RESEARCH REPORT
PORK SAFETY

Title: Longitudinal study to determine *Salmonella* serovars and identify risk factors associated with their dissemination in commercial swine farm – **NPB #12-001** revised

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Industry Summary

The purpose of this study was to determine what *Salmonella* serotypes are present in pigs and their environment on commercial swine farms, and to further characterize and compare *Salmonella* from different sources. *Salmonella* isolates were obtained during a longitudinal study sampling pigs and their environment from 30 commercial swine farms in North Carolina. This study followed ten cohorts (groups) of pigs from farrowing to slaughter, sampling the pigs, farm environment, pig carcasses and the slaughter environment at various stages of production. Sampling was carried out from October 2008 to December 2010 at various stages of production, including once at farrowing (7-10 day old), twice at each of nursery (4 and 7 weeks of age) and finishing stages (16 and 26 weeks of age), and finally once at slaughter. During the farrowing stage, a cohort of 35 healthy piglets per farm (4 piglets/sow) were selected and ear tagged for identification; subsequently, sampling followed the same cohort of pigs at different sampling stages during farm and slaughter stages. *Salmonella* isolates collected during this study were sent to the National Veterinary Services Laboratories (NVSL) to determine the serotype. A representative subset (n=272) of *Salmonella* was genotyped using Pulsed-Field Gel Electrophoresis (PFGE) to determine the specific strain, or PFGE fingerprint profile, at different stages of production. Comparisons were then made between PFGE strains and serotypes found in pigs and the farm environment. Even though the focus of this grant was at the farm level, for providing more in depth information to the scientific community we compared fingerprint profiles of *Salmonella* isolated from carcasses of the same pigs at slaughter with the farm isolates. Serotyping revealed 22 different serotypes found in pig and environmental samples on farm and at slaughter, with *Salmonella* Typhimurium being the most common. Genotyping analysis revealed 47 clusters containing 100% similar *Salmonella* isolates among pig and environmental isolates, including feed, water, soil, lagoon, floor swabs and slaughter lairage both within and between cohorts. In addition, 41 unique strains were also detected. This indicates that certain serotypes and strains are present in pigs and their environment throughout the pork chain, irrespective of the farm or stage of production. We also found evidence highlighting the clear role played by the environment in the persistence and dissemination of *Salmonella* to conventionally reared pigs at farm and slaughter.

Keywords

Salmonella, serotype, PFGE genotyping, swine, environment, slaughter,

Scientific Abstract
The aim of this study was to characterize and compare *Salmonella* isolates from pig and environmental sources in conventional swine farms on a phenotypic and genotypic level. Isolates were previously obtained during a longitudinal study examining the prevalence and distribution of *Salmonella* in ten cohorts of conventional swine and their environment throughout the production chain (farrowing to slaughter). Fresh fecal samples (10 g) were collected from piglets using sterile fecal loops (Webster Veterinary, Devens, MA), and from their respective sows, to aid in the determination of the transmission of *Salmonella* from sows to piglets at birth. Similarly, fecal samples were collected from the ear tagged pigs twice at each of nursery and finishing stages using gloved hands. Environmental samples also were collected at every stage of sampling to determine the role played by the environment as a reservoir and in the transmission of *Salmonella* to/from the pigs. Overall, we collected a total of 1650 fecal, 250 each of feed, water, floor swabs, 245 lagoon and 80 inter-farm truck samples from 30 (representing 10 cohorts of pigs) conventional farms and their environment. These isolates were submitted to the National Veterinary Science Laboratory for serotyping. We identified 22 different serotypes of *Salmonella*, with the predominate serotype being S. Typhimurium (33.7%), followed by S. Infantis (16.4%), S.Derby (11.4%), S. Anatum (9.1%) and S. Ouakam (7.8%). A representative subset of 272 isolates was also characterized on the genotypic level using the gold-standard method of PFGE. PFGE analysis identified 47 clusters with 100% genotypic similarity among pig, environment and slaughter isolates both within and between sample cohorts. The dynamics of *Salmonella* prevalence in pigs and carcasses were reciprocated in the farm and slaughter environment clearly indicating an exchange of this pathogen between the pigs and their surroundings. These results indicate the important role played by the farm and slaughter environment in the persistence and dissemination of *Salmonella* on conventional swine farms.

Introduction
Salmonella is among the leading causes of bacterial foodborne illness in the United States and globally. In the United States, *Salmonella* is responsible for the highest number of foodborne related illnesses with a reported 1.4 million illnesses, 15,000 hospitalizations and deaths of more than 500 people each year (1). It is important to note that the actual incidence of salmonellosis is estimated to be 38 times the number of reported cases (2). *Salmonella* is also an important pathogen in animal health. In particular, the emergence of antimicrobial resistant foodborne pathogens has raised public alarm. Thus, understanding the molecular epidemiology of *Salmonella* circulating in conventional swine farms is of great importance to both human and animal health. Phenotypic and genotypic analyses have shown that the environment and pre-slaughter handling, such as transport and lairage, play a significant role in the dissemination of this pathogen in pigs (3-5). It is quite evident that the environment plays a crucial role as a reservoir in transmission of AR pathogens to pigs all along the production chain, either directly or indirectly (4, 6, 7). Reducing *Salmonella* prevalence in conventional swine herds will both increase production and reduce costs in industry, but will also reduce the potential for outbreaks of human foodborne illness. Using phenotypic and genotypic methods to further characterize *Salmonella* isolated from pigs, the farm environment and at slaughter will allow for better and more targeted biosecurity and prevention measures to be put in place to reduce illness and burden.

Objectives
We proposed to serotype multiple isolates of *Salmonella* isolated from 30 commercial swine farms as part of a USDA funded longitudinal study that has recently been completed in North Carolina. The main aim was to fill some of the gaps that exist in our understanding of the distribution and persistence of *Salmonella* serovars in commercial swine farms.

The specific objectives were:
1) Determine the distribution and persistence of *Salmonella* serovars in pigs and their environment on commercial swine farms;

2) Characterize and compare the isolates from different sources on farm and the environment at the phenotypic (serotyping) and genotypic (Pulsed field gel electrophoresis profile) levels and;
3) Identify the risk factors based on questionnaire data that are associated with the dissemination and persistence of *Salmonella* serovars in pigs and their environment on farm.

Materials & Methods

Sample and *Salmonella* isolate origin

Salmonella isolates from pigs and their environment were collected as part of longitudinal study conducted from October 2008 to December 2011 on 30 conventional farms at different stages of production from farm to slaughter in North Carolina. In this production system, pigs were reared indoors and followed an all-in-all-out (AIAO) production system. These conventionally raised pigs were given antimicrobials for prophylaxis and therapeutic purposes. The details of the study design, sampling and microbiological methods, estimates of *Salmonella* prevalence in pigs and their environment at farm and slaughter, antimicrobial susceptibility profiles, and their phenotypic and genotypic characterizations has been reported elsewhere (8). Briefly, fecal samples from ten cohorts (35 pigs/cohort) and associated environmental samples (soil, water, feed, floor swabs, lagoon and truck floor swabs) were collected at various stages of production, including once at farrowing (7-10 day old) and twice each at the nursery (4 and 7 weeks of age) and finishing stages (16 and 26 weeks of age). Post-evisceration and post-chill swabs, along with mesenteric lymph nodes (MLN), were collected from the pig carcasses along with lairage and truck floor samples representing the slaughter environment.

Salmonella serotyping

We will follow the Kauffman-White scheme for serotyping 922 *Salmonella* where the isolates will be shipped to the National Veterinary Sciences Laboratories (NVSL) in Iowa. Briefly, the isolates will be cultured overnight at 37^{o}C on Luria-Bertani (LB) agar (Statens Serum Institute, Denmark). Polyvalent somatic antigen (O) testing will be completed from a concentrated isolate and saline mixture made by flushing the growth off of the previous TSI slant. After the O antigens have been identified, we will test for the polyvalent flagellar antigen.

