Molecular epidemiologic investigation of the role of gilts in the introduction and transmission of *Salmonella* in swine production systems

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Summary

Objective: To investigate whether purchased gilts introduce new *Salmonella* genotypes into a swine production system and acquire *Salmonella* infection from resident pigs.

Materials and methods: One multi-site and one single-site swine production system from Illinois were recruited on prior evidence of a high prevalence of *Salmonella* infection. Cohorts of 102 and 120 incoming gilts on the multi-site and single-site farms, respectively, were sampled longitudinally for five to eight visits until the gilts were introduced into the breeding herd. On each visit, fecal and floor samples were obtained from cohort gilts and an approximately equal number of resident pigs in the same or adjacent room or building. All samples were cultured to detect *Salmonella*. The 123 *Salmonella* isolates were genotyped using repetitive sequence polymerase chain reaction with REP, BOX, and ERIC primers. Cluster analysis classified the isolates by degree of genetic relatedness. The 45 isolates from the multi-site farm were serotyped.

Results: For both farms, the most closely genetically related *Salmonella* isolates were obtained from the same room on the same visit. For the multi-site farm, there was no evidence of transmission between gilts and resident pigs. For the single-site farm, there was evidence of transmission between gilts and resident pigs in the breeding barns. However, conclusive evidence that incoming gilts introduce new *Salmonella* genotypes into a swine production system was lacking. Serotyping information supported conclusions drawn from genotyping data.

Implications: Spatial separation of purchased gilts from other pigs by all-in, all-out pig flow reduces *Salmonella* transmission in swine production systems.
Keywords: swine, gilt, *Salmonella,* transmission, molecular epidemiology

Resumen - Investigación epidemiológica molecular sobre el papel de las primerizas en la introducción y transmisión de *Salmonella* en sistemas de producción porcina

Objetivo: Investigar si las primerizas adquiridas introducen nuevos genotipos de *Salmonella* en un sistema de producción porcina y si contraen la infección de *Salmonella* de los cerdos residentes.

Materiales y métodos: Un sistema de producción porcina de de sitios múltiples y uno de sitio único en Illinois se reclutaron ante la evidencia previa de una alta prevalencia de infección de *Salmonella*. Se tomaron muestras de manera longitudinal de grupos de 102 y 120 primerizas respectivamente, que entraron a un sistema multisitios y a uno de sitio único durante cinco a ocho visitas hasta que las primerizas se introdujeron al hato reproductor. En cada visita, se obtuvieron muestras fecales de primerizas y del piso del grupo en estudio y un número aproximadamente igual de cerdos residentes en el mismo cuarto o en el cuarto o edificio adyacente. Todas las muestras se cultivaron para detectar *Salmonella*. Los 123 aislados de *Salmonella* se genotipificaron utilizando la reacción en cadena de la polimerasa de secuencia repetitiva con primers de REP, BOX, y ERIC. El análisis de grupos clasificó a los aislados por su grado de relación genética. También se serotipificaron los 45 aislados de la granja de sitios múltiples.

Resultados: En ambas granjas, los aislados de *Salmonella* genéticamente más relacionados se obtuvieron del mismo cuarto durante la misma visita. En la granja de sitios múltiples, no hubo evidencia de transmisión entre las primerizas y los cerdos residentes. En la granja de sitio único, hubo evidencia de transmisión entre las primerizas y los cerdos residentes en los edificios de reproducción. Sin embargo, faltó evidencia concluyente de que las primerizas adquiridas introducen genotipos de *Salmonella* al sistema de producción porcina. La información de serotipificación apoyó las conclusiones obtenidas del análisis de genotipificación.

Implicaciones: La separación espacial de primerizas adquiridas de los cerdos mediante un flujo todo dentro-todo fuera reduce la transmisión de *Salmonella* en sistemas de producción porcina.

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Résumé - Enquête épidémiologique moléculaire sur le rôle des truies dans l'introduction et la transmission de *Salmonella* dans les structures de production porcine

Objectif: Déterminer si les cochettes achetées introduisent de nouveau génotypes de *Salmonella* dans une structure de production porcine et si elles contractent une infection à *Salmonella* à partir des porcs résidents.

Matériels et méthodes: Une structure de production multi-sites et une structure de production à site unique situées en Illinois, ont été sélectionnées compte tenu d'évidences d'une prévalence élevée d'infections par *Salmonella*. Des cohortes de 102 et 120 cochettes entrant, respectivement, sur la ferme à sites multiples et la ferme à site unique, ont été échantillonnées lors de cinq à huit visites jusqu'à ce que les cochettes soient introduites dans le troupeau reproducteur. À chaque visite, des échantillons fécaux et provenant du plancher ont été prélevés des cohortes de cochettes ainsi que d'un nombre approximativement égal de porcs résidents dans les mêmes chambres ou dans des chambres ou bâtisses adjacentes. Tous les échantillons ont été cultivés pour détecter la présence de *Salmonella*. Les 123 isolats de *Salmonella* obtenus ont été génotypés par réaction d'amplification en chaîne par la polymérase utilisant des séquences répétitives avec des amorces REP, BOX, et ERIC. L'analyse par agrégation a permis de classer les isolats en fonction de leur parenté génétique. Les 45 isolats provenant de la ferme à sites multiples ont été sérotypés.

