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Title	**Salmonella isolated in Sewage Sludge traced back to human cases of salmonellosis**
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Introduction

Sewage sludge contains pathogenic bacteria and if it is spread on arable land, there is a risk of spreading diseases to people and animals. The reduction of pathogens in sludge depends on treatment methods used to stabilise sludge in sewage treatment plants. Sludge can be treated using several methods: sedimentation; mesophilic or thermophilic anaerobic digestion (AD); composting; storage; and by combinations of these methods.

This study surveyed the content of *Salmonella* spp and indicator bacteria in raw and treated sewage sludge in eight Swedish sewage treatment plants (STPs) with four different treatment methods. Moreover, a possible link between salmonella isolated from infected humans and salmonella isolated from sewage sludge was investigated.

Materials and methods

Eight STPs (geographically spread throughout Sweden) were included in the survey; they differed in size and type of treatment. Samplings were performed from July 2000 to June 2001, resulting in 64 raw sludge samples and 69 treated sludge samples. At each STP, two additional samples were collected 6 and 12 months after the last sampling. The treated sludge had a dry matter content around 20-30%.

Human salmonella strains matching the salmonella strains isolated from sewage sludge were selected for Pulsed Field Gel Electrophoresis (PFGE) analysis in order to link salmonella isolated from infected humans to those isolated from sewage sludge. The criteria used for the search of human cases were as follows: same serotype and, if applicable the same phage type, resident in the area that the actual STP served and onset of disease before the Salmonella strain was isolated from the sewage sludge. Thirty-seven (37) human salmonella strains were selected for further analysis, including 13 different serotypes and representing four different geographic areas matching the serving area of four sewage treatment plants. The bacterial analysis were determined according to Nordic Committee on Food Analysis (NMKL) methods; NMKL 71:5: 1999 for Salmonella spp., NMKL 68:2:1992 for enterococci, NMKL 44:4:1995 for coliform bacteria and NMKL 125:3.1996 for thermo-tolerant coliform bacteria and E. coli. Serotyping of Salmonella was performed according to Kaufmann and White (WHO, 1997) at the National Veterinary Institute, Uppsala, Sweden.

The link between Salmonella isolated from humans and sewage sludge was investigated

using PFGE and antibiotic resistance monitoring. The macrorestriction enzyme analysis and PFGE was performed as described in Palmgren et al. 2000. Restriction enzymes used for each strain were: Xba1, Spe1 and Bln1. Strains were considered identical if they were undistinguishable with all three enzymes used.

Results

Salmonella was found in 67% (43/64) of the samples from raw sludge and in 55% (38/69) of the samples from treated sludge. Serotyping revealed 49 different serotypes out of 104 isolates of *Salmonella* from the sewage sludge.

There are differences in treatment methods concerning the reduction of pathogens and indicator bacteria. Concerning the treated sludge, most *Salmonella* was found in STPs with sedimentation only and in plants with mesophilic digestion where *Salmonella* was isolated in 17/24 samples and in 14/24 samples respectively. *Salmonella* was isolated once (1/8) in both sludge treated with thermophilic digestion and sludge treated by composting.

Eight out of the 37 analysed pairs of salmonella from humans and sewage sludge were identical regarding PFGE.

Discussion

The vast amount of Salmonella in sewage sludge in Sweden (Sahlström et al. 2004) was somewhat surprising, taking into consideration the zero tolerance of Salmonella infection in animal husbandry in Sweden, resulting in a low frequency of human domestically infected salmonella cases. Salmonellosis is a noticeable disease according to the Communicable Disease Act in Sweden. All notifications are submitted to the Swedish Institute for Infectious Disease Control (SMI). More than 90% of the salmonella infections among humans, which are reported to the authorities in Sweden, are not domestic but acquired abroad (SWEDRES). When an infection is introduced through the human body it can be transferred to the sewage sludge, survive in a STP and possibly be spread with the sludge in the environment. Thus, salmonellas may be transferred into the sewage treatment plant with the risk of being spread with the sewage sludge out in the environment, which may pose a threat in a re-cycling society.

Salmonella spp. was detected in 55% (38/69) of the samples from treated sludge. If sludge with a hygienic quality as presented in this study is spread on arable land, the environmental load of pathogens increases: this increases the risk for spreading diseases to people and animals.

Salmonella isolated from sewage sludge was traced back to Salmonella infected humans. This was proved by some strains of Salmonella isolated from the sewage sludge and from salmonella infected humans, respectively, which were identical when analysed by Pulsed Field Gel Electrophoresis. This link between strains from human infections and the salmonella isolates from the sewage sludge indicate a resistance of Salmonella to treatment of sewage sludge including mesophilic (approximately 35°C) anaerobic digestion.

The hygienic quality of sludge seemed to be influenced by the different treatment methods used at the STPs. The results indicate that thermophilic AD reduces pathogens and indicator-bacteria to a higher degree than conventional treatment such as sedimentation and mesophilic AD. In the thermophilically treated sludge, Salmonella was found once (1/8) in treated sludge. In the mesophilically treated sludge, Salmonella was found in 58% of the samples. In STPs with sedimentation, Salmonella was found in 71% of the samples. The treatment of sewage sludge in large-scale treatment plants seemingly fails to reduce the pathogen content, which was one of the primary reasons to establish the conventional sewerage system. Consequently contaminated sewage sludge used as fertiliser on arable land may transfer pathogens back to the ecocycle. Salmonella in sewage sludge can be a health hazard for both animals and humans if the sludge is used in agriculture.

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