Investigations into the Infection-Contamination-Infection Cycle of Zoonotic Salmonella on Swine Farms: Investigation into the Occurrence of *Salmonella* in the Environment on Four Selected Minnesota Swine Farms

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Introduction

Experience from the poultry industry (1, 2) and research results about the effect of segregated early weaning on the occurrence of Salmonella in swine (3), suggest that apart from the animal to animal transmission, other sources of salmonella infection may play an important role in the on-farm Salmonella epidemiology.

It was, therefore thought that, to test the hypothesis of the risk of permanently introducing *Salmonella* into a swine herd from its environment as an explanation for the recently identified changes of the *Salmonella* prevalence on swine farms over time (1), may add to the current knowledge on salmonella in swine. Therefore, an investigation into the *Salmonella* load of the environments of farms with a different *Salmonella* prevalence in their slaughter hogs was carried out. Four Minnesota farms out of a previous study (1) on the occurrence of *Salmonella* in slaughter hogs were selected: 1 farm with a high prevalence (HP farm = >5%), 2 farms with a medium prevalence (MP1 farm and MP2 farm = >1%, but < 5%), and 1 farm with a very low herd prevalence (LP farm = <1%).

This paper presents the findings of the *Salmonella* occurrence and the serovar pattern in the environment of these 4 selected Minnesota swine farms.

Material and Methods

Sampling the environment:

Samples from the environment were taken simultaneously with those of the study pigs on each farm. Additionally, samples from post-cleaned rearing environments were taken when the opportunity existed. These samples included feed, feces, organic matter, dust and swabs of organic and inorganic material in the environment. Samples were taken using aseptic technique. The environment samples were from the direct (intimate contact) and indirect (outside of the study animals' direct contact) environment of the study group pigs on each farm.

The environmental samples were categorized as E I, E II and E III:

E I - direct contact with the study group animals (i.e. pen surfaces, feeders, water source, gating, feces and other organic matter).

E II - indirect contact with the study animals, but in close proximity (water, feed and electrical lines/conduit, surfaces of ventilation and heating equipment, walk-ways within the study group rooms, any in-room gated-off surfaces).

E III - indirect contact with the study animals, but outside the rearing environment of the study pigs (organic matter outside the buildings, walk-ways between buildings, feed trucks, offices, etc.)

Culturing the samples:

All environment and pig samples were cultured within 24 hours post collection. Ten grams of the environmental samples (feces, feed, dust, soil, water etc.) were placed into sterile plastic bags. Lymph nodes were processed using 95% alcohol to flame on lymph node surface for a few seconds to decontaminate the surface and then placed into a sterile sample bag and disrupted using a hammer to expose the interior. The processed samples (environment and lymph node) were incubated with 100ml of freshly prepared Tetrathionate Broth (Tetrathionate Broth Base, Difco Laboratories, Detroit, MI 48232-7058) containing iodile (potassium iodide, certified A.C.S., Fisher Scientific, Fair Lawn, NJ 07410) in sterile sampling bags or in sterile 25X150 mm disposable tubes (Fisher Scientific, Pittsburgh, PA 15219) for 22-26 hours at 37°C. After initial incubation, a swab was used to transfer 100ul of the Tetrathionate broth culture to 10µl of Rappaport-Vassiliadis R10 Broth (Difco Laboratories, Detroit, MI 48232-7058) media in 16 X 150 mm sterile disposable tubes, and incubated at 37°C for 22-26 hours.10ml loops (Nalge Nunc International, Denmark) were used to strike the Rappaport-Vassilaidis media culture on to XLT-4 Agar (Difco Laboratories, Detroit, MI 48232-7058) and BG Sulfur Agar (Difco Laboratories, Detroit, MI 48232-7058) medium plates. Plates were incubated for 22-

26 hours at 37°C. *Salmonella* suspect colonies were identified, and a single colony was placed into biochemical test tubes, Triple Sugar Iron Agar, Lysine Iron Agar, MIO, and Urea Agar (Difco Laboratories, Detroit, MI 48232-7058) and incubated for 22-26 hours at 37°C.