Résultats: Pour les deux fermes, les isolats de *Salmonella* les plus apparentés génétiquement provenaient de la même chambre au cours de la même visite. Pour la ferme multi-sites, il n'y avait aucune évidence de transmission entre les cochettes et les porcs résidents. Pour la ferme à site unique, il y avait évidence de transmission entre les cochettes et les porcs résidents dans les bâtiments de reproduction. Toutefois, il n'y avait pas d'évidences claires que les nouvelles cochettes ont introduit de nouveaux génotypes de *Salmonella* dans les structures de production. Les résultats du sérotypage corroboraient ceux obtenus par génotypage.

Implications: Une ségrégation spatiale entre des cochettes achetées et les autres porcs par une pratique de tout-plein, tout-vide réduit la transmission de *Salmonella* dans les structures de production porcine.

Salmonellosis in humans is a serious food-borne disease characterized by acute and chronic gastroenteritis, septicemia, and sometimes death. The United States Department of Agriculture (USDA) has placed *Salmonella* at the top of its priority list for elimination of food-borne pathogens, with pork identified as an important source of infection.[1] Molecular biological analysis comparing the genotypes of human and pig isolates of *Salmonella* has repeatedly implicated pigs as a source of human infection.[2-8]

There have been numerous epidemiologic investigations of *Salmonella* in swine production systems. *Salmonella* has been isolated from samples of swine feces,[9-15] pen floor contents,[12-17] feed,[12,13,15,16,18] water,[14-16] boot swabs,[14-16] flies,[14-16] intestinal contents of rodents,[14-16] and feces of birds[14,15] and cats.[14,15] Although these studies have identified numerous sources of *Salmonella* within the environment of the swine-production ecosystem, most of the focus on external sources for introduction of *Salmonella* into a production system has been on the role of feed, with the implication that this is the main conduit for introduction of *Salmonella* into a production system.[10,18,19] However, several epidemiologic studies have found little or no *Salmonella* in feed.[9,13,14] Thus, other sources for the introduction of *Salmonella* into a production system need to be investigated.

Purchase of gilts may be an important mechanism for introducing *Salmonella* into a herd without apparent clinical signs. Modern swine production systems typically replace 40% to 50% of sows each year.[20,21] Monitoring suppliers of replacement breeding stock is a key component of national *Salmonella* control programs for swine production in Sweden and Denmark.[20]

In a prior study of the role of gilts in *Salmonella* transmission in swine production systems, the movement of two cohorts of 6-month-old gilts (n = 202) from a gilt-development farm to a breeding farm was monitored.[22] Fecal samples were collected from gilts within 1 week before leaving the gilt-development farm and again within 2 weeks after arrival at the breeding farm. The rate of fecal shedding of *Salmonella* by the gilts was higher after transport and introduction to the breeding herd (> 20% after versus < 5% before). Serotyping of *Salmonella* isolates indicated that 55% of isolates from gilts at the breeding farm were serotypes not identified from gilt samples on the development farm. It was noted that new serotypes may have been identified only at the breeding farm because of increased shedding of *Salmonella* by gilts after transport, but that this may have also been due to acquisition of new infections from the environment of the breeding farm. Regardless of the source of infection, transportation of gilts was implicated as responsible for increasing the *Salmonella* load in the environment and thus maintaining a high prevalence of *Salmonella* infection in the herd. Transportation of pigs has been recognized repeatedly as a factor increasing the rate of *Salmonella* shedding,[23-25] so the acquisition of gilts from an external source is likely to be a risk factor for introduction of new *Salmonella* into a herd.

The previous study by Davies et al[22] suggests that introduction of 6-month-old gilts may contribute to the introduction and maintenance of *Salmonella* infections in swine herds. This increase in prevalence might be attributed to incoming gilts acquiring infection from resident pigs or vice versa. However, the role of gilts in transmitting *Salmonella* infections to herd residents was not investigated because resident pigs were not sampled. Also, a common practice in gilt acquisition is to purchase replacement breeding stock at weaning.[26] Several studies have indicated that weaning-age pigs are a relatively minor source of *Salmonella* infection,[11,12,27,28] most likely due to maternally acquired immunity,[29] which provides justification for acquiring replacement gilts at weaning.

The present study was designed to extend the analysis of the role of acquired gilts in introducing *Salmonella* into a herd by also examining *Salmonella* shedding in resident pigs. Evaluation of transmission between incoming gilts and resident pigs was made more precise by using molecular genotyping[30] and statistical methods developed for inferring transmission from genetic relatedness.[15]

Materials and methods
The project protocol was approved for animal use by the University of Illinois Institutional Animal Care and Use Committee, which was accepted by USDA for their purposes. One multi-site (Farm M) and one single-site (Farm S) commercial swine production facility in Illinois were investigated. Both farms had total confinement housing with concrete floors in the growing-finishing and breeding areas and slotted wood floors in the nursery. The farms were selected on the basis of prior evidence of a high prevalence of *Salmonella*. Farm M had the highest prevalence of *Salmonella* infection in a previous study of 18 swine farms in Illinois.[15] Farm S had been identified through the Illinois Department of Agriculture because of outbreaks of enteric disease in the nursery due to *Salmonella enterica* within the previous year. Both farms acquired gilts at 2 to 3 weeks

of age at intervals of ≤ 3 months, with at least 100 gilts per shipment. No *Salmonella* vaccine was used on either farm during the course of the study. On both farms, nursery-stage pigs received antibiotics in feed to promote growth.

