that most sub-saharan peoples have a positive aversion to any contact with human excreta). However, significant effort has been expended in the construction of sanitary disposal facilities which lend themselves ideally to the composting and reuse of faecal waste. Since 1979 many thousands of double chamber ventilated pit latrines have been constructed in Botswana. These latrines are based on Building Research Establishment designs for the Permanent Improved Pit (PIP) or Ventilated Improved Double Pit (VIDP) latrine (van Nostrand and Wilson 1983), but are referred to as Revised Earth Closet (RECII) latrines in Botswana.

The majority of REC II latrines have been installed in'site and services' developments in urban areas. The principle of the double pit relies on users restricting defaecation to one chamber until it is full. The chamber is subsequently sealed for a period of 1-2 years before emptying, during which time the alternate chamber is used. When the second chamber is full, the first is ready for reuse, and the cycle may be repeated. The chamber contents are removed by a purpose-designed sludge suction appliance such as the BREVAC tanker (Carroll, 1984). The design should ensure continuous use of the facility and the generation of a microbiologically safe product for disposal or reuse.

Unfortunately, the REC II latrines are not always properly used. A tendency for some house-holders to empty wastewater into latrines leads to rapid, premature filling and the creation of very aqueous raw sludges. In addition, regardless of its age and quality, latrine waste is not reused, but disposed of into waste stabilisation ponds or landfill.

METHODS

Bacteriology

Bacteriological analysis was undertaken according to a modified Most Probable Number (MPN) technique. Media were those specified in UK standard procedures (DHSS, 1982).

Sludge was weighed into sterile 25 compartment Replidishes (Sterilin, UK) in the following sequence: 5 x 0.01g, 5 x 0.1g, 5 x 1.0g, and where necessary 5 x 0.001g. Primary isolation media were Minerals Modified Glutamate Broth (Oxoid CM607 and CM607G) for total and thermotolerant coliforms, Kanamycin Aesculin Azide Broth (Oxoid CM771B) for faecal streptococci, and Buffered Peptone Water (Oxoid CM509B) for salmonellae. Secondary and confirmatory media were Brilliant Green Bile Broth (Oxoid CM031B) and tryptone water (10g/l tryptone and 5g/lNaCl) for total and thermotolerant coliforms, Kanamycin Aesculin Azide Agar (Oxoid CM591B) for faecal streptococci, and Rappaport Broth (Oxoid CM669B) and XLD Medium (Oxoid CM469B) for salmonellae. Suspect Salmonella colonies on XLD Medium were screened on Urea Agar (Oxoid CM053B) and TSI Agar (Oxoid CM277B) before further biochemical and serological confirmation (Enterotube II, Roche Diagnostica, and polyvalent 'O' and 'H'

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Primary broth media were pipetted onto sludge samples in Replidishes. Incubation temperatures were 37°C for total coliforms, 44°C for thermotolerant coliforms and faecal streptococci, and 37°C for Salmonella. Selective enrichment for Salmonella was conducted at 41.5°C and plating on XLD Medium at 37°C. Subculture and confirmation of other bacteria was undertaken at 37°C for total coliforms and 44°C for thermotolerant coliforms and faecal streptococci.

Parasitology

Parasitological analysis was undertaken by the Liverpool School of Tropical Medicine Department of Parasitology under the direction of Dr. W. Crewe. 100 ml of each sample was mixed with water and worked using more water through a coarse nylon mesh (a tea strainer) into a 1000 ml measuring cylinder. The suspended sample was left to settle overnight, and the supernatant fluid was then poured off and replaced with clean water. This process was repeated every 24 hours for a week, until the supernatant fluid was clear. The final clear supernatant fluid was discarded, and the material remaining in the cylinder (the sieved, washed and settled sludge in a small volume of water) was divided into aliquots of 5-10 ml.