Pulsed Field Gel Electrophoresis (PFGE) analysis

A subset of 272 *Salmonella* will be genotypically analyzed by PFGE as recommended previously (9). Briefly, overnight culture cells will be mixed with an equal volume of agarose and dispensed into a mold to form agarose plugs to extract the DNA. The agarose embedded DNA will be digested with the specific restriction enzyme. The restriction digested DNA will then be separated using CHEF-DRIII pulsed field gel electrophoresis (Biorad, CA) apparatus. The gel will be stained in ethidium bromide. Gel Doc 2000 (Biorad) CCD camera will be used to capture fingerprint images. Analysis of PFGE data will be performed using Bionumerics 4.0 software (Applied Maths, Belgium) and the patterns will be compared by the Dice coefficient and the UPGMA method.

Statistical Analysis

Statistical analysis was carried out using STATA version 12.1 (Stata Corp, College Station, Texas). Each farm type stage of production and type of sample collected for isolation of *Salmonella* was considered for descriptive analysis before forcing these variables for multivariable modeling. Contingency table analyses without adjustments for clustering by cohort were carried out using likelihood ratio (LR) χ^2 test statistics for each of the variable/s types and used to examine their association with *Salmonella* prevalence. The LR χ^2 test for *Salmonella* prevalence was also carried out for the source of sampling and stages of production. Separate multivariable analyses for pigs versus their environment were carried out using the logistic regression procedure (XTLOGIT) with either random effects (RE) or generalized estimating equation (GEE) models. The XTLOGIT procedure was used instead of XTMELOGIT (multi-level hierarchical logistic regression) because of problems achieving convergence in XTMELOGIT given the high numbers of zero cells in the ABF farm type. The main effects of stage of production and sample type along with their 2-way interaction terms were tested. The final

full factorial RE or GEE model was constructed for both main effects and their interaction terms. The final significant model (all variable sets $P < 0.05$) was selected based on the associations of these variables and their interaction terms with the prevalence of *Salmonella*. The same procedure was repeated by forcing cohorts for robust variance estimation and compared. From the final model marginal predictions were obtained for the proportion of positive *Salmonella* and these were estimated with 95% confidence intervals. The marginal means were plotted using final predictions from the full factorial RE or GEE models for: 1) *Salmonella* prevalence among pigs by different stages of production, 2) *Salmonella* prevalence in environmental samples and different stages of production, and 3) *Salmonella* prevalence in environmental samples and different stages of production accounting for sample type differences.

Results

Salmonella serotype distribution

In total, 922 *Salmonella* isolates from pigs (n=189), the farm environment (n=439), carcasses (n=197) and the slaughter environment (n=97) were serotyped using one of two methods. Initially, a multiplex PCR was performed to identify *Salmonella* Typhimurium using published primers and protocol. In this method a total of 238 isolates were serotyped. Remaining isolates (n=684) were sent to the National Veterinary Services Laboratories (NVSL) for serotyping. In this study we identified 22 different serotypes among pigs and their environment (Table 1). At the farm level, S. Typhimurium (pig: 28.5%, environment: 35%), S. Infantis (pig: 16.4%, environment: 13.8%), S. Anatum (pig: 15.8%, environment: 12%) and S. Rissen (pig: 3%, environment: 8.8%) were the most commonly detected serotypes. At slaughter, S. Typhimurium (carcass: 37%, environment: 30%), S. Derby (carcass: 35.5%, environment: 30%) and S. Infantis (carcass: 6.5%, environment, 48.4%) were most prevalent. Our study highlights the identification of S. Rissen in pigs and their environment. S. Rissen has not been documented in animals in the US, yet is the most common non-human serotype in Asian countries.

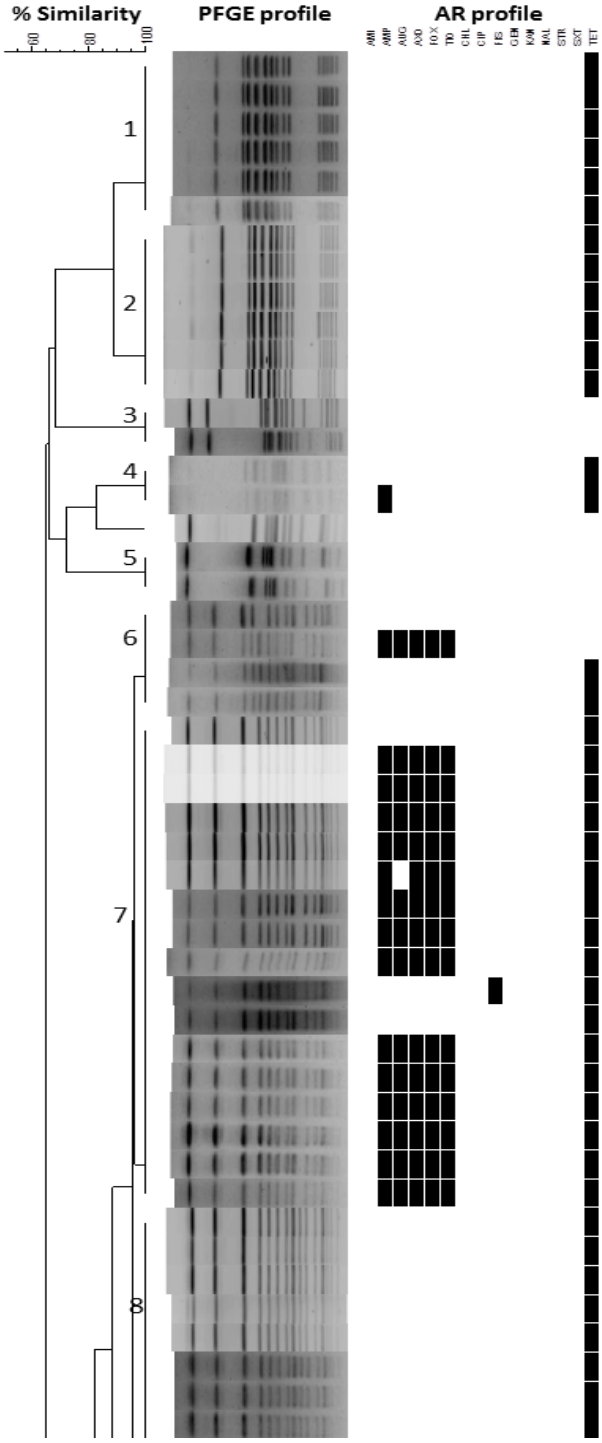
Table 1. Distribution of *Salmonella* serotypes from pigs and the environment at farm and slaughter.

Serotypes Identified	Farm		Slaughter	
	Pigs n=189	Environment n=439	Carcasses n=197	Environment n=97
S. Agona	0[a]	0	1 (0.5)	0
S. Anatum	30 (15.8)	53 (12)	6 (3)	15 (15.3)
S. Braenderup	0	1 (0.2)	0	0
S. Cerro	12 (6.3)	3 (0.6)	0	0
S. Derby	4 (2.1)	31 (7)	70 (35.5)	1 (1)
S. Heidelberg	0	17 (3.8)	0	0
S. Infantis	31 (16.4)	61 (13.8)	13 (6.5)	47 (48.4)
S. Inverness	0	2 (0.4)	0	0
S. Johannesburg	0	1 (0.2)	5 (2.5)	0
S. London	0	3 (0.6)	0	2 (2)
S. Mbandka	2 (1)	2 (0.4)	3 (1.5)	1 (1)
S. Muenchen	3 (1.5)	0	0	0
S. Newport	0	1 (0.2)	0	0
S. Ohio	20 (10.5)	24 (5.4)	6 (3)	1 (1)
S. Ouakam	23 (12.1)	41 (9.3)	8 (4)	0
S. Rissen	6 (3.1)	39 (8.8)	1 (0.5)	0
S. Rough_O:r:1,5	0	0	9 (4.5)	0
S. Schwarzengrund	0	2 (0.4)	0	0
S. Senftenberg	4 (2.1)	2 (0.4)	1 (0.5)	0
S. Typhimurium	54 (28.5)	154 (35)	73 (37)	30 (30.1)
S. Typhimurium Var 5	0	0	1 (0.5)	0
6,7, Non motile	0	2 (0.4)	0	0

