Brief communication **Peer reviewed**

Survey of *Salmonella* populations from swine waste-treatment technologies

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Summary

Salmonella presence, populations, serotypes, and antibiotic susceptibilities in untreated and treated swine manure were determined for farms implementing environmentally superior waste-treatment technologies. The waste-treatment systems surveyed showed potential in reducing *Salmonella* populations.

Keywords: swine, *Salmonella*, waste management, manure

Received: June 11, 2010
Accepted: August 27, 2010

Resumen - Monitoreo de la población de *Salmonella* a partir de tecnologías de tratamiento de desechos porcinos

Se determinaron la presencia, población, serotipos, y susceptibilidad a antibióticos de la *Salmonella* en excretas de cerdo tratadas y no tratadas provenientes de granjas que implementan tecnologías medioambientalmente superiores de tratamiento de desechos. Los sistemas de tratamiento de desechos investigados mostraron potencial para reducir las poblaciones de *Salmonella*.

Résumé - Enquête sur les populations de *Salmonella* provenant d'unités de traitement du lisier de porc

La présence, les populations, les sérotypes, et les patrons de sensibilité aux antibiotiques d'isolats de *Salmonella* provenant d'échantillons de lisier de porc non-traités et traités ont été déterminés pour des fermes mettant en place des technologies de traitement du lisier supérieures d'un point de vue environnemental. Les systèmes de traitement étudiés ont démontré du potentiel pour réduire les populations de *Salmonella*.

Swine manure is generally used as fertilizer and applied to fields for growing agricultural commodities. *Salmonella* and other pathogens have frequently been isolated from swine wastes.[1-3] Land application of *Salmonella*-contaminated manure may pose an environmental risk if movement occurs to surface and groundwaters.[4-6] Sustainable swine production requires the development of innovative and cost-effective waste-management systems to address these and other environmental concerns.[7]

Much emphasis has been placed on odor and air-quality issues when developing new swine waste-management technologies.[8,9] However, the presence, populations, and diversity of *Salmonella* in the liquid- and solid-waste effluents from different waste-treatment technology systems has received less attention. Thus, the objectives of this field survey were to evaluate the effects of several promising swine waste-management and treatment technologies on reducing *Salmonella* presence and populations and to characterize the diversity of *Salmonella* isolates recovered from the waste streams using serotyping and antibiotic-susceptibility analysis.

Materials and methods

In this field study, five environmentally superior waste-treatment technologies were evaluated for *Salmonella* presence, populations, serotypes, and antibiotic susceptibilities. Technologies included ambient temperature anaerobic digester, solid separation constructed wetland, up-flow biofiltration, multi-step biological and chemical, and high-solids anaerobic digester (HSAD) treatment systems,[6,7] as well as a traditional lagoon treatment system on a conventional swine-production farm. Each treatment technology was implemented on a single swine-production site and was operational for a limited period of time; thus, each farm was sampled only once between August 2002 and March 2003. At the time of sampling, each technology had been in operation between 3 and 6 months.

The ambient temperature anaerobic digester treatment system was designed to "close the loop" with regard to the on-farm processing of swine wastes (Figure 1). This farm was a farrow-to-wean swine operation with approximately 4000 sows in six houses: two farrowing houses and four gestation houses. A pit-recharge system was used to first collect the manure from the farrowing and gestation swine houses. The ambient digester system was installed with an impermeable cover over an in-ground digester that was used for primary waste treatment, with the effluent discharged into one end of a storage pond. The methane biogas produced in the digester was

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This article is available online at http://www.aasv.org/shap.html.

Payne JB, Li X, Santos FBO, et al. Survey of *Salmonella* populations from swine waste-treatment technologies. *J Swine Health Prod.* 2011;19(2):100–106.

used for generating electricity. Moreover, the ammonia generated in the anaerobic effluent was oxidized to nitrate in the first biofilter. The nitrified water was stored in a tank and then recycled to recharge the pits inside the swine rearing houses. Tomato plants housed in adjacent greenhouses utilized a significant portion of the nutrients recovered from the storage pond after further oxidation of ammonia to nitrate in the second biofilter. In this system, a composite sample was collected from each of the following: fresh feces recovered from the farrowing and gestation pigs, effluent streams exiting the farrowing and gestation swine houses, the ambient digester, the first biofilter, the west storage pond, the second biofilter, the liquid-storage tank in the tomato greenhouse, and the medium used for growing the tomato plants (Figure 1).

