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Title	**Effects of mesophilic temperature and alkaline additives on the persistence of *Ascaris suum*, *S. typhimurium* DT 104 and indicator organisms in dry-collected faeces**
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Effects of mesophilic temperature and alkaline additives on the persistence of *Ascaris suum*, *S. typhimurium* DT 104 and indicator organisms in dry-collected faeces

Introduction

Evaluation of the hygienic risks correlated to different sanitation practices is of main interest to assess the efficiency of the primary treatment within ecological sanitation. The impact of different latrine alternatives on parasitic infections in rural areas of El Salvador was presented in the EcoSan conference in Lübeck (Corrales, *et al*., 2003). Double-vaulted, urine-diverting desiccating latrines were found to be significantly associated with an increased prevalence of *Ascaris* and *Trichuris*. Increased contact with inadequately treated human excreta might explain the increased risk for Ascaris infection observed. Ascaris is regarded as one of the most resistant pathogens in the environment (Feachem, et al., 1983). Their development in wastewater sludges has been found to be inhibited at 40 °C (Ghiglietti, *et al*., 1995) however a persistence of *Ascaris* in sludge in the range of month to year is often quoted (Haug, 1993). Laboratory pilot studies were therefore performed to determine the influence of mesophilic temperatures and alkaline pH as found in the El Salvador study, on the inactivation of *Ascaris* ova and *S. typhimurium*. Furthermore, microbial indicators of treatment efficiency were evaluated as surrogates for pathogen inactivation under these same conditions.

Methods

The persistence of the pathogens *Ascaris suum* and *Salmonella typhimurium* DT104, the bacteria *E. coli* and *E. faecalis,* and bacteriophages Φx174 and *S. typhimurium* 28 B in dry collected faecal material was evaluated under different moisture, pH and temperature storage conditions. The pathogens were exposed to 25 °C and 35 °C at 70% moisture levels and the indicators at 20 °C, 30 °C and 40 °C at 45% and 18% moisture levels. By successive samplings, including measurements of pH and moisture content, the persistence of the seeded organisms was evaluated. Lime in selected doses (10 and 20 g kg^{-1}) was mixed to adjust the initial pH in the material. In control experiments the effect of wood ash (pH 10.6, 250 g kg^{-1}) was tested for *Salmonella* and a glycine buffer (pH 10.5) was tested for the indicator organisms. The viability of *Ascaris* eggs, stored in nylon bags in the material, was determined by their capacity to develop into the L-2 larval stage within 3 weeks at room temperature. The reduction of a multi-resistant strain of *Salmonella typhimurium* DT104 was determined directly by culture on two selective substrata, using gentamycin to suppress the overgrowth of atypical bacterial colonies. A most probable number (MPN) method was used

to detect low levels of *Salmonella* in the material until undetectable levels were reached. The indicator reduction was determined for the bacteria directly by culture on selective agars and for the bacteriophages by a plaque assay method. Mathematical models using analysis of variance were used to predict the survival rates (T_{90}, days) and the impact of moisture, temperature and lime dose on the long-term persistence of the indicators.

Results

The initial viability of the *Ascaris* eggs (93%) remained stable until day 92 in 0.05 M sulphuric acid at 4 °C (89%), but was reduced to 9% at 25 °C and 6% where lime was added. At 35 °C however, the viability fell to about 4% after 4 days storage and no viable eggs were detected at day 10. The effect given by the higher temperature was dominating compared to the pH set by initial lime additions of 20 g kg^{-1}. The persistence of *Salmonella* was highest at 25 °C (T_{90} = 3.8 d) and similar when ash was mixed with the material (T_{90} = 3.5 d). Lime addition gave a high initial reduction (T_{90} = 1.8 d), though a smaller fraction was surviving the treatment. At 35 °C *Salmonella* fell below detectable levels within 7 d. Similarly to the salmonellae, indicator organisms, preadapted prior to seeding, were instantly log-reduced by the alkaline additions. When added in the higher dose (20 g kg^{-1}) lime was the most efficient additive. The reduction effect was lower for bacteria (enterococci 0.5-2 log; faecal coliforms 1.3 log) compared to bacteriophages (Φx174 1.0-1.2 log and *S. typhimurium* phage 28 B 2.0-3.7 log). The initial decay however was followed by a secondary phase with slower reduction that was studied until 58 d after commencement.

For all indicator organisms, temperature was the dominating effect for their reduction. For the bacteriophages the survival (T_{90}) at 20 °C ranged from 16 d to 144 d and was varying between the two species, moisture content and lime addition. At 30 °C the survival times ranged within the interval 4-44 d and at 40 °C in the interval 0.5-7 d. At low moisture (18%) the survival was higher for the *Salmonella*-phage compared to Φx174 but at medium moisture (45%) the rates were similar. For the bacteria a rapid decay was observed for *E. coli* with a maximum T_{90} at 20 °C of 2.1 d for non-adapted cells and 4.3 d for starved cells, reflecting its unsuitability for total assessment of microorganism decay in faeces. The faecal enterococci (*E. faecalis* and indigenous bacteria) were more persistent compared to the *E.coli* and the moisture content of 18% and 45% did not have any significant effect on the reduction ($p>0.1$). The long-term survival was reflected by T_{90} in the interval 37-51 d at 20 °C. Generally the persistence was similar to the *Salmonella*-phage, and a rapid reduction was calculated at 40 °C with T_{90} of 3-4 d.

Conclusions

The persistence of human pathogens that may pose a health risk in the use of dry faecal material in agriculture was evaluated in the laboratory by the pathogen models. The results indicate that a temperature change in the interval 25 to 35 °C gave a predominant effect in reducing *Ascaris* and *Salmonella* in dry collected faecal material to safe levels. Results for the indicator organisms emphasised the role of temperature in microorganism reduction. The bacteria as well as the bacteriophages were instantaneously log-reduced in contact with lime and to a smaller extent by wood ash and glycine buffer. Dosages of these alkaline additives tested however were not sufficient to ensure a total reduction and did not demonstrate a long-term effect on organism inactivation.

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