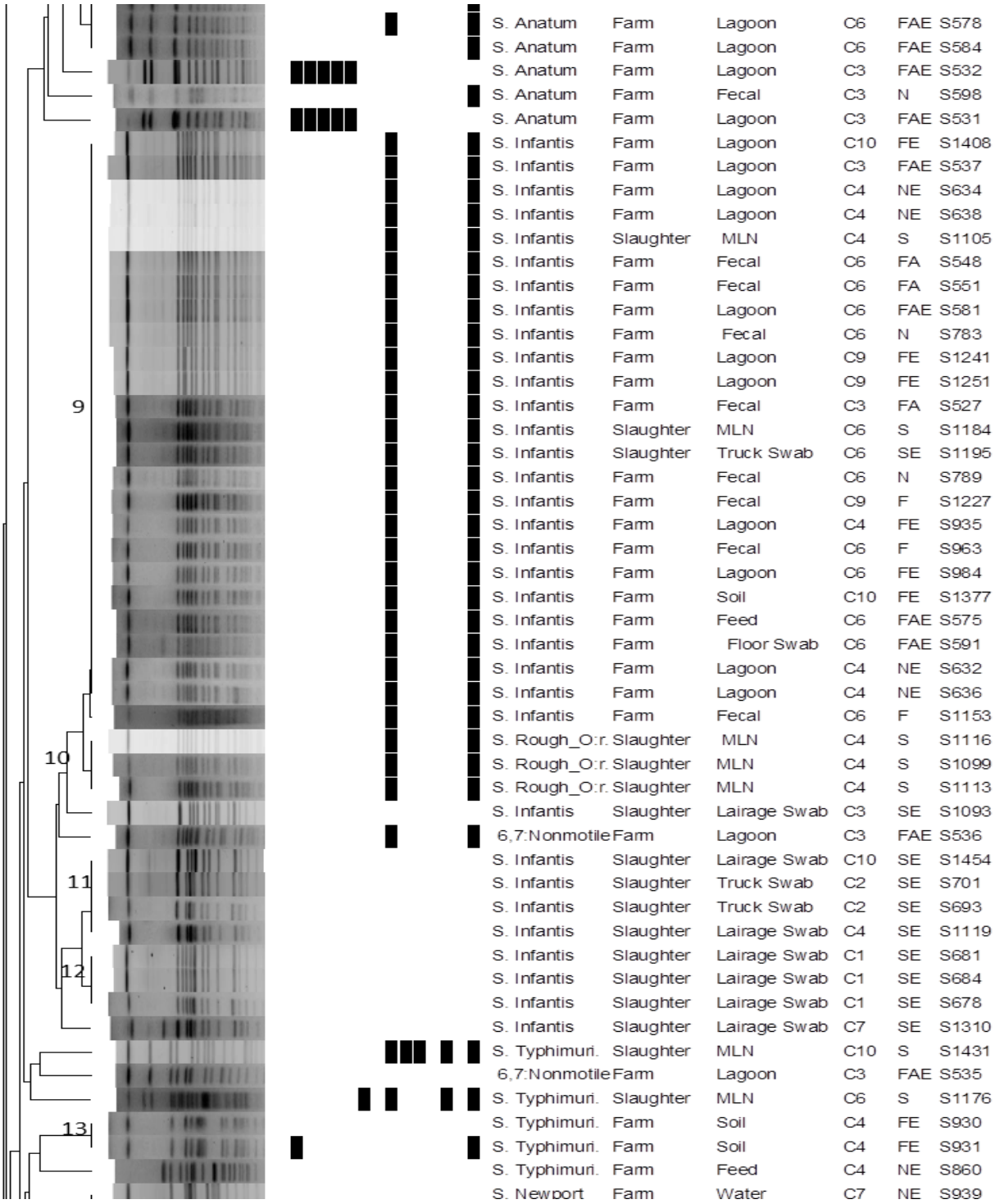
[a] Number (Percent)

***Salmonella* genotyping profiles**

A subset of 272 *Salmonella* isolates from pigs and environment, which were representative of different sampling stage, type of samples, serotype and antimicrobial resistant profiles, were genotyped using PFGE according to the PulseNet protocol. Restriction analysis by *Xba*I produced on average 10-16 bands and distributed the 272 isolates into 47 clusters, consisting of isolates with similar PFGE profiles, and another 41 unique PFGE patterns represented by a single isolate each (Fig. 1). Genotyping results showed 100% genotypic similarity among fecal and environmental isolates including feed, water, soil, lagoon, floor swab and lairage with in the cohort and also between the cohorts with similar resistance pattern and serotype.