The solid separation constructed wetland treatment system consisted of house effluent that was initially pumped to a mechanical solids separator (Figure 2). The liquid was then pumped into a settling basin before flowing into either of two parallel constructed wetlands (inner cell and outer cell) and then to a storage pond. Periodically, settled solids were pumped from the settling basin back to the separator. Some of the treated liquid from the storage pond was used to recharge the house pits. Excess liquids from the storage pond and excess solids from the solids separator were land applied to cropland. The constructed wetland system was designed to convert ammonia contained in the house effluent to nitrate, which was subsequently utilized by the wetland's cattails. The system was a simple, low-energy alternative to a conventional anaerobic lagoon system. It was installed on a 3520-head swine finishing facility composed of four swine houses. A composite sample was collected from each of the following: fresh swine feces, house effluent, separated solids, inner and outer cell wetland influent, inner and outer cell wetland effluent, and the storage pond (Figure 2).

The up-flow biofiltration treatment system incorporated solids separation and aerobic biological treatment of the flushed prescreened liquid manure for the purpose of reducing chemical oxygen demand, odor, and ammonia emission by promoting nitrification (Figure 3). Five finishing barns with a total of 4300 pigs were connected to the system. Separated solids were pumped into the anaerobic lagoon. Following solids separation, the liquid waste stream was pumped into an equalization tank to allow settling, and then the liquid was pumped upwards into the sequential and aerated biofilters. Plastic fixed media contained within the series of up-flow biofilters provided sufficient surface area for the formation of a bacterial biofilm that aerobically stabilized the organics and converted ammonia to nitrate. The treated effluent was then pumped into the anaerobic lagoon for barn-pit recharge or recirculated into the equalization tank via the solids separator. The biofilters were periodically cleaned through air agitation and a backwash procedure. It should be noted that this waste treatment technology treated only a portion of the total waste generated on the farm. There were other barns on the farm where the wastes were treated conventionally with an anaerobic lagoon system. On this farm, the anaerobic lagoon that received manure from the barns

Figure 1: Ambient temperature anaerobic digester waste-treatment system installed on a six-house farrow-to-wean swine operation housing 4000 sows. Each sample-collection point (•) represents a composite sample that was analyzed for *Salmonella* presence and most probable number population estimates.