An aliquot was transferred to a 50 ml centrifuge tube, and the tube was then filled with water and centrifuged at 600g for 1 minute. The supernatant water was discarded and replaced with 40 ml of flotation solution. Two flotation solutions were used, $MgSO_4$ (sp. gr. 1.275) and $ZnSO_4$ (sp. gr. 1.375). The sludge and the flotation solution were poured into a conical flask and thoroughly mixed, then returned to the centrifuge tube, allowed to stand for 5 minutes, then centrifuged at 600g for 1 minute. The supernatant solution was then poured off into a series of 15 ml centrifuge tubes.

Enough solution was poured into each tube to form a convex meniscus, and a coverslip was placed on this meniscus. The tube and coverslip were then left to stand for at least 30 minutes, and then the coverslip was lifted off the tube, placed on a microscope slide, and examined under the microscope. The slides were scanned using a magnification of x 60, and confirmation of the identity of any eggs found was made using a magnification of x 240. Fifty coverslips were examined for each of the 8 samples.

Virology

Virological analyses were undertaken according to standard techniques for sludge samples by the Welsh Water Authority under the supervision of Dr J.M. Tyler.

Sampling Programme

Fourteen samples of sludge from REC II type latrines were taken at depths of 150-1500mm. Because the visual inspection of sludges and the results of interviews with latrine users revealed a variable pattern of use of the facilities, samples were divided into two categories according to the following criteria:

CATEGORY A - Sludge from an apparently well operated latrine:
Age believed to be 1 year or greater;
Texture slightly moist (through liquefaction) or dry;
Smell not unpleasant.

CATEGORY B - Sludge from an incorrectly operated latrine - for example not sealed after filling, containing large amounts of water or domestic refuse etc.;
Age less than 1 year or indeterminate;
Texture moist - very wet;
Smell septic.

RESULTS

Results of bacteriological analyses are based on conventional statistical tables for most probable numbers (DHSS, 1982) and are expressed as MPN per gram weight of original sludge. Due to unavoidable limitations in the experimental technique (the lowest weight of sludge which could be realistically analysed was 0.001g wet weight), many bacteriological results exceeded the maximum feasible count of 1800 MPN/g. They are quoted as >1800 MPN/g. Properly stored Category A sludges gave such a result on only one occasion (for faecal streptocci), but Category B sludges gave such results on three (out of seven) occasions for thermotolerant coliforms, on three occasions for total coliforms, and on four occasions for faecal streptococci.

Because of the relatively low number of samples, these results necessarily lead to caution in making comparisons, particularly because they limit the scope for quoting mean values. Nevertheless ranges and medians have been quoted (Table 1), and some direct numerical comparisons may be drawn.

For all indicators, the minimum levels of indicator bacteria present in samples of Category A sludge were less than equivalent minimum levels detected in Category B sludge by at least one $\log_{10}$ order of magnitude. Median levels of thermotolerant and total coliforms were more than two $\log_{10}$ orders of magnitude lower in Category A sludges compared with improperly stored Category B sludges.

TABLE 1 Summary of Most Probable Number counts of faecal indicator bacteria in latrine sludge, Gaborone 1986

Indicator	Category A Sludge			Category B Sludge		
	Range per g	Median per g	n	Range per g	Median per g	n
FC	<0.2 - 1600	2.3	7	24 ->1800	280	7
TC	0.2 - 1600	2.3	7	2.3 ->1800	920	7
FS	1.7 ->1800	35	7	350 ->1800	>1800	7

FC: Thermotolerant coliforms; TC: Total coliforms; FS:Faecal streptococci.
Category A : Sludge age >1 year; Category B : Sludge age <1 year.

Results of parasitological and virological analysis are shown below. Six samples of properly stored (Category A) sludge were analysed. Ascaris levels in this material ranged from 0 to 0.32 ova per gram (mean 0.08, n = 6). Levels of Schistosoma were low, but enteroviruses, rotaviruses and Taenia were consistently present.