Farm M had 3500 sows. Incoming gilts were isolated in a nursery on a separate site reserved for purchased gilts. At approximately 9 to 10 weeks of age, these gilts were moved as a cohort to a vacant room on one of several available finishing sites, where they remained until about 5 to 6 months of age. They were then moved to a gestation barn on one of two breeding sites, where cohort gilts were mixed in pens with "resident sows" already circulating in the breeding herd.

Farm S had 6000 sows. A cohort of incoming gilts from a single source was segregated from other pigs in one of two nursery buildings containing only one room. At 6 to 8 weeks of age, the cohort of gilts was moved to a single pen in a growing facility consisting of one large room with multiple pens housing pigs from various sources segregated by age. Pigs were moved as a cohort from pen to pen as they advanced in age, and moved to a similar finishing building at 14 to 16 weeks of age. At 5 to 6 months, each finishing-age gilt showing signs of estrus was moved into a pen shared with approximately 15 to 25 other gilts, in one of two breeding barns.

Sample collection

The first visit to a farm was on the day of gilt arrival. A cohort of incoming gilts was selected and ear-tagged for follow-up. Cohort size was 102 on Farm M and 120 on Farm S. On each visit, samples were collected from cohort pigs and from an approximately equal number of resident pigs that were the nearest neighbors (same room, adjacent room, or adjacent building). Subsequent farm visits were made as indicated in Tables 1 and 2. Samples collected included feces and swabs of pen floors. Feces were obtained from nursery pigs using feline fecal loops. From older pigs, a 1-g fecal sample was obtained by digital insertion into the anus, with the collector wearing a latex glove that was changed between pigs. Pen floors were sampled to obtain at least 1 g of floor contents by swabbing with one 4 × 4-inch cotton gauze for each pen, collecting in several parts of the floor where feces was deposited. Fecal samples were transferred immediately into tubes containing 9 mL tetrathionate broth (TTB) with iodine, and floor samples

Table 1: Characteristics of samples collected from cohorts of replacement gilts for isolation of *Salmonella* at each visit to Farm M, a 3500-sow, multi-site commercial swine-production facility in Illinois*

Visit	Age of cohort gilts (weeks)	Cohort gilt location	Other pigs sampled	Time of sample collection
1	2	Nursery, Site 1	Adjacent room	Day of arrival
2	3	Nursery, Site 1	Adjacent room	1 week later
3	9	Finisher, Site 2	Adjacent room	Day of movement of cohort gilts to finisher
4	10	Finisher, Site 2	Adjacent room	1 week later
5	22	Breeding, Site 3	Same room	3 days after movement into breeding building; cohort and resident pigs mixed in pens

* Replacement gilts were acquired at 2 to 3 weeks of age at intervals of ≤ 3 months, with ≥ 100 gilts per cohort. Cohort pigs were ear-tagged on the day of arrival. On each visit, fecal samples were collected from all cohort pigs and from approximately equal numbers of the nearest resident pigs.

Table 2: Characteristics of samples collected for *Salmonella* isolation at each visit to Farm S, a 6000-sow, single-site commercial swine-production facility in Illinois*

Visit	Age of cohort gilts (weeks)	Cohort gilt location	Other pigs sampled	Time of sample collection
1	2	Nursery building 2	Nursery building 1 (adjacent building)	Day of arrival
2	10	Growing stage	Grower, nursery 1	1 day after movement of cohort gilts into grower
3	14	Growing stage	Grower	1 day after next batch of gilts moved to grower
4	22	Grower-finisher	Grower, finisher	1 day after 50% of cohort gilts moved to finisher
5	26	Finisher	Finisher	1 day after all cohort gilts moved to finisher
6	29	Finisher & gestation	Finisher, gestation	After 50% of cohort gilts moved to gestation
7	31	Finisher & gestation	Finisher, gestation	After 75% of cohort gilts moved to gestation
8	36	Gestation	Gestation	After 100% of cohort gilts moved to gestation

* Replacement gilts were acquired at 2 to 3 weeks of age at intervals of ≤ 3 months, with ≥ 120 gilts per cohort. Cohort pigs were ear-tagged on the day of arrival. On each visit, fecal samples were collected from all cohort pigs and from approximately equal numbers of the nearest resident pigs.

were placed in tubes with 25 mL TTB with iodine for enrichment of *Salmonella*. Tetrathionate broth tubes were placed on ice packs in coolers and transported to the laboratory the same day.

