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Survival of human and animal pathogens in soil amended with biowaste

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Ongoing research at the Swedish National Veterinary Institute deals with questions concerning human and animal infectious diseases in recycling of biowaste. The research concerns the ability of microorganisms to survive in biowaste and biosecurity consequences of the use of biowaste as fertilizer in agriculture. Interdisciplinary work in the fields of soil science and veterinary epidemiology is applied to solve clinical veterinary problems.

Cattle manure and biowaste containing, slaughterhouse offal, household and restaurant swill, is a useful resource in agriculture in order to maintain soil nutrient levels and stimulate various aspects of soil fertility. Thus, biowaste including manure are recycled back as fertilizer to agricultural land used for crop or silage production and cattle grazing. However, this procedure poses a risk of transmission of pathogens affecting humans and animals. Cattle manure is not routinely treated to reduce their pathogenic content, and despite a separate pasteurisation step at 70C˚ for 60 min of biowaste before anaerobic digestion, some heat resistant viruses, bacterial- and fungal spores can still be present in the final product. However, while soil and vegetation can be expected to directly influence the survival of pathogens, relatively little is known concerning the role of soil as a vector and reservoir.

A set of incubation studies were undertaken to evaluate the survival of pathogenic microorganisms in soil amended with infected cattle manure and digested residues, respectively. Two zoonotic bacterial strains; a non-toxigenic strain of *Escherichia coli* O157 and a *Salmonella* Typhimurium strain 178 and one viral strain; Porcine parvovirus (PPV) strain 893/76 were used. Different filed soils were collected, prepared and mixed with inoculated cattle manure or digested residues. The biowaste-amended

soil were placed in aerated microcosms (0.5dm^3) and incubated at constant temperature regimes. The recovery and enumeration of *E. coli* O157 and S. Typhimurium 178 were determined by plating ten-fold dilutions onto selective agars of sorbitol MacConkey and xylose lysine deoxycholate, respectively. PPV strain 893/76 was extracted with physiological saline, filtered through 0.45μm followed by passage through a PD 10 column with Sephadex G-25. Virus titre TCID_{50} was determined by end-point titration using PK-15 cells and immunoperoxidase staining, followed by calculation according to Kärber.

We found both *E. coli* O157 and S. Typhimurium as well as the porcine parvovirus to survive well in soil amended with biowaste. For *E. coli* O157 and S. Typhimurium approximately 1-$\log_{10}$ and 0.5-$\log_{10}$reductions occurred over an 80-day period of storage at 4˚C in cattle manure. The reduction of PPV strain 893/76 was approximately 1-$\log_{10}$ over a 56-day period of storage at 15˚C in digested residue. The influence of some soil factors was studied in more detail with *E. coli* O157. Hence, we found the reduction rate of *E. coli* O157 to be significantly lower in the clay loam soil compared with the sandy loam and loamy sand soils used. Moreover, we found a clear relationship between temperature and reduction rate of *E. coli* O157 during storage at 4, 15 or 21˚C respectively.

The results this far indicate that different pathogens have the ability to survive for extended periods in soil and thus we address the importance of adequate treatments of biowaste before use on arable land.

Reference: KÄRBER G. (1931) Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Archive für experimentelle Pathologie und Pharmakologie, 162, 480-483.