TABLE 2 Summary of parasitological and virological data from latrine sludges, Gaborone 1986

Sludge Category	Estimated Solids per cent	Ascaris ova per g	Taenia ova per g	Schistosoma ova per g	Total Enterovirus pfu* per g	Total Rotavirus fffu**per g
A	25	0	1.45	0	1.50	NR
A	30	0	2.44	0	1.50	>180
A	8	0	0.40	0.02	0.32	>270
A	30	0.10	0.24	0	1.50	>727
A	10	0.32	2.98	0	1.50	>360
A	10	0.10	2.17	0	0.30	> 90
B	10	0.56	0.15	0	0.30	>270
B	30	0	0	0	0.76	>180

NR: No result due to cytotoxicity of sample; * pfu:plaque forming unit (per g original sample); **fffu: fluorescent focus forming unit (per g original sample)
Category A: Sludge age >1 year; Category B: Sludge age <1 year.

DISCUSSION

Human faecal waste would normally be expected to contain approximate microbial loadings of the following orders of magnitude (typical numbers per gram faecal material):

Thermotolerant coliforms	10^6 - 10^9
Total coliforms	10^6 - 10^9
Faecal streptococci	10^4 - 10^6
Salmonellae	up to 10^6 (for carriers)
Ascaris	up to 10^4 (for carriers)
Taenia	up to 10^4 (for carriers)
Schistosoma	up to 10^2 (for carriers)
Enterovirus	up to 10^6 (for carriers)
Rotavirus	up to 10^{10} (for carriers)

In simple quantitative terms, it is clear that the density of bacterial indicators in properly stored pit latrine waste can be very low indeed. In the case of thermotolerant coliforms, reductions of between six and nine $\log_{10}$ orders of magnitude can be achieved compared with levels which might be found in fresh faecal material. The highest density of thermotolerant coliforms detected in properly stored sludge was still several orders of magnitude less than fresh faecal material.

Equivalent reductions for faecal streptococci were substantially less. This reflects the generally longer survival of these organisms in the environment and in certain cases may also be due to the ability of some faecal streptococci eg some strains of Streptococcus faecalis to multiply in decaying organic matter. Nonetheless, reductions in properly stored sludge were substantial. In contrast, where sludge has been improperly treated, reductions were not as great, except perhaps in the lowest margins of the stored material where age and anaerobic liquefaction can militate against the survival of enteric bacteria.

However, the usefulness of faecal indicator bacteria alone as indices of pathogen risk from sanitary waste can be questioned. Ratios of indicator bacteria to pathogens can vary enormously according to the original quality and subsequent treatment of the waste. Thus the use of persistent helminth ova eg Ascaris has been suggested as a more appropriate index of the quality of stored or composted faecal material (Feachem et al., 1981). Ascaris has been used for precisely this purpose in China (McGarry and Stainforth, 1978).

Certainly, helminth ova (and possibly some enteric viruses) are the most hardy of the pathogens of interest in faecal waste intended for handling and reuse. Fortunately, most evidence suggests that provided storage exceeds one year, the number of even these pathogens is likely to be very low. Although some studies have demonstrated the survival of nematode and cestode eggs for up to three years in stored sludge (Schwartzbrod et al., 1986), it has been shown that even the most persistent eggs eg Ascaris are usually rendered non-viable by storage for more than one year in sludge at moderate temperature eg 25°C (O'Dornel et al., 1984).

This is important both for occupational risk assessment and public health considerations. If it may be assumed that faecal waste stored for a minimum of 12 months will generally contain loadings of Ascaris of eg less than 10 viable ova per 100g (less than 0.1 ova per g), such storage may be used as a guideline for assuring a product of little parasitological hazard to occupational or public health on handling or reuse.

With respect to the actual numbers of parasite eggs detected in the sludge in this study, it is clear that contamination levels were several log orders of magnitude lower than might be expected in the worst case of fresh faecal material from an infected individual. Ascaris was detected in five of the eight samples of sludge for which parasitological analysis was undertaken, confirming the endemicity of the infection in the communities investigated. It is interesting to note therefore, that of the eight samples of stored sludge assayed for parasite ova, only two had an Ascaris level greater than 10 per 100g (0.1 per g). And in both of these samples, levels of faecal indicator bacteria were slightly elevated (all groups $> 10^2$ per g) compared with minimum levels detected in properly stored material. However, all other samples had Ascaris levels of less than 1 per 100g (0.01 per g). And all but one of these six samples were taken from latrines which were thought to have been operated more or less correctly.