Serotype	Location	Sample Type	Cohort	Stage	ID
S. Rissen	Farm	Fecal	C3	N	S812
S. Rissen	Farm	Feed	C3	NE	S823
S. Rissen	Farm	Floor Swab	C3	NE	S824
S. Rissen	Farm	Water	C3	NE	S833
S. Rissen	Farm	Lagoon	C3	NE	S836
S. Rissen	Farm	Floor Swab	C3	NE	S604
S. Rissen	Farm	Floor Swab	C3	NE	S829
S. Rissen	Farm	Floor Swab	C3	NE	S830
S. Rissen	Farm	Lagoon	C3	NE	S841
S. Rissen	Farm	Floor Swab	C3	FE	S1004
S. Rissen	Farm	Floor Swab	C3	FE	S1009
S. Rissen	Slaughter	MLN	C3	S	S1031
S. Inverness	Farm	Truck Swab	C3	NE	S542
S. Inverness	Farm	Truck Swab	C3	NE	S543
S. Heidelberg	Farm	Feed	C2	NE	S429
S. Derby	Farm	Feed	C2	NE	S430
S. Infantis	Slaughter	Carcass Swab	C10	PE	S1448
S. Infantis	Slaughter	Carcass Swab	C10	PC	S1446
S. Infantis	Slaughter	Lairage Swab	C11	SE	S1449
S. Anatum	Slaughter	Lairage Swab	C3	SE	S1049
S. Anatum	Slaughter	Lairage Swab	C3	SE	S1090
S. Anatum	Farm	Floor Swab	C10	NE	S1220
S. Anatum	Farm	Fecal	C7	F	S1259
S. Anatum	Farm	Lagoon	C10	FAE	S1170
S. Anatum	Farm	Fecal	C5	FA	S596
S. Anatum	Farm	Fecal	C5	N	S732
S. Anatum	Farm	Floor Swab	C5	NE	S762
S. Anatum	Farm	Lagoon	C5	NE	S774
S. Anatum	Farm	Fecal	C5	N	S864
S. Anatum	Farm	Fecal	C3	N	S813
S. Anatum	Farm	Fecal	C5	N	S863
S. Anatum	Farm	Floor Swab	C5	NE	S869
S. Anatum	Farm	Lagoon	C10	FAE	S1169
S. Anatum	Farm	Lagoon	C10	FAE	S1174
S. Anatum	Farm	Fecal	C5	N	S740
S. Anatum	Farm	Floor Swab	C5	NE	S759
S. Anatum	Farm	Water	C5	NE	S767
S. Anatum	Farm	Lagoon	C5	NE	S768
S. Anatum	Farm	Lagoon	C5	NE	S772
S. Anatum	Farm	Fecal	C5	FA	S595
S. Anatum	Farm	Fecal	C6	FA	S562
S. Anatum	Farm	Fecal	C6	FA	S567
S. Anatum	Farm	Lagoon	C6	FAE	S587
S. Anatum	Farm	Lagoon	C6	FE	S980
S. Anatum	Farm	Lagoon	C6	FE	S983
S. Anatum	Farm	Fecal	C6	FA	S557
S. Anatum	Farm	Soil	C6	FAE	S569
S. Anatum	Farm	Feed	C6	FAE	S572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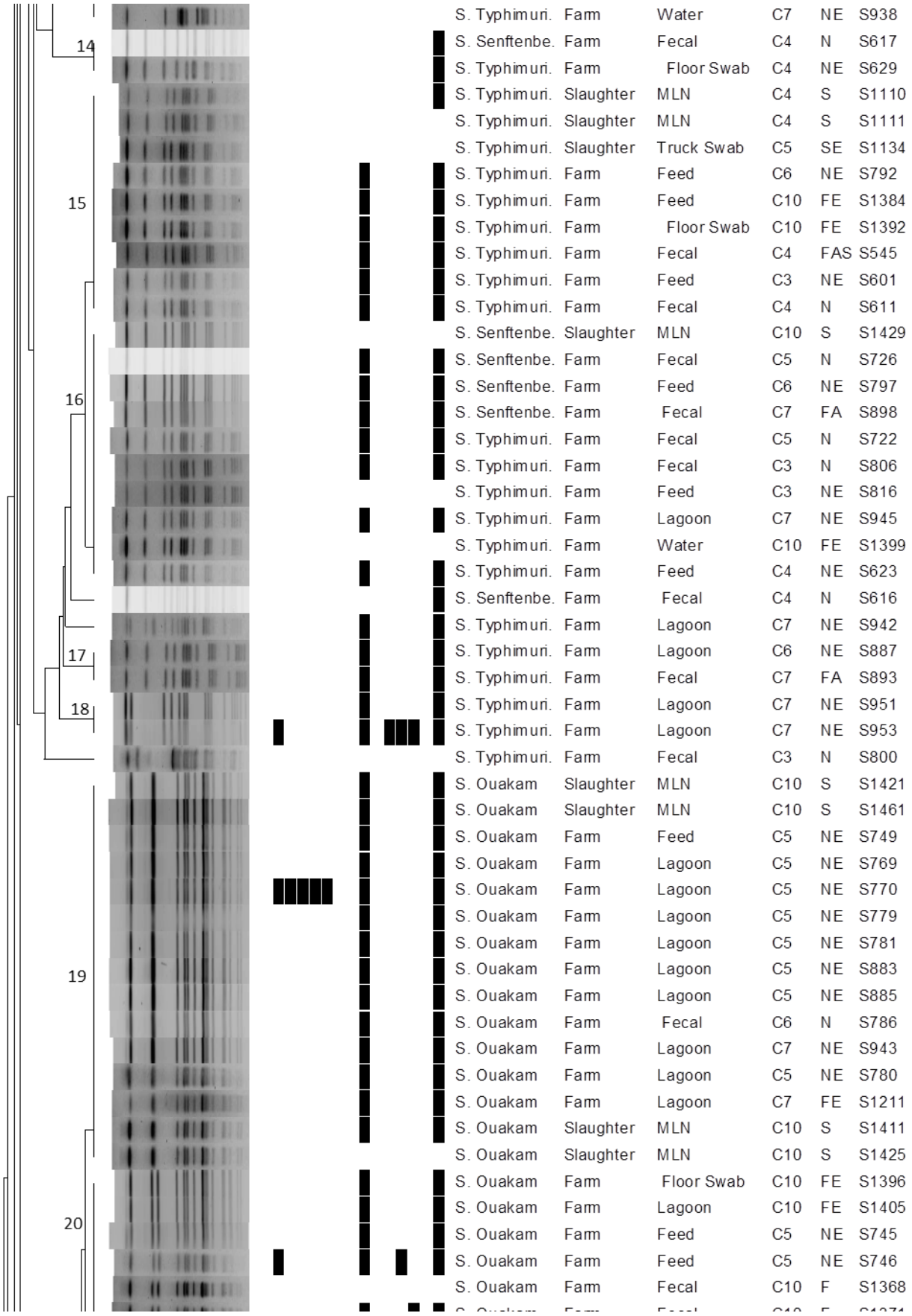
S. Anatum Farm Fecal C3 N S598
S. Anatum Farm Lagoon C3 FAE S531
S. Infantis Farm Lagoon C10 FE S1408
S. Infantis Farm Lagoon C3 FAE S537
S. Infantis Farm Lagoon C4 NE S634
S. Infantis Farm Lagoon C4 NE S638
S. Infantis Slaughter MLN C4 S S1105
S. Infantis Farm Fecal C6 FA S548
S. Infantis Farm Fecal C6 FA S551
S. Infantis Farm Lagoon C6 FAE S581
S. Infantis Farm Fecal C6 N S783
S. Infantis Farm Lagoon C9 FE S1241
S. Infantis Farm Lagoon C9 FE S1251
S. Infantis Farm Fecal C3 FA S527
S. Infantis Slaughter MLN C6 S S1184
S. Infantis Slaughter Truck Swab C6 SE S1195
S. Infantis Farm Fecal C6 N S789
S. Infantis Farm Fecal C9 F S1227
S. Infantis Farm Lagoon C4 FE S935
S. Infantis Farm Fecal C6 F S963
S. Infantis Farm Lagoon C6 FE S984
S. Infantis Farm Soil C10 FE S1377
S. Infantis Farm Feed C6 FAE S575
S. Infantis Farm Floor Swab C6 FAE S591
S. Infantis Farm Lagoon C4 NE S632
S. Infantis Farm Lagoon C4 NE S636
S. Infantis Farm Fecal C6 F S1153
S. Rough_O:r. Slaughter MLN C4 S S1116
S. Rough_O:r. Slaughter MLN C4 S S1099
S. Rough_O:r. Slaughter MLN C4 S S1113
S. Infantis Slaughter Lairage Swab C3 SE S1093
6,7:Nonmotile Farm Lagoon C3 FAE S536
S. Infantis Slaughter Lairage Swab C10 SE S1454
S. Infantis Slaughter Truck Swab C2 SE S701
S. Infantis Slaughter Truck Swab C2 SE S693
S. Infantis Slaughter Lairage Swab C4 SE S1119
S. Infantis Slaughter Lairage Swab C1 SE S681
S. Infantis Slaughter Lairage Swab C1 SE S684
S. Infantis Slaughter Lairage Swab C1 SE S678
S. Infantis Slaughter Lairage Swab C7 SE S1310
S. Typhimuri. Slaughter MLN C10 S S1431
6,7:Nonmotile Farm Lagoon C3 FAE S535
S. Typhimuri. Slaughter MLN C6 S S1176
S. Typhimuri. Farm Soil C4 FE S930
S. Typhimuri. Farm Soil C4 FE S931
S. Typhimuri. Farm Feed C4 NE S860
S. Newport Farm Water C7 NE S939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S. Typhimuri. Slaughter Truck Swab C5 SE S1134
S. Typhimuri. Farm Feed C6 NE S792
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S. Typhimuri. Farm Fecal C3 N S806
S. Typhimuri. Farm Feed C3 NE S816
S. Typhimuri. Farm Lagoon C7 NE S945
S. Typhimuri. Farm Water C10 FE S1399
S. Typhimuri. Farm Feed C4 NE S623
S. Senftenbe. Farm Fecal C4 N S616
S. Typhimuri. Farm Lagoon C7 NE S942
S. Typhimuri. Farm Lagoon C6 NE S887
S. Typhimuri. Farm Fecal C7 FA S893
S. Typhimuri. Farm Lagoon C7 NE S951
S. Typhimuri. Farm Lagoon C7 NE S953
S. Typhimuri. Farm Fecal C3 N S800
S. Ouakam Slaughter MLN C10 S S1421
S. Ouakam Slaughter MLN C10 S S1461
S. Ouakam Farm Feed C5 NE S749
S. Ouakam Farm Lagoon C5 NE S769
S. Ouakam Farm Lagoon C5 NE S770
S. Ouakam Farm Lagoon C5 NE S779
S. Ouakam Farm Lagoon C5 NE S781
S. Ouakam Farm Lagoon C5 NE S883
S. Ouakam Farm Lagoon C5 NE S885
S. Ouakam Farm Fecal C6 N S786
S. Ouakam Farm Lagoon C7 NE S943
S. Ouakam Farm Lagoon C5 NE S780
S. Ouakam Farm Lagoon C7 FE S1211
S. Ouakam Slaughter MLN C10 S S1411
S. Ouakam Slaughter MLN C10 S S1425
S. Ouakam Farm Floor Swab C10 FE S1396
S. Ouakam Farm Lagoon C10 FE S1405
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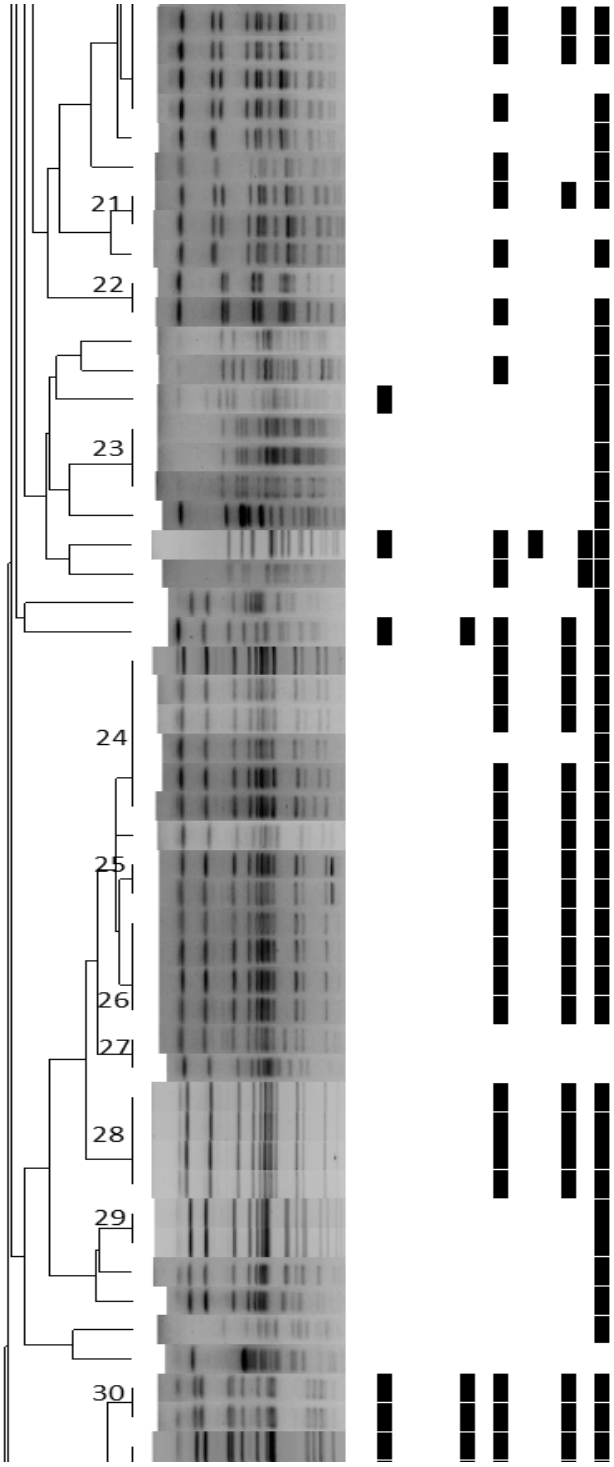
S. Ouakam Farm Feed C5 NE S746
S. Ouakam Farm Fecal C10 F S1368