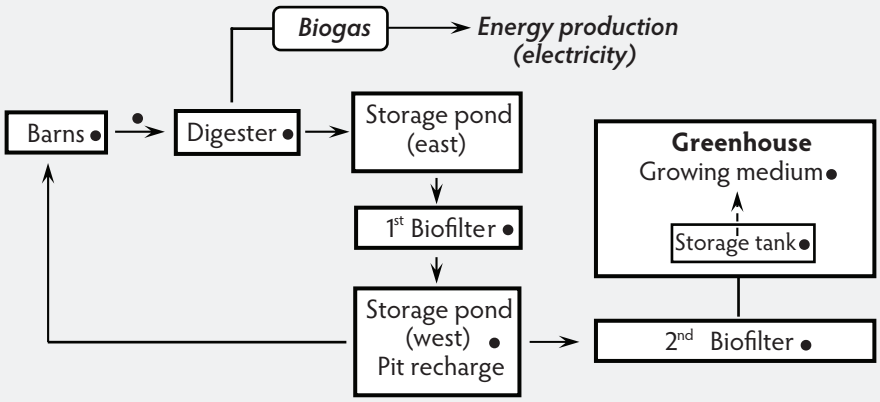
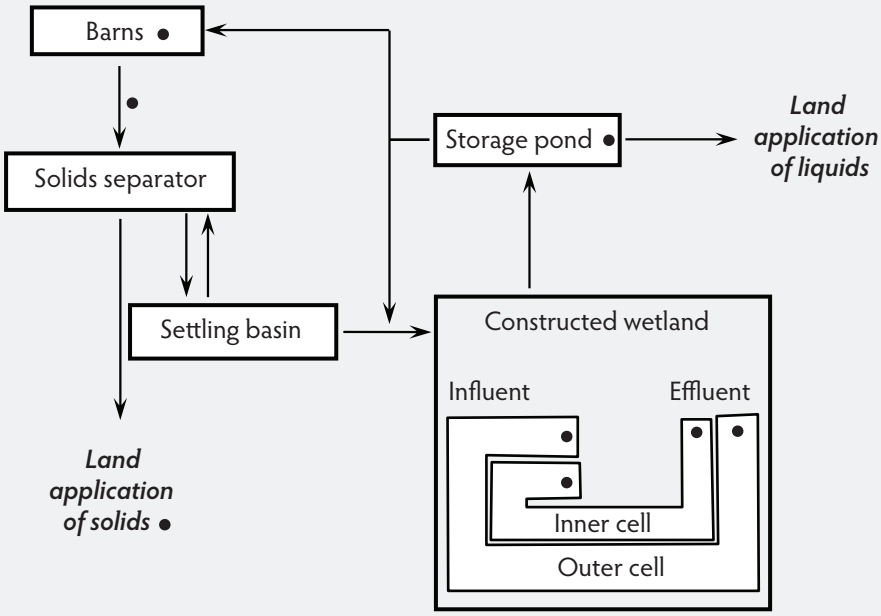


Figure 2: Solid separation constructed wetland waste-treatment system installed on a four-house finishing swine operation housing 3520 finisher pigs. Each sample-collection point (•) represents a composite sample that was analyzed for *Salmonella* presence and most probable number population estimates.

