Title	Salmonella isolated in Sewage Sludge traced back to human cases of salmonellosis
Keywords	Salmonella, Sewage sludge, health, disease transmission
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Photograph attached (jpg)	

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. Sludge can be stabilised with several different methods including: sedimentation; mesophilic or thermophilic anaerobic digestion (AD); composting; storage; and by combinations of these methods. The reduction of pathogens in sludge is dependent on the treatment method used to stabilise the sludge in sewage treatment plants.
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 was surveyed (Sahlström et al, 2004). Moreover, a possible link between *Salmonella* isolated from infected humans and *Salmonella* isolated from sewage sludge has been investigated.

MATERIALS AND METHODS

Eight STPs, which were different in size and type of treatment and geographically spread throughout Sweden, were included in the survey (Sahlström et al, 2004). The treatments used at the STPs were: one plant with thermophilic anaerobic digestion (AD), three using Mesophilic AD, one treated the sludge in windrow composts and the others used sedimentation only. Samplings were performed every other month 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 untreated sludge had a dry matter content of approximately 2-3% and the treated sludge had a dry matter content around 20-30%. The untreated sludge was collected from taps and the treated sludge was collected with a spade after the dewatering phase (centrifuge).
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, not more than 12 months, 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. The macrorestriction enzyme analysis and PFGE was performed as described by Palmgren et al. (2000). Restriction enzymes used for each strain were: *Xba* I, *Spe* I and *Bln* I (Amersham Biosciences, Uppsala, Sweden). Strains were considered identical if they were undistinguishable with all three enzymes used.

RESULTS

Salmonella was isolated from 67% (43/64) of the samples from raw sludge and from 55% (38/69) of the samples from treated sludge (Sahlström et al., 2004). 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 *Salmonella* and indicator bacteria. Concerning the treated sludge, most *Salmonella* was found in STPs with sedimentation only and in plants with mesophilic AD 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 AD and sludge treated by composting.
Indicatorbacteria were not reduced after sedimentation. When thermophilic AD was used coliform bacteria were reduced with approximately 4 log10 units, however, in the unwatered sludge the bacteria count reached approximately 4 log10 cfu/g.
Comparison of restriction patterns with all three enzymes used, revealed eight salmonella isolates from humans, which were identical with a *Salmonella* isolate from sludge.

DISCUSSION

Salmonella spp. was detected in more than half (55%) of the samples from treated sludge. If sludge with a hygienic quality like this is spread on arable land, the environmental load of pathogens increase, as well as the risk for spreading diseases to people and animals. The vast amount of *Salmonella* in sewage sludge in Sweden (Sahlström et al. 2004) was somewhat surprising, taking into consideration the low tolerance of *Salmonella* infection in animal husbandry in Sweden, resulting in a low frequency of human domestically infected salmonella cases. Salmonellosis is a notifiable disease according to the Communicable Disease Act in Sweden. All notifications are submitted to the Swedish Institute for Infectious Disease Control (SMI). More than 80% of the *Salmonella* infections among humans, which are reported to the authorities in Sweden, are not domestic but acquired abroad (SWEDRES). An epidemiology study from Norway (Kapperud et al, 1998) presented similar figures considering salmonella infected humans. 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 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 studied by restriction enzyme analysis and Pulsed Field Gel Electrophoresis. This link between the *Salmonella* from humans and sludge also demonstrate a possible spread of imported *Salmonella* to the environment through the sludge. Moreover, 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 different STPs. Thermophilic AD reduces pathogens and indicator-bacteria more than conventional treatment such as sedimentation and mesophilic AD. In the thermophilically treated sludge, *Salmonella* was found once (1/8) in treated sludge. In contrast, from 58% of the samples from the mesophilically treated sludge *Salmonella* could be isolated. In STPs with sedimentation only, *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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