Bacteriologic culture and polymerase chain reaction analysis

Upon return to the laboratory (maximum of 8 hours after collection), all samples were incubated at 37°C for 48 hours. Thereafter, 100 μL of TTB broth from each tube was transferred to 10 mL Rappaport's medium (R-10) and incubated for 24 hours at 37°C. Samples from R-10 were streaked onto xylose-lysine-tergitol 4 agar (XLT-4) plates and incubated for 24 hours at 37°C. Red colonies with black centers were picked and streaked onto brilliant green agar (BGA) plates. Red non-lactose-fermenting suspected *Salmonella* colonies from BGA plates were transferred to tryptic soy agar (TSA) plates and analyzed using polymerase chain reaction (PCR) with primers for the *invA* gene,[31] using methods described previously.[32] DNA was extracted by boiling for 5 minutes the cells obtained from an isolated colony from a TSA plate. Polymerase chain reaction-positive isolates were frozen at -80°C in tryptic soy broth for storage. *Salmonella* isolates were genotyped using repetitive sequence PCR (rep-PCR) methods with three different primers (REP, BOX, and ERIC) as described previously.[30] Resulting DNA amplicons were separated by size by gel electrophoresis.

All *Salmonella*-positive isolates from Farm M were submitted to the National Veterinary Diagnostic Laboratory (Ames, Iowa) for serotyping.

Data analysis

Data analysis consisted of pairwise comparison of sizes of PCR amplicons to calculate the percentage of fragments matching by size (Dice coefficient) as an estimate of genetic distances between isolates.[30] A 5% tolerance level for matching was used. Hierarchical cluster analysis, using the complete linkage algorithm, was conducted to determine the overall pattern of genetic similarity among isolates.[33] On the basis of statistical principles of identification of clusters from the distribution of linkage distances,[33] previous studies investigating cluster formation from a matrix of genetic distances calculated from three-primer rep-PCR had identified isolates within a genetic distance ≤ 0.15 as "similar."[15,30] In the present study, isolates within this distance were considered to have a recent common ancestor, thus implicating transmission from one source to another. Linkage of genetically similar isolates was examined with respect to their similarity in time of sampling (farm visit), location (same room or not), and whether a sample was from a resident pig or incoming gilt, using the binomial test,[34] in an analytic model developed previously.[15] For Farm M, the associations of serotypes with time of sampling, farm site, and porcine source of sample were tested using 2 × 2 contingency tables, as described previously.[15] Due to use of multiple statistical tests, an alpha level for significance equal to .01 was used.

Results

There was no evidence of clinical salmonellosis on either farm during the course of the study, ie, no widespread signs of enteric disease. On Farm M, 856 samples were collected and cultured for *Salmonella*, 45 (5.6%) of which were positive (Table 3). *Salmonella*-positive fecal samples were found only in 22-week-old pigs at the breeding site. None of the fecal samples collected from 2- to 3-week-old nursery pigs or the floor samples from their pens were positive. However, *Salmonella* was detected in the floor samples after pigs were moved to the growing-finishing sites at 9 weeks of age. Not differentiating by location, pig source, or time of visit, *Salmonella* prevalence was higher in floor samples (44.0%) than in fecal samples (2.0%).

Serotypes of *Salmonella* isolates were clearly differentiated by site on Farm M. Three serotypes were identified from samples collected on finishing site 2 during visits 3 and 4: Alachau (n = 12), Anatum (n = 8), and Minnesota (n = 1). Three different serotypes were identified from samples collected on breeding site 3 during visit 5: Derby (n = 20), Typhimurium (n = 3), and Infantis (n = 1). There was also some differentiation of *Salmonella* serotype by pig source, with Typhimurium found only in the feces of 22-week-old cohort gilts, and Alachau found only on the pen floors of 9- and 10-week-old resident pigs.

On Farm S, 2345 samples were collected and cultured for *Salmonella*, 78 (3.3%) of which were positive (Table 4). Positive samples were found on each visit. There was no apparent differentiation of positive samples by location, pig source, or time of visit. Overall, *Salmonella* prevalence was higher among floor samples (5.8%) than fecal samples (2.5%). Serotyping information was not available for *Salmonella* isolated on Farm S.

Table 3: *Salmonella* prevalence in a total of 856 fecal and floor samples collected from Farm M*

Visit	Age of cohort gilts (weeks)	Cohort gilt fecal samples		Cohort gilt floor samples		Resident pig fecal samples		Resident pig floor samples		Mixed-pen floor samples	
		n	No. positive (%)	n	No. positive (%)	n	No. positive (%)	n	No. positive (%)	n	No. positive (%)
1	2	102	0 (0.0)	5	0 (0.0)	ND	NA	ND	NA	NA	NA
2	3	99	0 (0.0)	4	0 (0.0)	100	0 (0.0)	10	0 (0.0)	NA	NA
3	9	95	0 (0.0)	2	2 (100.0)	97	0 (0.0)	15	10 (66.7)	NA	NA
4	10	91	0 (0.0)	2	1 (50.0)	97	0 (0.0)	15	8 (53.3)	NA	NA
5	22	67	10 (14.9)	NA	NA	42	6 (14.3)	NA	NA	13	8 (61.5)
Totals		**454**	**10 (2.2)**	**13**	**3 (23.1)**	**336**	**6 (1.8)**	**40**	**18 (45.0)**	**13**	**8 (61.5)**

* Farm M and sampling schedule described in Table 1.
ND = not done; NA = not applicable.