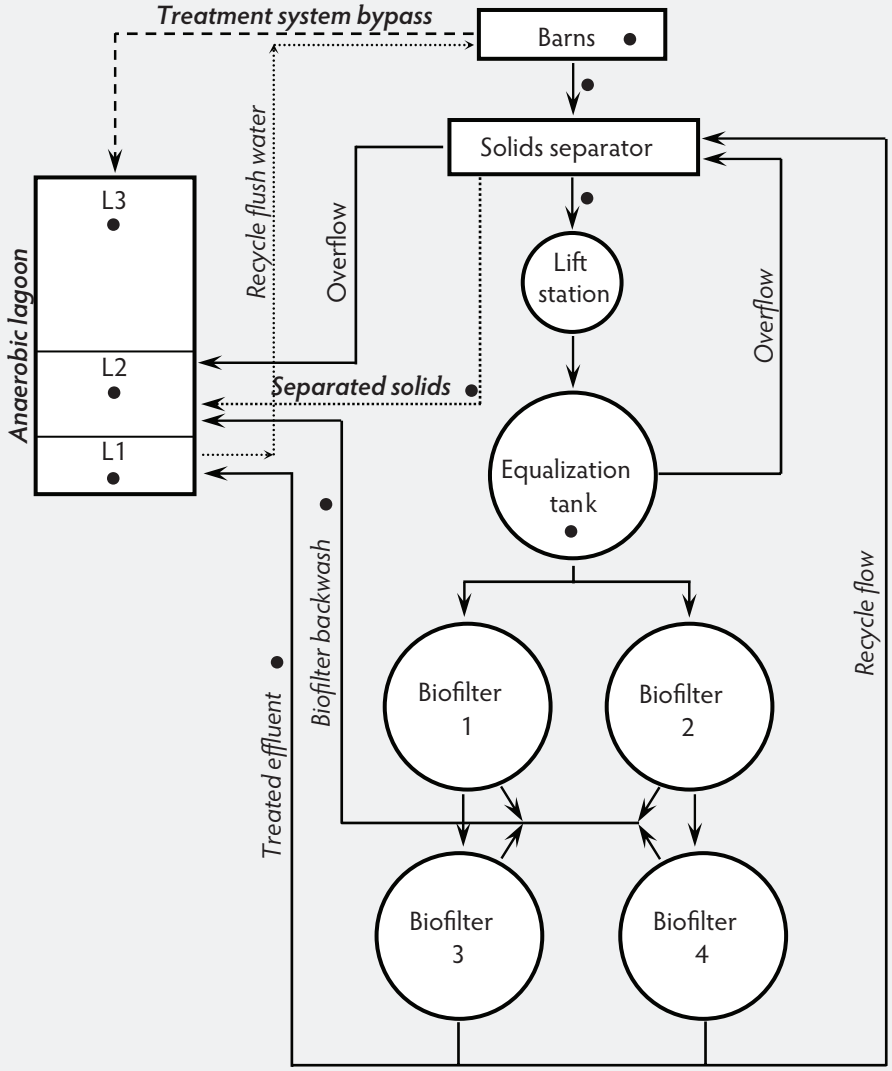


was partitioned by plastic curtains into three sections (L1, L2, and L3), with one section (L3) much larger than the other two; the relative surface areas were 13%, 16%, and 71% of total area, respectively. The L3 section received manure from barns not connected to the treatment system. The L2 section received overflow from the solids separation basin, separated solids, and backwashed biosolids removed from the biofilters. The L1 section received the treated effluent from the biofilters. A composite sample was collected from each of the following: fresh swine feces, house effluent, separated solids, separated liquids, the equalization tank, biofilter effluent, biofilter backwash, the solids reservoir (L2), liquid storage (L1), and the lagoon (L3) (Figure 3).

The multi-step biological and chemical treatment system incorporated solids separation, with the liquid waste stream subjected to nitrification, de-nitrification, and phosphorus extraction processes (Figure 4). The system was installed on a 4400-head finishing facility composed of six swine houses. First, flushed manure was pumped into a homogenization tank and then separated in the solid-liquid separation module. The liquid-waste stream flowed through nitrification and de-nitrification tanks and then to a settling tank where it was further treated for phosphorus precipitation to calcium phosphate. Treated effluent was stored in the existing lagoon prior to land application. The complete treatment consisted of solids, nitrogen, and phosphorus removal. The separated solids were then subjected to an HSAD treatment system, where swine waste organics were converted to methane biogas. The solids were further composted for conversion to a value-added fertilizer product. For the multi-step biological and chemical treatment system, a composite sample was collected from each of the following: fresh swine feces, house effluent, the homogenization tank, the solid-liquid separation module, the nitrification and de-nitrification tanks, the settling tank, treated effluent, and calcium phosphate fertilizer. For the HSAD treatment system, a composite sample was collected from each of three effluent ports of the HSAD (Figure 4).

The conventional swine-production system consisted of one finishing farm employing a traditional lagoon treatment system. The four-house farm housed 5000 pigs, and the wastes were discharged by flushing to an anaerobic lagoon. A composite sample was collected from each of the following: fresh swine feces, house effluent, and the lagoon.

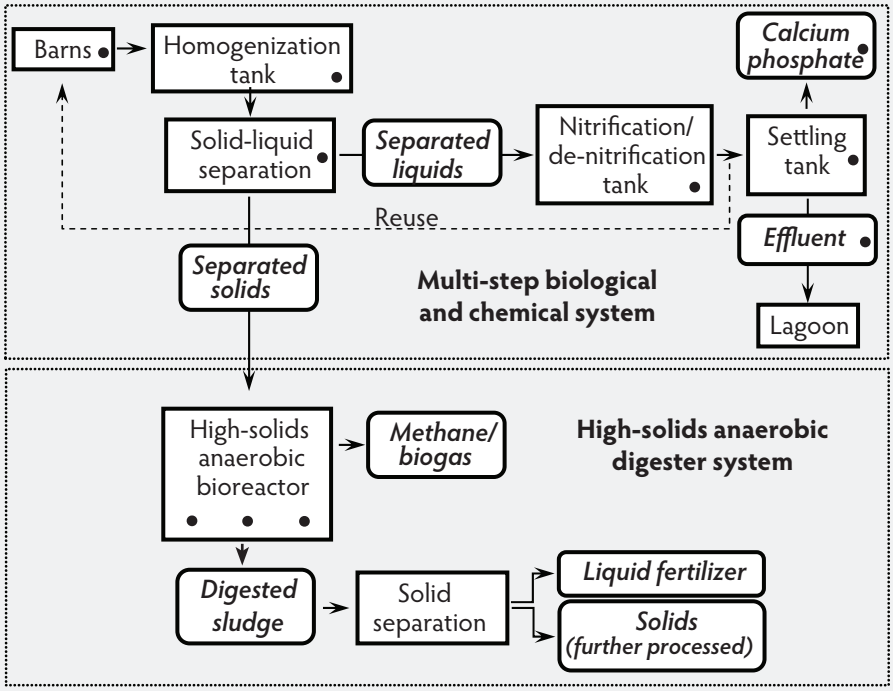
Figure 3: Up-flow biofiltration waste-treatment system installed on a five-house finishing swine operation housing 4300 finisher pigs. Each sample-collection point (•) represents a composite sample that was analyzed for *Salmonella* presence and most probable number population estimates.