S. Ouakam	Farm	Fecal	C10	F	S1371
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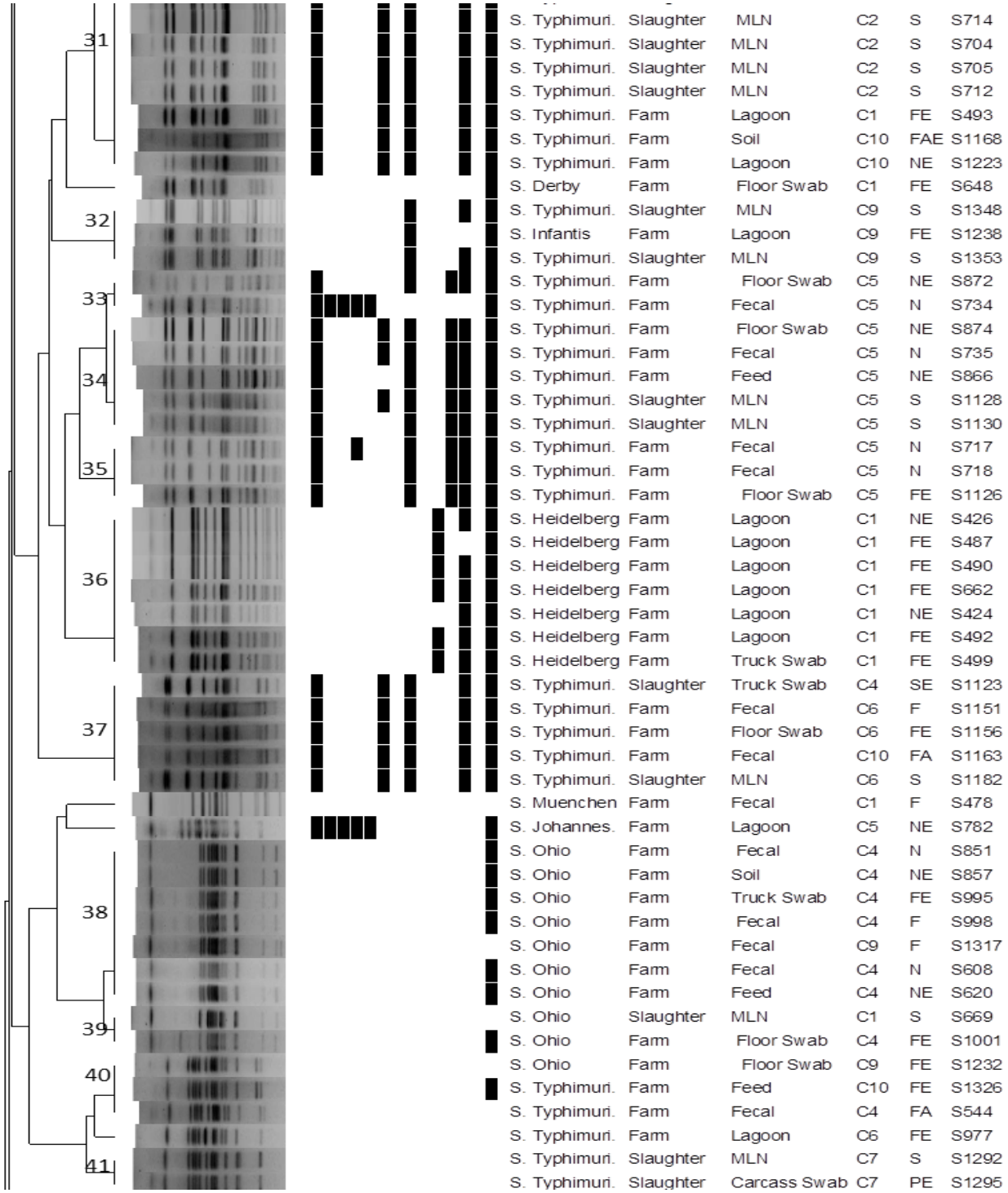
S. Ouakam	Farm	Floor Swab	C10	FE	S1397
S. Ouakam	Farm	Lagoon	C10	FE	S1400
S. Schwarze.	Farm	Feed	C6	FE	S967
S. Ouakam	Farm	Lagoon	C5	NE	S878
S. Ouakam	Farm	Fecal	C7	FA	S891
S. Ouakam	Farm	Fecal	C7	FAS	S911
S. Ouakam	Farm	Floor Swab	C7	FAE	S914
S. Ohio	Farm	Feed	C9	FE	S1228
S. Typhimuri.	Farm	Water	C9	FE	S1323
S. Anatum	Farm	Fecal	C2	FA	S319
S. Ouakam	Farm	Floor Swab	C6	FE	S970
S. Anatum	Farm	Fecal	C2	FA	S320
S. Anatum	Farm	Feed	C7	FE	S1199
S. Anatum	Farm	Floor Swab	C7	FE	S1202
S. Johannes.	Slaughter	Carcass Swab	C7	PE	S1298
S. Typhimuri.	Farm	Truck Swab	C1	FE	S496
S. Heidelberg	Farm	Lagoon	C3	NE	S607
S. Typhimuri.	Farm	Lagoon	C1	FE	S481
S. Typhimuri.	Farm	Soil	C4	NE	S644
S. Typhimuri.	Farm	Floor Swab	C2	FE	S647
S. Derby	Farm	Floor Swab	C2	NE	S329
S. Derby	Farm	Floor Swab	C2	NE	S324
S. Derby	Farm	Fecal	C2	N	S427
S. Infantis	Farm	Lagoon	C2	NE	S435
S. Derby	Farm	Floor Swab	C2	FE	S502
S. Derby	Slaughter	Lairage Swab	C7	SE	S1313
S. Derby	Farm	Floor Swab	C2	NE	S432
S. Derby	Slaughter	MLN	C3	S	S1019
S. Derby	Slaughter	Carcass Swab	C3	PE	S1082
S. Derby	Farm	Lagoon	C3	FE	S1010
S. Derby	Slaughter	MLN	C3	S	S1048
S. Derby	Farm	Lagoon	C3	FE	S1018
S. Derby	Slaughter	MLN	C3	S	S1034
S. Derby	Slaughter	Carcass Swab	C3	PE	S1085
S. Derby	Slaughter	MLN	C4	S	S1102
S. Derby	Farm	Lagoon	C3	FE	S1013
S. Derby	Slaughter	MLN	C3	S	S1023
S. Derby	Slaughter	MLN	C3	S	S1039
S. Derby	Slaughter	MLN	C3	S	S1044
S. Derby	Farm	Floor Swab	C1	FE	S651
S. Derby	Slaughter	MLN	C1	S	S672
S. Derby	Farm	Lagoon	C1	FE	S657
S. Derby	Farm	Water	C1	FE	S654
S. Infantis	Farm	Soil	C2	FAE	S322
S. Typhimuri.	Farm	Lagoon	C4	FE	S932
S. Typhimuri.	Farm	Lagoon	C4	NE	S633
S. Typhimuri.	Farm	Lagoon	C4	NE	S637
S. Typhimuri.	Slaughter	MLN	C2	S	S710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S. Typhimuri. Slaughter MLN C2 S S704
S. Typhimuri. Slaughter MLN C2 S S705
S. Typhimuri. Slaughter MLN C2 S S712
S. Typhimuri. Farm Lagoon C1 FE S493
S. Typhimuri. Farm Soil C10 FAE S1168
S. Typhimuri. Farm Lagoon C10 NE S1223
S. Derby Farm Floor Swab C1 FE S648
S. Typhimuri. Slaughter MLN C9 S S1348
S. Infantis Farm Lagoon C9 FE S1238
S. Typhimuri. Slaughter MLN C9 S S1353
S. Typhimuri. Farm Floor Swab C5 NE S872
S. Typhimuri. Farm Fecal C5 N S734
S. Typhimuri. Farm Floor Swab C5 NE S874
S. Typhimuri. Farm Fecal C5 N S735
S. Typhimuri. Farm Feed C5 NE S866
S. Typhimuri. Slaughter MLN C5 S S1128
S. Typhimuri. Slaughter MLN C5 S S1130
S. Typhimuri. Farm Fecal C5 N S717
S. Typhimuri. Farm Fecal C5 N S718
S. Typhimuri. Farm Floor Swab C5 FE S1126
S. Heidelberg Farm Lagoon C1 NE S426
S. Heidelberg Farm Lagoon C1 FE S487
S. Heidelberg Farm Lagoon C1 FE S490
S. Heidelberg Farm Lagoon C1 FE S662
S. Heidelberg Farm Lagoon C1 NE S424
S. Heidelberg Farm Lagoon C1 FE S492
S. Heidelberg Farm Truck Swab C1 FE S499
S. Typhimuri. Slaughter Truck Swab C4 SE S1123
S. Typhimuri. Farm Fecal C6 F S1151
S. Typhimuri. Farm Floor Swab C6 FE S1156
S. Typhimuri. Farm Fecal C10 FA S1163
S. Typhimuri. Slaughter MLN C6 S S1182
S. Muenchen Farm Fecal C1 F S478
S. Johannes. Farm Lagoon C5 NE S782
S. Ohio Farm Fecal C4 N S851
S. Ohio Farm Soil C4 NE S857
S. Ohio Farm Truck Swab C4 FE S995
S. Ohio Farm Fecal C4 F S998
S. Ohio Farm Fecal C9 F S1317
S. Ohio Farm Fecal C4 N S608
S. Ohio Farm Feed C4 NE S620
S. Ohio Slaughter MLN C1 S S669
S. Ohio Farm Floor Swab C4 FE S1001
S. Ohio Farm Floor Swab C9 FE S1232
S. Typhimuri. Farm Feed C10 FE S1326
S. Typhimuri. Farm Fecal C4 FA S544
S. Typhimuri. Farm Lagoon C6 FE S977
S. Typhimuri. Slaughter MLN C7 S S1292
S. Typhimuri. Slaughter Carcass Swab C7 PE S1295