Table 4: *Salmonella* prevalence in a total of 2345 fecal and floor samples collected from Farm S*

Visit	Age of cohort gilts (weeks)	Cohort gilt fecal samples		Cohort gilt floor samples		Resident pig fecal samples		Resident pig floor samples	
		n	No. positive (%)	n	No. positive (%)	n	No. positive (%)	n	No. positive (%)
1	2	120	4 (3.3)	6	1 (16.7)	60	3 (5.0)	6	1 (16.7)
2	10	120	2 (1.7)	4	3 (75.0)	120	6 (5.0)	10	1 (10.0)
3	14	114	1 (0.9)	4	0 (0.0)	120	7 (5.8)	6	2 (33.3)
4	22	106	1 (0.9)	8	0 (0.0)	120	2 (1.7)	8	0 (0.0)
5	26	113	0 (0.0)	22	0 (0.0)	106	2 (1.9)	50	1 (2.0)
6	29	120	0 (0.0)	58	5 (8.6)	121	1 (0.8)	65	1 (1.5)
7	31	90	8 (8.9)	61	3 (4.9)	120	2 (1.7)	89	3 (3.4)
8	36	93	3 (3.2)	83	7 (8.4)	117	2 (1.7)	105	6 (5.7)
Totals:		**876**	**19 (2.1)**	**246**	**19 (7.7)**	**884**	**25 (2.8)**	**339**	**15 (4.4)**

* Farm S and sampling schedule described in Table 2.

Figures 1 and 2 show the dendrograms from the cluster analysis of genetic distances between *Salmonella* isolates. For Farm M, of the three largest clusters formed (separated at a distance approximately equal to 0.7, or less than 30% similarity in rep-PCR fragmentation), two were from the breeding site (W) and one was from the finishing site (K). Within the large finishing-site cluster, there were two smaller clusters distinct at a distance approximately equal to 0.55, or about 45% rep-PCR similarity. One cluster included only isolates from the pen floors of resident pigs, consisting entirely of *Salmonella* isolates with the Alachau serotype. The other cluster included isolates from samples from the floors of both cohort gilts and resident pigs, all with the Anatum serotype, except for one isolate with the Minnesota serotype (Figure 1). Although this differentiation of serotypes by genetic cluster was clear, there were a few cases of isolates of different serotypes (Minnesota and Anatum; Derby and Typhimurim; Derby and Infantis) being more closely related to each other than to isolates of the same serotype. The genetic clusters of *Salmonella* isolates from Farm S were generally mixed with respect to farm visit and building (Figure 2).

Statistical analysis of the spatial, temporal, and pig characteristics of the most genetically similar isolates (genetic distance ≤ 0.15) revealed the following. For all 11 of the closest isolate pairs from Farm M, both isolates were collected from the same room on the same visit, and both isolates were from the same pig source, ie, either both from resident pigs or both from cohort gilts (*P* < .001, all cases). For Farm S, of the eight closest isolate pairs, six (75%) were identical for time of visit (*P* < .001), and six (75%) were identical for location (room) of sampling (*P* = .001). Three were different for pig source (ie, paired isolates were from both gilt and resident), but this association was not significant (*P* = .35). The mixed-source (gilt and resident) close isolate pairs were all sampled in the breeding barns.

For Farm M, where serotyping information was available, there were associations of the Derby serotype with the breeding site (*P* < .001) and the Alachau (*P* < .001) and Anatum (*P* < .001) serotypes with the finishing site. Also, samples from the pen floors of resident pigs were more likely to have *Salmonella* of the Alachau serotype than were samples from the pen floors of cohort gilts (*P* < .001).

Discussion

For both farms studied, the most genetically similar *Salmonella* isolates were found at the same farm visit and in the same housing unit. For the multi-site farm, *Salmonella* serotypes were differentiated by site. Isolates obtained on the same visit from different locations were genetically different, although not necessarily differentiated by serotype. The genotyping results are further confirmation of previous results,[15] indicating the importance of physical barriers in inhibiting *Salmonella* transmission. One important difference between the two farms was that for the single-site farm only, there were several genetically similar isolate pairs from both gilt and resident pig samples, all from the gestation area. This indicates transmission of *Salmonella* between purchased gilts and resident pigs, although the direction of transmission is unknown. There was mixing of resident pigs and purchased gilts within the breeding barns on both farms. Transmission of *Salmonella* between gilts and residents on the single-site farm may have been due to earlier mixing within the same room in the grower stage. Only two farms were studied here, therefore limiting the ability to generalize. Nevertheless, the consistent finding, here and in the previous study,[15] that physical barriers reduce the probability of transmission, suggests that the practice of mixing pigs from different sources in growing and finishing stages facilitates the transmission of *Salmonella* that is present within the swine production system. However, even when purchased gilts are moved as segregated cohorts through several housing facilities in the different stages of production (eg, nursery to grow-finish to gestation), the success of preventing *Salmonella* transmission between housing units depends upon additional factors, such as disinfection procedures and limiting the flow of air, water, human traffic, and animal vectors such as rodents, birds, cats, and insects.[14]