Low levels of Schistosoma ova (detected in only one out of eight samples) allow the conclusion that either the rate of Schistosoma carriage in the community investigated was low or that the persistence of the ova on storage is low. It is difficult to draw a more specific conclusion based on the results of a relatively small number of samples, but it appears that the sludges sampled (if representative) do not pose any quantifiable hazard to human health from schistosomiasis, especially if they are not returned to an aquatic environment.

Taenia ova were detected in low-moderate numbers in seven of the eight samples. Where Taenia was detected it was present in numbers 3-4 log orders of magnitude lower than might be expected in fresh faecal material from infected individuals. An investigation of the type of ova (whether derived from beef or pork tapeworms) or their viability was not possible. However, the presence of these ova does emphasise the need for care in reuse policy. No other types of parasitic ova eg Trichuris or hookworm were detected in sludge samples.

Levels of enterovirus detected in the sludges were relatively consistent and provided good evidence of the efficacy of long term storage. Numbers were all less than 2 pfu per g. This confirms that although all latrines sampled had been exposed to enteroviral contamination, the remaining levels were approximately 6 $\log_{10}$ orders of magnitude less than might be expected in fresh faecal material from an infected individual.

Less quantitative information may be derived from the rotavirus results. Levels were substantially higher than those observed for enteroviruses. In all cases the number of fluorescent foci was beyond the upper limit of detection. However, because the potential rate of excretion of rotavirus particles from infected individuals is so high (greater than 10^{10} per g in some individuals), the detection of numbers greater than 10^{2} in stored sludge is not surprising. In a more detailed study the actual persistence of human rotavirus in sludge would merit further study. But the endemicity of rotavirus in many communities in less developed countries and the dominance of the person-person transmission route in children suggests that stored latrine waste would not be a major source of rotaviral infection provided that good sanitary practice is followed by those responsible for handling and reuse of the material as well as those using the facilities.

The principal factors affecting the decay of enteric bacteria in pit latrine waste have been reviewed (Strauss, 1985). Natural degenerative processes will be enhanced by unfavourable storage conditions eg low or decreasing humidity (water activity), high temperature, low pH etc.

The practice of adding some water to pit latrines may promote some beneficial microbiological effects eg anaerobic digestion and liquefaction. But in non-permeable soils, if excessive water is added the latrine will fill too quickly and may therefore require emptying before digestion and elimination of enteric microorganisms has occurred. In permeable soils, the addition of excessive water may not result in premature filling and emptying, but it could substantially increase the transport of undesirable compounds, eg nitrates and nitrites, and microorganisms, thereby increasing burdens on groundwater.

If little or no water is added to latrines, digestive and liquefactive processes may be retarded, but the benefits of dessication (immobilisation of organics and destruction of enteric microorganisms) will be maximised. This greatly increases the value and manageability of latrine waste as an agricultural resource (both as a fertiliser and soil conditioner) as well as reducing pollutant burdens on the aquatic environment and health hazards associated with the handling of wastes.

It must be noted that the number of samples assayed for bacteriological quality was small and for parasites and viruses even less. The results cannot therefore be used for extrapolation, or to draw general conclusions about the efficacy of certain types of latrine waste storage. However, the results do confirm the validity of extrapolations and tentative guidelines based largely on data from industrialised countries. Thus, the principle that dry storage of latrine waste for a minimum period of one year is likely to result in a product of substantially improved microbiological quality is supported by the data presented here.

It remains to be seen if the powerful arguments for increased reuse of sludge will be enhanced by the required social and educational research to ensure that well designed and constructed facilities can be used to the maximum public health and economic benefit of the recipient community. In countries which have no experience of such practices it is clearly vital that programmes of public education be intimately linked to construction if those benefits are to be realised.

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