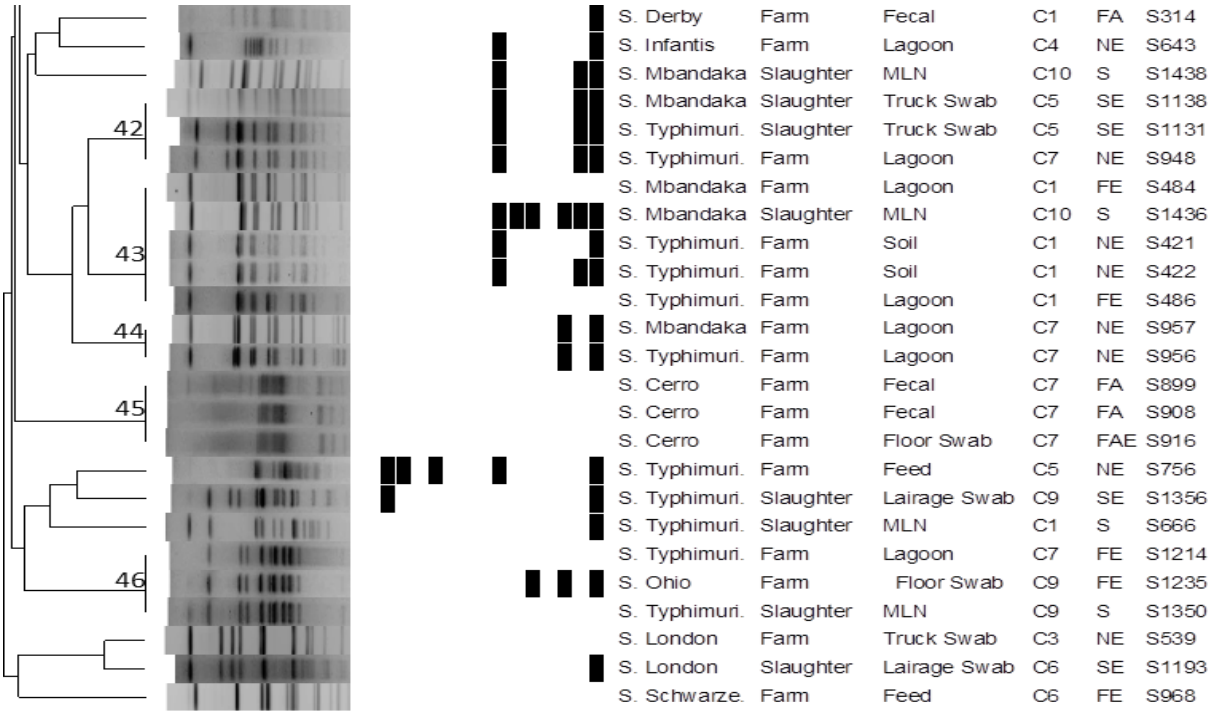

Figure 1. PFGE fingerprint profile for the overall 272 *Salmonella* isolated from conventional production farms