Serotyping information on *Salmonella* isolates from the multi-site farm was insufficient to provide accurate data on transmission. Three

Figure 1: Dendrogram from cluster analysis of genetic distances among *Salmonella* isolates from Farm M (described in Table 1), as determined by repetitive sequence polymerase chain reaction. Sample identification code: FV[S-B-R-P]ET/X#[Z], where F = farm code, V = visit number, S = site (K = grower-finisher, W = breeding), B = building code, R = room number, P = pen number, E = stage of production (G = growing, F = finishing), T = sample class (F = fecal, R = floor), X = pen type (g = cohort gilts only, r = resident pigs only, m = mixed-cohort gilts and resident pigs), # = isolate sequence number within pen for pens with more than one isolate. [Z] indicates serotype code: D = Derby; I = Infantis, L = Alachau, M = Minnesota, N = Anatum, T = Typhimurium.
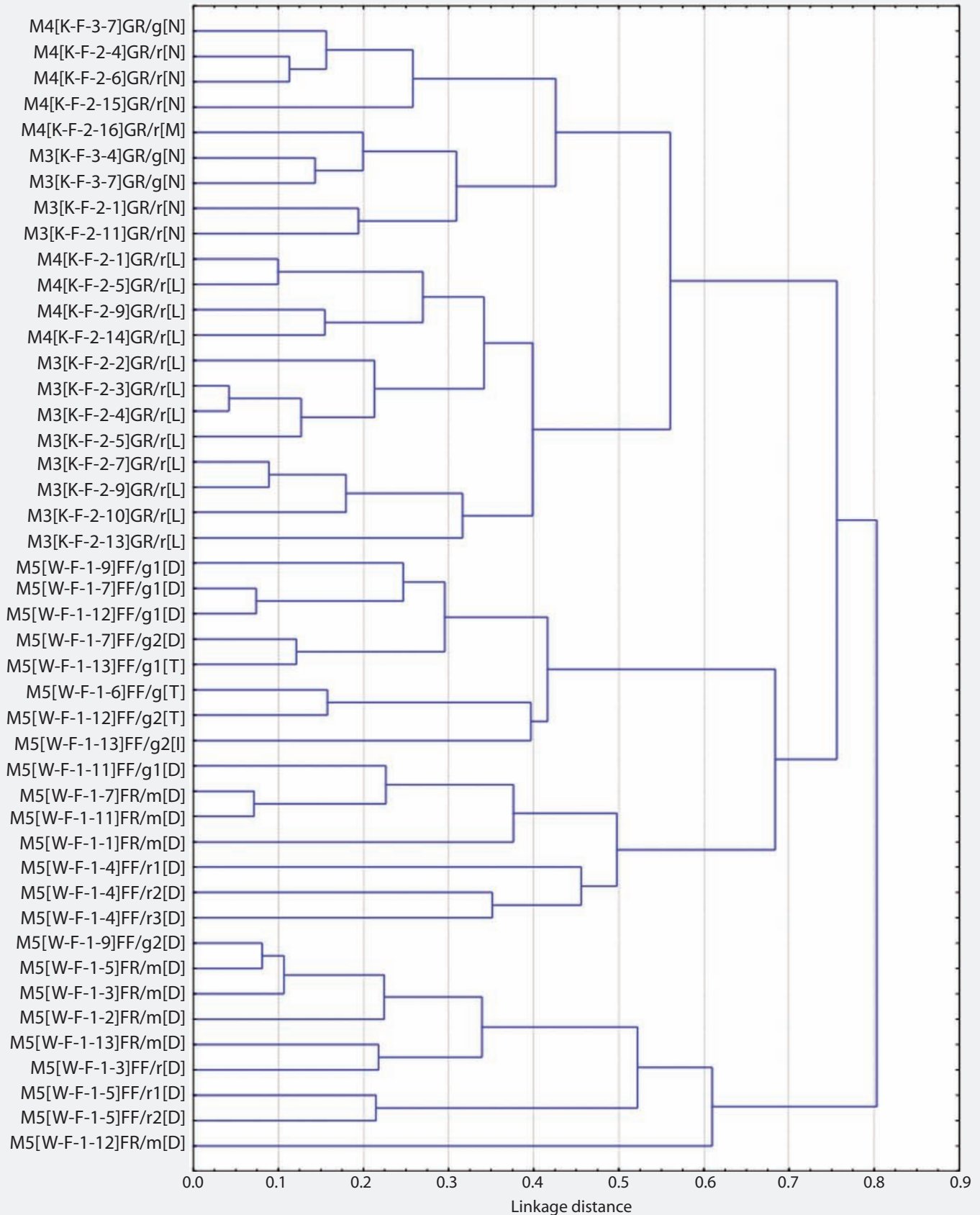
Figure 2: Dendrogram from cluster analysis of genetic distances among *Salmonella* isolates from Farm S (described in Table 2), as determined from repetitive sequence polymerase chain reaction. Sample identification code: FV[B-P-#]ET/Xt, where F = farm code, V = visit number, B = building code (N1, N2 = nurseries; GR = growing stage, FN = finishing stage; G1, G2 = gestation), P = pen number, # = sample sequence number, E = stage of production (G = growing stage, F = finishing stage, S = gestation), T = sample class (N = nursery pig, G = growing-stage pig, F = finishing-stage pig, L = sow, R = floor), X = pen type (g = pen with cohort gilts only, r = pen with resident pigs only, m = mixed-cohort gilts and resident pigs), t = cohort gilt ear-tag number.
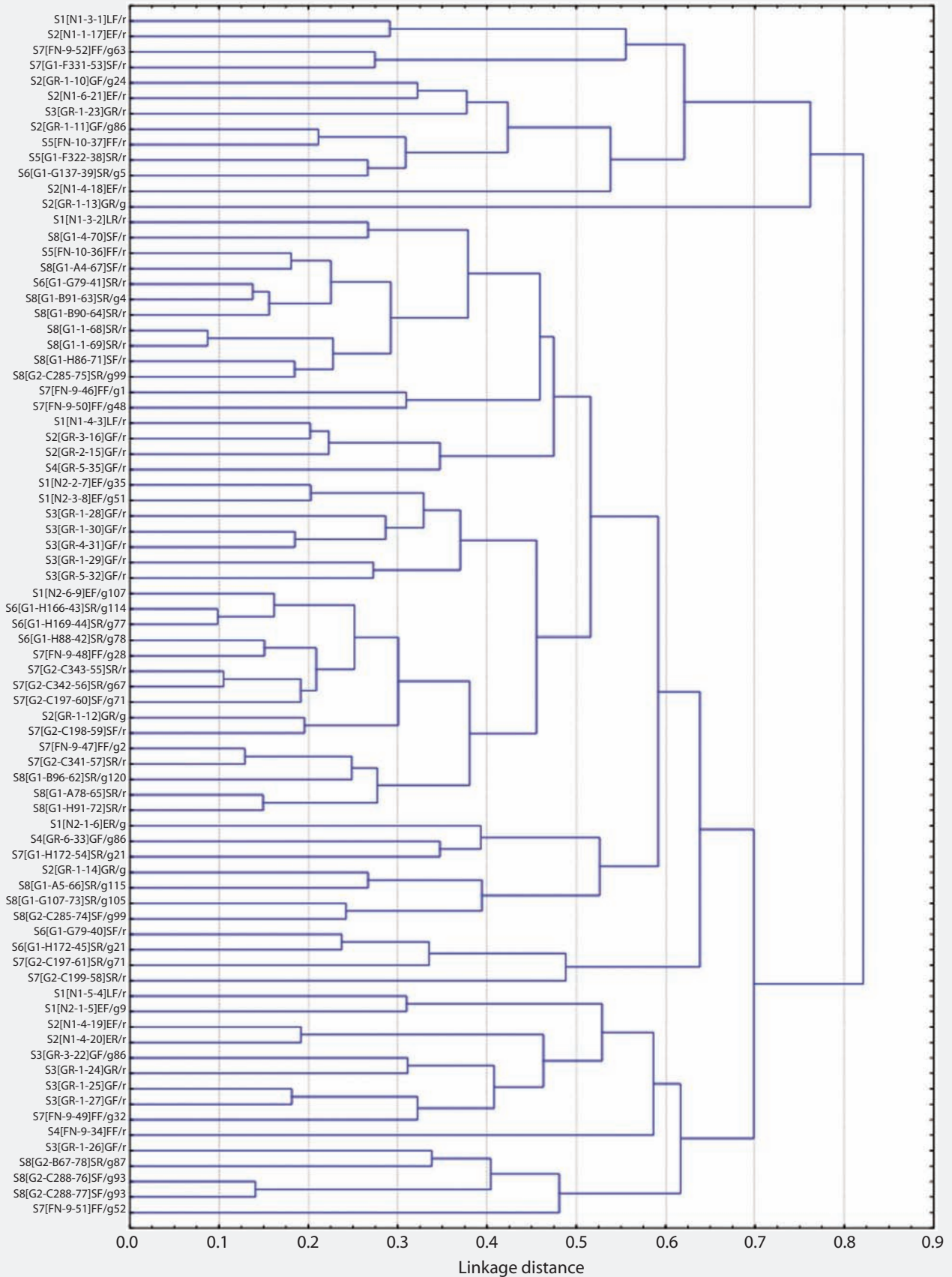
isolates obtained from cohort gilts on the finishing site shared the Anatum serotype with five isolates obtained from resident pigs on the same site; however, their genetic distances based on rep-PCR were < 60% similarity in DNA fragment matching, indicating they were unlikely to be related to a recent common source.