Solid and liquid samples were aseptically collected in sterile plastic bottles. Following collection, all samples were stored on ice in a transport cooler and processed in the laboratory within 2 hours. Twenty-five-gram solid samples and 25-mL liquid samples were used to determine *Salmonella* presence and most probable number (MPN) population estimates using culture methods in Rappaport-Vassiliadis broth (Oxoid Ltd, Ogdensburg, New York) and modified lysine iron agar (Oxoid Ltd) as described by Santos et al.[10] The three-tube MPN technique employed serial dilution in triplicate tubes. Both culture methods were plated in duplicate. Suspect colonies were confirmed using triple sugar iron agar (Difco, Lawrence, Kansas) and poly-O antiserum (Difco) as previously described.[10]

Fifty *Salmonella* isolates were successfully serotyped, originating from the ambient temperature anaerobic digester treatment system (four), the constructed wetland treatment system (nine), the up-flow biofiltration treatment system (18), the multi-step biological and chemical treatment system (12), the HSAD treatment system (two), and the conventional swine operation unit (five). One isolated colony was randomly picked from each sample plate,

Figure 4: Multi-step biological and chemical waste-treatment system installed on a six-house finishing swine operation housing 4400 finisher pigs. Separated solids were subjected to a high-solids anaerobic digester waste-treatment system. Each sample-collection point (•) represents a composite sample that was analyzed for *Salmonella* presence and most probable number population estimates.



transferred onto tryptic soy agar (Difco) slants, grown overnight at 37°C, and submitted to the United States Department of Agriculture National Veterinary Service Laboratories in Ames, Iowa, for serotype determination. Serotyping was based on the Kauffmann-White scheme.

The antimicrobial susceptibilities of the 50 serotyped *Salmonella* isolates were determined using the Sensititre Susceptibility System (TREK Diagnostic Systems Inc, Westlake, Ohio) as previously described by Santos et al.[11] Antimicrobials selected for these assays reflect the recommendations of the National Committee for Clinical Laboratory Standards.[12] The susceptibility tests contained the following 15 antimicrobial agents (dilution ranges are indicated in parenthesis): amikacin (0.5 to 64 µg per mL), amoxicillin-clavulanic acid (1 to 32 and 0.5 to16 µg per mL, respectively), ampicillin (1 to 32 µg per mL), cefoxitin (0.5 to 32 µg per mL), ceftiofur (0.12 to 8 µg per mL), ceftriaxone (0.25 to 64 µg per mL), chloramphenicol (2 to 32 µg per mL), ciprofloxacin (0.015 to 4 µg per mL), gentamicin (0.25 to 16 µg per mL), kanamycin (8 to 64 µg per mL), nalidixic acid (0.5 to 32 µg per mL), streptomycin (32 to 64 µg per mL), sulfisoxazole (16 to 256 µg per mL), tetracycline (4 to 32 µg per mL), and trimethoprim-sulfamethoxazole (0.12 to 4 and 2.38 to 76 µg per mL, respectively).