S. Infantis isolated from pig fecal, lagoon, soil, feed, truck swab and MLN originating from five separate cohorts (C3, C4, C6, C9, C10) had 100% similar fingerprint profiles (Fig. 1). Identical fingerprint patterns were detected (cluster 9; Fig. 1) among *S.* Infantis isolates from pig and environmental samples of the same flow (C6) at different stages including farrowing (isolate ID: S548, 551, 575, 581and 591), nursery 1 (isolate ID: S783 and 789), finishing 1 (isolate ID: S963 and 984) and slaughter (isolate ID: S1184 and 1195). Furthermore, we found 100% genotypic similarity among *S.* Infantis (FIS TET pattern) isolated from the conventional production system at farrowing, nursery 1, finishing 1 and slaughter including slaughter truck samples (cluster 9; Fig. 1). Within the conventional production system, we found 100% genotypic similarity among *S.* Rissen isolated from pig fecal, MLN, environmental samples including feed, water, floor swab and lagoon at nursery 1, nursery 2, finishing 2 and slaughter representing the same flow (C3; Fig 1; cluster 1, 2). Interestingly, we detected 100% genotypic similarity among S. Rissen isolated from pig fecal, carcass, feed, water, floor swabs and lagoon at the nursery, finishing and slaughter.

Statistical Analysis

A total of 922 *Salmonella* isolates were isolated from the all samples collected in the study. The overall proportion of samples that were positive for *Salmonella* was lower in pigs (66/1,650, 4%) compared to the environment (156/1,325, 11.7%). The multivariable analysis using logistic regression generated the final significant model (all variables $P < 0.05$), which was selected based on the associations of these variables and their interaction terms with the estimated prevalence of *Salmonella* and plotted along with 95% confidence intervals (Fig 2-4). The proportion of samples positive for *Salmonella* in the conventional (66/1,650, 4%) pigs at the following different sampling stages: farrowing (6.7%), nursery 1 (8.8%), nursery 2 (7.2%), finishing 1 (6.7%), finishing 2 (7.7%) (Fig. 2).

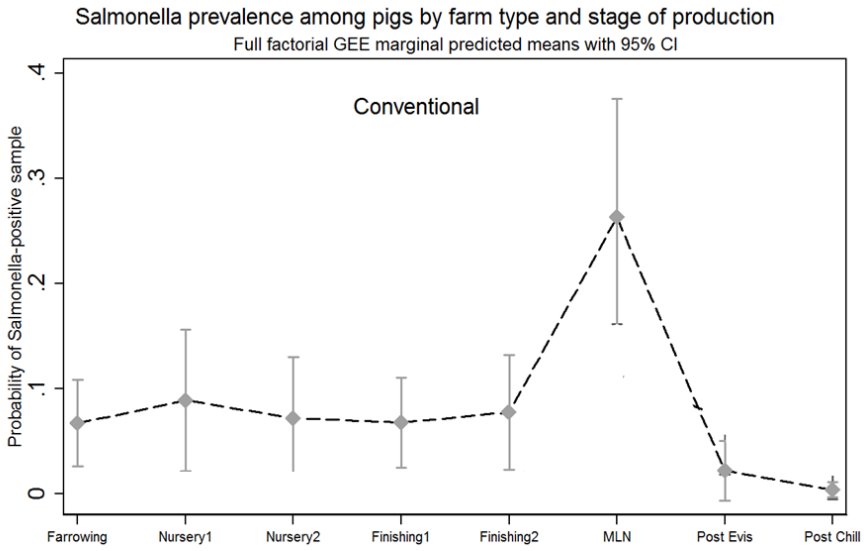

Figure 2: *Salmonella* prevalence among conventional pigs at farm and slaughter

The overall *Salmonella* prevalence in the environmental samples on conventional farms was 11.7% (156/1,325). In the environment, *Salmonella* was successfully recovered from water, soil, feed, floor swabs, lagoons, and truck samples. Among all the environmental samples, the *Salmonella* mean prediction was higher in lagoons when compared to other environmental samples (Fig 3).

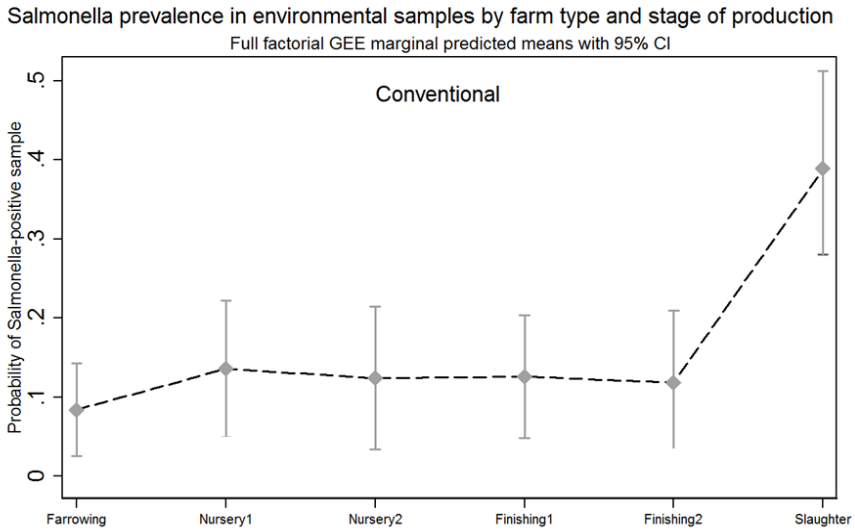

Figure 3: *Salmonella* prevalence in the conventional environment at farm and slaughter

Salmonella were also isolated from the slaughter environment (Fig. 4). The overall prevalence of *Salmonella* in the slaughter environment was 38.8%, with the highest marginal prediction in lairage (46%) followed by truck (30%) samples.

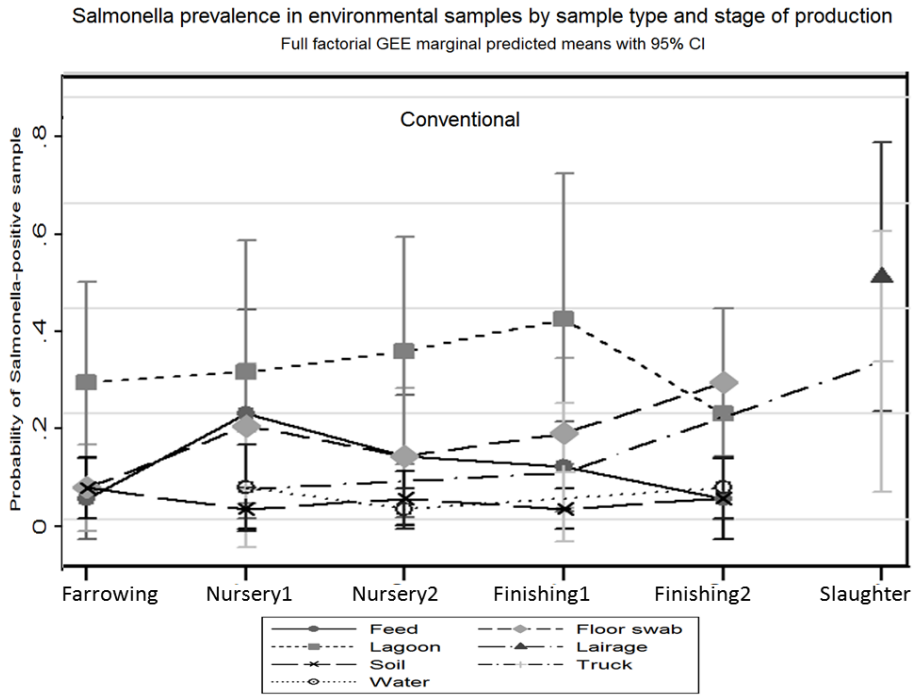

Figure 4: *Salmonella* prevalence among conventional environmental samples at farm and slaughter

Risk Factor Analysis

Farm management data was collected using questionnaires to fulfill objective #3 to identify the risk factors that are associated with the dissemination and persistence of *Salmonella* serovars in pigs and their environment on farm. However, the prevalence of *Salmonella* in the ABF farms was low which significantly reduced the chances of conducting risk factor analysis. As such, we had to curtail conducting detailed statistical analysis. The presence of "zero" value in majority of the cells in both the production systems, especially the outdoor farms was the major challenge. In addition, there was tremendous co linearity among the risk factor variables even within the conventional farms that it was not possible for us to distinguish between different farms thereby making it difficult to conduct any risk factor analysis.