Definitive evidence for introduction of new genotypes of *Salmonella* into a swine production system through importation of gilts was not provided by the present study. The two farms studied provided contrasting evidence on the risk of purchased gilts being infected with *Salmonella*. Failure to detect shedding of *Salmonella* from nursery-stage pigs on the multi-site farm suggests that pigs of this age may be at low risk for contributing to the *Salmonella* load in the production system. In contrast, detection of *Salmonella* in fecal and floor samples from newly arrived gilts on the single-site farm identifies purchased gilts as a possible source of new *Salmonella* infection for a production system. Previous studies have reported low rates of *Salmonella* shedding in nursery-age pigs.[13,29] These low shedding rates may be attributable either to maternally acquired immunity or to the smaller amounts of feces collected from 2- to 3-week-old piglets.[35] Application of antibiotics to feed is also likely to inhibit *Salmonella* excretion. However, smaller pigs defecate less, and therefore shed less *Salmonella* into a production system environment, all other things being equal. Thus, recently weaned gilts with maternal antibodies, less history of exposure to pathogens, receiving oral antibiotics, and with lower fecal output, are less likely to introduce new *Salmonella* genotypes into a production system than are gilts acquired as replacements at 4 to 5 months of age. Purchase of early-weaned gilts has been recognized as a strategy for reducing *Salmonella* transmission.[27]