Serotyping isolates:

Confirmed *Salmonella* isolates, based on the biochemical test results, and were serotyped using the *Salmonella* O- and H-antigen sera (polyvalent, group, and monovalent, Difco Laboratories, Detroit, MI 48232-7058

Results

In total, 620 of 6572 environmental samples were positive (10.6 %). Adding E I-, E II- and E III-samples per farm, the environmental *Salmonella* load "mimicked" to a certain degree the high, medium and low herd prevalence of the study herds. However, excluding the E I-samples (E I is dependent on the *Salmonella* shedding by the animals) from the analysis, all four farms had a quite similar *Salmonella* load in their E II- and E III-samples: about 5.9 % of the samples that were from material with only a potential, indirect contact with the animals were positive (Table 1).

Twenty different serovars were identified in the four farm environments (Table 2): 16 on the high prevalence farm, 6 on each of the medium prevalence farms and 5 on the low prevalence farm. Some of the serovars (e.g. *S. Mbandaka* and *S. Montevideo* and *S. Give*) predominate on only one farm, whereas others (e.g. *S. Typhimurium*, *S. Agona* and *S. Derby*) were found on all four farms.

Discussion

The results of culturing environmental samples on the four farms show an unexpected high percentage of material on swine farms being contaminated by *Salmonella spp.* The number of different serovars identified per farm was also unexpectedly high, which leads to the assumption that there is a multitude of sources for the introduction of *Salmonella spp.* into swine herds, which could be confirmed by subtyping the *S. Typhimurium* strains (5). The most striking result, however, is that the environments, to which the animals have no direct contact (E II and E III), were as highly contaminated on the farms with medium and low salmonella prevalence in the slaughter hogs as on the high prevalence farm. This suggests that the daily working procedures determine to which extend and how often the *Salmonella spp.* of the environment are introduced into the herd.

Table 1: Environmental samples positive by farm

Type of Sample	HP farm	MP1 farm	MP2 farm	LP farm
Feces	22.0 %(116/525)	7.3 % (8/110)	5.8 % (6/104)	0.8 % (1/131)
Feed (trough)	4.1 % (7/172)	2.7 % (3/106)	0.0 % (0/93)	3.3 % (1/58)
Dust	16.1 % (26/162)	12.0 % (13/108)	2.2 % (2/91)	5.4 % (7/129)
Spilled feed	45.% % (5/11)	54.5 % (6/11)	0.0 % (0/9)	13.9 % (5/36)
Water source	0.0 % (0/12)	18.2% (2/11)	0.0 % (0/18)	16.7 % (1/6)
Organic material	14.0 % (7/50)	38.5 % (5/13)	27.3 % (3/11)	19.1 % (4/21)
Boot swab	16.7 % (25/150)	28.6 % (2/7)	0.0 % (0/5)	13.9 % (5/36)
Mice	0.0 % (0/1)	7.7 % (1/13)	12.5 % (1/8)	0.0 % (0/9)
E I + E II + E III	14 %(186/1303)	10.7 % (41/385)	4.0 % (14/347)	4.9 % (20/409)
E II + E III	6.7 % (42/627)	11.6 % (31/268)	3.8 % (9/328)	5.8 % (14/242)

Table 2: Serovars by Environment Sample and Farm

Sample Type	HP farm	MP1 farm	MP2 farm	LP farm
Feces	Agona, Anatum, Benfica, Cubana, Derby, Havana, Mbandaka, Newington, Typhimurium, untypable	Cerro, Typhimurium, Senftenberg, Bredeney, Give	Derby, Infantis, Montevideo, Tennessee, Typhimurium	Agona, Derby, Give, Typhimurium
Feed (trough)	Cubana, Derby, untypable	Cerro, Typhimurium		Derby
Dust	Agona, Cerro, Cubana, Derby, Mbandaka, Montevideo, Ohio, Rissen, untypable	Cerro, Typhimurium, Bredeney	Typhimurium	Bredeny, Derby, Give
Spilled feed	Cerro, Typhimurium, Bredeney	Cerro, Typhimurium, Bredeney	Agona, Infantis, Typhimurium	Agona
Water source	Agona, Typhimurium	Agona, Typhimurium		Derby
Organic material	Agona, Derby, Mbandaka	Cerro, Typhimurium	Typhimurium	Derby, Typhimurium
Boot swab	Agona, Anatum, Cerro, Cubana, Derby, Budapest, Mbandaka, untypable	Typhimurium		Derby, Give
Mice		Bredeney	Infantis	
E I + E II + E III	16	6	6	5
E II + E III	13	4	3	4

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