Discussion

This longitudinal study was conducted to determine and compare AR *Salmonella* at their phenotypic and genotypic levels, isolated from pigs and the environment at different stages of production from farm to slaughter. We observed an increase in the prevalence of *Salmonella* in the final stages of production (finishing 1&2), similar to previous reports of higher prevalence of *Salmonella* among finishing herds. The likely reasons include a previously infected group of pigs at the farm, contaminated transport vehicles or handling and close contact of pigs during transportation (6, 7, 10). Higher prevalence of *Salmonella* in pigs during the final stages of production is of greater concern from a public health and food safety perspective. A higher number of environmental samples were positive for *Salmonella,* in spite of strict AIAO practices. This highlights the potential role of the farm environment as a reservoir, which is in accordance with studies highlighting the persistence of *Salmonella* in the farm environment for several months to years (11, 12).

In our study, we found a higher prevalence of *Salmonella* at slaughter in both pigs and the environment when compared to prevalence at the farm. Factors contributing to the increased prevalence of *Salmonella* at slaughter likely include cross contamination at peri-harvest stage by trucks involved in transfer to the slaughtering facilities, stress experienced by the pigs during transport, cross contamination at lairage and at post-harvest stages (4, 5, 13) . In addition, a previous study highlighted that contaminated feed at the end stage of production have significant role in dissemination of *Salmonella* (14) . The MLN samples from had a higher

prevalence of *Salmonella* when compared to fecal samples at the farm, which is in accordance with previous reports suggesting occurrence of *Salmonella* in the gastrointestinal (GI) tract and lymphatic tissue in carrier pigs(5, 6, 15) . Even though the MLN and gut contents are not used for consumption, occurrence of *Salmonella* in the MLN may act as a reservoir in contaminating carcasses during the post-evisceration stage. We also isolated *Salmonella* from the post-evisceration carcasses, which were cleansed with water before they were stored in the chilling facility. This indicates possible cross contamination during the evisceration process along the slaughter chain. Interestingly, we isolated *Salmonella* in post-chill swabs. The occurrence of *Salmonella* in post-chill swabs (16) is of critical importance to public health and food safety, as this sample closely represents the final retail product. In this study, we also isolated *Salmonella* from truck floors, which are used to transport conventional pigs from farm to slaughter. The most significant contribution to positive samples for *Salmonella* at slaughter was from lairage swabs, where pigs rest for about two hours before they are slaughtered. It takes less than two hours for a particular *Salmonella* serotype to establish in the GI tract of pigs and to be shed in their feces (4, 5). Clusters 7 and 8 (Fig. 1) highlight similar PFGE fingerprint profiles among *Salmonella* isolates from ABF carcass and lairage swabs.

In this study we identified a total of 22 different serotypes among conventional pigs and their environment. Based on both phenotypic and genotypic data, it was clear that specific serotypes, including S. Anatum, S. Infantis, S. Tyhimurium, S. Ouakam, S. Give and S. Ohio, were able to persist in both pigs and the environment throughout the production chain, from farrowing to slaughter. This is in accordance with a Centers for Disease Control and Prevention (CDC) report of the top four predominant serotypes in swine (17) . We identified for the first time *S.* Rissen in pigs and the environment. *S.* Rissen is one of the top ten serotype most commonly isolated from pigs since 2004 in Europe (18) and the most common nonhuman serotype in Asian countries. According to the CDC annual report, *S.* Rissen was isolated from humans (< 20 isolates per year) from 1999 to 2007 and there were no reports of its occurrence in food animals in the US (18, 19). This serotype is uncommon in the US; it was reported to have entered the US in late 2008 and early 2009 through imported white pepper, resulting in a human outbreak in northern California and Nevada (20) . We identified this serotype in our samples collected in late 2009.

Overall, *Salmonella* isolates exhibited a high frequency of AR (80%). The use of antimicrobials for treatment and growth purposes likely results in a higher prevalence of *Salmonella* as previously reported (21, 22). MIC distribution was similar for all the antimicrobials tested except TET, which was highest in *Salmonella* isolates of pig origin (MIC > 32 µg/mL; resistance: 80%) when compared to environmental isolates. The possible reasons may be use of tetracyclines as growth promoters administered in feed of growing pigs in our study, which has been reported extensively in the swine industry (23, 24). We detected a high frequency of MDR isolates (27%). *S.* Typhimurium was broadly associated with the common MDR pattern of AMP CHL FIS STR TET at farm and slaughter as previously reported (6, 25). This penta-resistant pattern is common to the *S.* Typhimurium phage type DT104 (26) . Identification of this phage type, both at farm and slaughter, is of significant public health concern because this phage type is commonly associated with human foodborne outbreaks worldwide (26, 27). Another important MDR pattern with β-lactams, including third generation cephalosporins (AXO TIO), was associated with *S.* Typhimurium and *S.* Anatum only in pigs and the environment at farm level, as previously reported (6). Emergence of these MDR patterns resistant to β-lactams is of concern because β-lactams (third generation cephalosporins) are extensively used to treat human clinical *Salmonella* infections (28) .

PFGE was used to genotype a representative subset of *Salmonella* isolates from pigs and the environment. PFGE is considered the gold standard test to determine the source of *Salmonella* in epidemiological studies (7, 15). Therefore, we used this genotyping method to determine whether a similar *Salmonella* genotype is disseminated from farm to slaughter along the production chain. Based on similar fingerprint profiles, *Salmonella* isolates in our study were grouped in 47 major clusters. Clustering was consistent with serotypes and resistance patterns as reported by a previous study (14). In addition, we observed fingerprint profile diversity among the same *Salmonella* serotypes representing different clusters as previously reported (11, 14, 29). Within the conventional production system, 100% genotypic similarity was observed among *S.* Rissen serotype isolates from pig fecal and environmental samples at different stages of production at

farm and slaughter from a single cohort (C3; Fig. 1). This result highlights the dissemination of relatively new *S.* Rissen serotype in pigs all along the production chain in the US. It was evident that specific serotypes, including *S.* Anatum, *S.* Infantis, *S.* Tyhimurium, *S.* Ouakam, *S.* Give and *S.* Ohio, were able to persist in the pigs and environment at different stages of production based on phenotypic and genotypic evidence (Table 1; Fig. 1). In an epidemiological study it is difficult to determine the exact mechanism and direction of pathogen transmission between pigs and the environment. However, detection of the same genotype among pigs and environment within the production system clearly suggests the exchange of *Salmonella* strains.

To summarize, this study demonstrates the presence of AR *Salmonella* conventional production systems at farm, slaughter and the environment. The phenotypic and genotypic fingerprint profile results underscore the potential role played by the environment in the persistence and dissemination of transmission of AR *Salmonella*. We detected MDR isolates throughout all the production stages and the environment. At the phenotypic level, *Salmonella* isolates from the lairage floor, carcass and MLN had similar resistance patterns and serotypes, which were not detected at the farm level. This highlights the importance of the farm and slaughter environment as separate but important reservoirs and as a crucial link to determining the dissemination of AR *Salmonella* among pigs. Future research should focus on environmental factors to develop a better understanding of the molecular epidemiology of this pathogen in the swine production environment and to reduce the burden of AR *Salmonella* on public health.

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