Results

Presence, populations, and serotypes of *Salmonella* determined from the waste-treatment technologies and the conventional swine-production system are summarized in Table 1. *Salmonella* populations in the final treated liquid waste streams of the ambient temperature anaerobic digester, constructed wetland, and multi-step biological and chemical treatment systems were under the detection limit (1 log MPN per mL), while in the final treated liquid wastes of the HSAD and the up-flow biofiltration treatment systems, populations were above the detection limit. *Salmonella* populations in the separated solids wastes from the constructed wetland, multi-step biological and chemical, and up-flow biofiltration treatment systems were also above the detection limit (1 log MPN per g). For each treatment technology, *Salmonella* populations from fresh swine feces were either low or below the detection limit.

A total of nine serotypes were identified, including *Salmonella* Derby (15 of 50 isolates, 30%), *Salmonella* Typhimurium (var Copenhagen, 12 of 50, 24%), *Salmonella* Johannesburg (8 of 50, 16%), *Salmonella* Anatum (5 of 50, 10%), *Salmonella* Infantis (3 of 50, 6%), *Salmonella* Muenchen (3 of 50, 6%), *Salmonella* Senftenberg (2 of 50, 4%), *Salmonella* Heidelberg (1 of 50, 2%) and *Salmonella* Worthington (1 of 50, 2%).

The antimicrobial agents to which *Salmonella* isolates (n = 50) were most commonly resistant were tetracycline (29 of 50 isolates, 58%), streptomycin (28 of 50, 56%), ampicillin (10 of 50, 20%), chloramphenicol (6 of 50, 12%), trimethoprim-sulfamethoxazole (3 of 50, 6%), and kanamycin (3 of 50, 6%). Sixty-eight percent of the isolates were resistant to ≥ 1 antimicrobial agent. No *Salmonella* isolates were resistant to amikacin, amoxicillin-clavulanic acid, cefoxitin, ceftriaxone, ceftiofur, ciprofloxacin, gentamicin, nalidixic acid, or sulfisoxazole.

Discussion

Salmonella populations throughout the waste-treatment systems were similar to those that Vanotti et al[3] reported when testing a pilot multi-step biological and chemical treatment system. The initial spike in *Salmonella* populations following the fresh fecal sampling point may have been due to flushing the feces out of the house with water, thus creating a more favorable environment for *Salmonella* proliferation. Most treatment technologies showed potential in reducing *Salmonella* populations in treated liquid manure.

According to the Centers for Disease Control National *Salmonella* Surveillance System Annual Summary for 2006,[13] *S* Typhimurium was the most frequently reported serotype from human clinical sources. *Salmonella* Infantis, Muenchen, and Heidelberg were also among the 14 most frequently reported serotypes in 2006. Additionally, the United States Department of Agriculture Food Safety Inspection Service recently reported that *S* Derby, *S* Typhimurium (var Copenhagen), *S* Johannesburg, *S* Infantis, and *S* Anatum were among the five most commonly isolated serotypes from swine-processing establishments for calendar year 2009.[14] These published statistics are in agreement with the profile of *Salmonella* serotypes isolated in the present

Table 1: Presence, populations, and serotypes of *Salmonella* recovered from five environmentally superior waste-treatment technologies and a conventional swine-production farm employing a traditional lagoon system*