From 1996 to 2000, the replacement rate of breeding herd females in US commercial swine herds averaged near 50%; average herd parity of culled sows ranged from 3.1 to 3.7.[21] High culling rates result in purchase of more breeding gilts. Swine represent the largest reservoir of *Salmonella* on any farm, and incoming gilts represent a large external biomass introduced into any swine-production ecosystem, therefore increasing the risk of bringing new infections onto the farm. Thus, gilt purchase becomes an important factor in making decisions about disease control.

In the previous study[22] of the role of gilts in introducing *Salmonella* into a herd, cultures of fecal samples from recently transported 6-month-old gilts provided evidence for the appearance of serotypes of *Salmonella* not present on the source farm. From this it was inferred that transmission of *Salmonella* from resident pigs to gilts had occurred, although this was recognized as inconclusive because resident pigs had not been sampled.[22] The higher *Salmonella* shedding rate (> 20%) for 6-month-old gilts in the previous study[22] might be indicative of an overall higher risk of acquiring and shedding *Salmonella* in this age group than in the recently weaned pigs in the present study, in which *Salmonella* shedding was not observed. If evidence from future studies confirms these age differences in *Salmonella* shedding rate after transportation, the case for reducing *Salmonella* infection in a herd by acquisition of gilts at an early age will be strengthened.

Compared to the previous study[22] of gilt introduction to the herd, in which pathways of transmission were inferred from the results of serotyping, an important improvement in the present study is the use of molecular genotyping to link *Salmonella* isolates of a common origin. Previous studies[15,30,36] have reported that rep-PCR provides better discriminative ability among *Salmonella* isolates than serotyping, with the latter often failing to differentiate genetically distinct isolates. The comparison of rep-PCR and serotyping results from the multi-site farm in the present study provides additional evidence indicating that *Salmonella* serotyping lacks the precision necessary to definitively track *Salmonella* transmission in swine production systems.

In the study of *Salmonella* transmission in a complex ecosystem such as a swine production unit, only a small fraction of the *Salmonella* present can be detected because of practical sampling limitations. Even for infected pigs, due to intermittent shedding, standard sampling of feces and pen floor contents can only identify recent *Salmonella* excretion. Thus, infected pigs not excreting *Salmonella* at the time of sampling, but which may have been the source or destination for *Salmonella* transmission, may not be detected. Another limiting factor is the imperfect sensitivity of standard laboratory culturing methods for detection of *Salmonella*.[37] Several PCR procedures for more direct detection of *Salmonella* in feces have been developed, but they have low sensitivity for field

samples with low *Salmonella* loads, and thus an enrichment stage is necessary.[37-39] Several studies have reported that, after enrichment, various PCR methods have high sensitivity and agree well with standard culture methods.[40-42] However, an evaluation of field samples of bovine feces using the same *invA* PCR as that used in the present study, and culture methods similar to those used in the present study, found standard bacterial culture of *Salmonella* to have only 75% sensitivity compared to PCR on samples processed through enrichment media.[39] These results indicate that reserving the *invA* PCR until *Salmonella* isolates have been obtained may result in falsely classifying some samples as negative. Complete culture, including plating on differential and selective media, is still required to recover *Salmonella* for genotyping, which is needed for the study of transmission. Use of PCR immediately after selective enrichment broth culture not only increases sensitivity in detecting *Salmonella*, but provides savings by not processing negative samples on differential and selective agar media.[39] This savings is offset somewhat by use of PCR on the large number of negative samples expected in field investigations.

The analysis conducted here provides confirmatory evidence for transmission of *Salmonella* between pigs from different sources, although it was not possible to determine whether the *Salmonella* transmitted was from outside the production system or was already circulating within the environment. As this study included only two swine production systems, additional study is needed to determine the specific risk of introducing *Salmonella* when 2- to 3-week-old gilts are purchased.

Implications

- Transmission of *Salmonella* occurs primarily within the same housing unit; thus, spatial separation of purchased gilts from other pigs by all-in, all-out pig flow reduces *Salmonella* transmission in swine production systems.
- Although transportation stress induces *Salmonella* shedding, gilts purchased at weaning age are less likely to shed *Salmonella* than older gilts and thus are preferred for preventing introduction of *Salmonella* into a production system.
- Mixing pigs from different sources in gestation is likely to increase *Salmonella* prevalence and genetic diversity

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