Sample type	Positive/negative for *Salmonella*	Log MPN/g or mL	Serotype
Ambient temperature anaerobic digester			
Fresh fecal (farrowing houses)	Neg	1.03	Untypeable
Fresh fecal (gestation houses)	Neg	< 1	NA
House effluent (farrowing houses)	Pos	2.70	Anatum (2)
House effluent (gestation houses)	Pos	3.88	Johannesburg (1)
Digester	Pos	2.56	Anatum (1)
1st biofilter	Pos	< 1	Untypeable
Storage pond (West)	Neg	< 1	NA
2nd biofilter	Neg	< 1	NA
Greenhouse storage tank	Neg	< 1	NA
Tomato medium	Pos	< 1	Untypeable
Solid separation constructed wetland			
Fresh fecal	Neg	1.56	Untypeable
House effluent	Pos	3.26	Johannesburg (2)
Separated solids	Pos	3.26	Johannesburg (2)
Inner cell influent	Pos	3.26	Johannesburg (1) Typhimurium (1)
Outer cell influent	Pos	3.26	Johannesburg (1) Typhimurium (1)
Inner cell effluent	Neg	< 1	NA
Outer cell effluent	Neg	< 1	NA
Storage pond	Pos	< 1	Typhimurium (1)
Up-flow biofiltration			
Fresh fecal	Neg	< 1	NA
House effluent	Pos	3.26	Derby (2)
Separated liquids	Pos	3.26	Derby (2) Heidelberg (1)
Separated solids	Pos	4.06	Derby (2)
Equalization tank	Pos	2.56	Derby (2) Typhimurium (1)
Biofilters effluent	Pos	1.76	Derby (1) Johannesburg (1) Typhimurium (1)
Biofilters backwash	Pos	2.18	Derby (1)
Liquid storage (L1)	Pos	2.56	Typhimurium (1)
Solids reservoir (L2)	Pos	1.75	Muenchen (1)
Lagoon (L3)	Pos	1.96	Muenchen (2)
Multi-step biological and chemical			
Fresh fecal	Pos	1.43	Derby (3)
House effluent	Pos	1.00	Typhimurium (1)

Table 1: continued

Sample type	Positive/negative for *Salmonella*	Log MPN/g or mL	Serotype
Homogenization tank	Pos	2.73	Typhimurium (1) Infantis (2) Worthington (1)
Solid-liquid separation module	Pos	3.43	Derby (2)
Nitrification/de-nitrification tanks	Pos	1.76	Typhimurium (1)
Settling tank	Neg	< 1	NA
Treated effluent	Pos	< 1	Infantis (1)
Calcium phosphate	Neg	< 1	NA
High-solids anaerobic digester			
Effluent port 1	Neg	< 1	NA
Effluent port 2	Neg	< 1	NA
Effluent port 3	Pos	4.06	Senftenberg (2)
Conventional swine-production farm			
Fresh fecal	Neg	1.00	Anatum (1)
House effluent	Pos	1.28	Typhimurium (2)
Lagoon	Pos	2.89	Typhimurium (1) Anatum (1)

* Each treatment technology was implemented on a single commercial swine-production site and was operational for a limited period of time. Each farm was sampled once and each sampling point represents a composite sample. Composite samples were used to determine *Salmonella* presence and most probable number (MPN) population estimates using culture methods in Rappaport-Vassiliadis broth and modified lysine iron agar. The three-tube MPN estimation technique employed serial dilution in triplicate tubes. Both culture methods were plated in duplicate. Presence or absence results are reported as positive (Pos) or negative (Neg) for *Salmonella*. Log MPN results are reported as the base10 logarithm of the MPN of *Salmonella* detected per g or mL of sample. The minimum detection limit for the MPN procedure was 10 cells/g or mL of sample (1 log MPN/g or mL). Serotyping was based on the Kauffmann-White Scheme. All *Salmonella* serotype Typhimurium isolates were classified as var Copenhagen.

NA = not applicable. *Salmonella* was not recovered by either procedure.

study. Similar to the findings of this study, *Salmonella* serotypes with multiple-drug resistance characteristics have been previously isolated from production animals and foods of animal origin.[15,16]

This multiple-farm survey provides an initial assessment of populations, serotypes, and antibiotic resistance of *Salmonella* recovered from environmentally superior swine waste-treatment technologies which would benefit future risk-assessment studies directed at these waste-management practices. Limitations of this study include single sampling of each site due to the brevity of the evaluation period, and variations in the production stage (finisher pigs versus sows) evaluated across the different waste-treatment technologies. Future pathogen research should include repeated sampling of swine waste-treatment technologies across seasons.

Implications

- The technologies surveyed appear promising for reducing swine-manure *Salmonella* populations.
- Further study over an extended time frame is warranted prior to drawing any conclusions about the efficacy of these waste-treatment technologies in reducing *Salmonella* populations.

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