Transmission Dynamics of a Multidrug-Resistant *Salmonella* Typhimurium Outbreak in a Dairy Farm

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Abstract

Cattle are recognized as an important source of foodborne *Salmonella* causing human illness, particularly for antimicrobial-resistant strains. The transmission dynamics of multidrug-resistant (MDR) *Salmonella* after the onset of a clinical outbreak in a dairy farm has been rarely monitored. The early transmission of a pathogen influences the outbreak size and persistence of the pathogen at the farm level and, therefore, how long the herd represents a risk for *Salmonella* zoonotic transmission. The objective of this study was to describe the transmission dynamics of MDR *Salmonella* Typhimurium after the onset of a clinical outbreak in a dairy herd. For that purpose, fecal shedding and serological response to MDR *Salmonella* were monitored in a longitudinal study conducted in a dairy herd after a few cases of salmonellosis, and a stochastic transmission model was developed to predict *Salmonella* persistence at the pen level. The outbreak was limited to five clinical cases, and only 18 animals out of 500 cows shed *Salmonella* in feces. The longest shedder was culture-positive for *Salmonella* for at least 68 days. The isolates ($n = 27$) were represented by four pulsed-field gel electrophoresis patterns; three patterns were similar. With one exception, isolates were resistant to nine or more antimicrobial drugs. Simulations of the transmission model indicated that approximately 50% of the outbreaks were likely to die out within 20 days after the first animal was infected. The simulation studies indicated that salmonellosis outbreaks with few clinical cases were likely due to the extinction of the pathogen in the premises in the early phase of the outbreaks. Small population size and group structure within the farm decrease the on-farm persistence of the pathogen.

Introduction

NONTYPHOIDAL STRAINS OF *Salmonella enterica* are a major cause of foodborne illness in humans. In 2007, the incidence of *Salmonella* infection in the United States was 14.92 cases per 100,000 people, a value twofold higher than the national *Salmonella* target incidence set for 2010 (CDC, 2008). Although most *Salmonella* infections in humans are self-limiting, infections with multidrug-resistant (MDR) *Salmonella* can be particularly serious. Patients infected with MDR strains are at greater risk of bacteremia, hospitalization, and death compared to patients infected with susceptible strains (Varma *et al.*, 2005).

Ground beef and dairy products have been identified as important sources of MDR *Salmonella* (Dechet *et al.*, 2006; Varma *et al.*, 2006). Designing control strategies to reduce human exposure to MDR *Salmonella* requires a better understanding of their transmission and epidemiology within the cattle reservoir. Although, in dairy farms, MDR *Salmonella* strains are commonly associated with outbreaks that result in a limited number of salmonellosis cases (Veling *et al.*, 2002;

Cummings *et al.*, 2009), MDR *Salmonella* have also been isolated in feces and in the farm environment long after a clinical outbreak (Gay and Hunsaker, 1993; Cobbold *et al.*, 2006). The transmission dynamics in the early phase of an outbreak greatly determines the severity of the outbreak and persistence of the pathogen on the premises. The number of infectious animals in the early phase of an outbreak is small, and therefore the probability that the outbreak fades out due to stochastic events is high (Keeling and Rohani, 2008). For MDR *Salmonella*, the transmission dynamics after the onset of a clinical outbreak has been rarely monitored in a dairy farm. Therefore, a longitudinal study was conducted in a dairy herd after a few cases of salmonellosis (caused by an MDR *Salmonella* Typhimurium) were confirmed to assess the *Salmonella* transmission dynamics at the early phase of the outbreak. The infection status of the herd was monitored by fecal culture and serology. In addition, we developed a stochastic *Salmonella* transmission model to determine the probability of infection persistence in the dairy farm, and to evaluate the influence that factors such as the proportion of infectious animals with clinical disease and pen size have on infection persistence at the pen level.

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Materials and Methods

Herd description

The herd was a freestall-housed Holstein dairy that milked 500 cows three times a day with a rolling herd average of over 11,300 kg of milk per year. All the facilities for adult cattle were on-site. Figure 1 displays the pen layout and describes the cattle movements among pens. Cows were housed in six-row freestall barns bedded with sawdust twice per week. Cows that were near parturition were housed separately, and were given access to a drylot area. Their bed consisted of either newspaper or sawdust. Approval for the study was obtained from the Institutional Animal Care and Use Committee of Cornell University.

Sampling procedures

The farm was first enrolled in the study after three salmonellosis cases, which were diagnosed on October 20, 2005. There was no known history of *Salmonella* before that event. The farm did not vaccinate for *Salmonella*, but did vaccinate dry cows with a commercially available Gram-negative vaccine, Upjohn J-5 Bacterin (Pfizer Animal Health, Exton, PA) to prevent coliform mastitis. Sampling took place from November 02, 2005, to January 23, 2006 (12 weeks). The herd was visited every week to obtain fecal and blood samples. In each visit, all the animals in the fresh cow pen (where the outbreak started) or the early lactation pen 1 were sampled (Fig. 1). In addition, a convenience sample from the remaining pens was taken during each visit. Overall, approximately 120 fecal samples for *Salmonella* culture were taken during each visit; 60 to 80 samples were obtained from the fresh cow or the early lactation pen 1, and approximately eight cows were sampled in each remaining pens. Table 1 details the number of fecal samples by pen that were taken in each sampling day. Fecal samples were taken from the rectum using a clean glove for each animal. Individual fecal samples were placed into a 30 mL disposable vial filled with 15 mL modified Cary-Blair transport medium (Meridian Bioscience Inc., Cincinnati, OH). Blood was drawn from about half of the animals that

had a fecal sample taken. Sample selection for blood testing was based upon ease of getting a sample. Animals were placed in restraint chutes for both fecal and blood collection. If an animal had to be released because it had to be milked or it had been locked for a long period of time, that event determined which animals were sampled for blood testing. Blood from the caudal vein was taken using blood collection needles, which deposited blood into 10 mL blood serum collection vacuum tubes (BD Vacutainer, Franklin Lakes, NJ). Environmental samples were collected on three herd visits (once a month) from the fresh cow pen floor, hospital pen floor, early lactation pen 1 floor, and manure pit. At each location, environmental samples were collected by wiping the area (10.16×10.16 cm) with four sterile gauzes that had soaked in sterile skim milk. Each gauze was wiped eight times, and the four gauzes were placed in a Whirl-Pak bag (Nasco, Fort Atkinson, WI) as a composite sample.

Salmonella culture

Bacteriologic culture procedures included enrichment of sample material in tetrathionate broth (Difco, Detroit, MI), selection of potential *Salmonella* isolates by use of brilliant green novobiocin agar (Becton Dickinson and Company, Franklin Lakes, NJ) and xylose-lysine-tergitol-4 agar, and biochemical identification of suspect isolates with Kligler iron agar slants (Becton Dickinson and Company) and an automated identification system (Sensititre Microbiology System Division, Westlake, OH). *Salmonella* isolates were identified by somatic serogroup classification (B, C1, C2, D1, and E) by means of a slide agglutination test. Confirmed *Salmonella* isolates were sent to the National Veterinary Services Laboratory of the U.S. Department of Agriculture (USDA) for serotyping. Confirmed *Salmonella* cultures were stored at room temperature (~22°C) on tryptic soy agar slants until they were lyophilized in skim milk.

Antimicrobial susceptibility testing

A broth microdilution method was used to determine the minimal inhibitory concentration (MIC) of all isolates using

FIG. 1. Cattle distribution and main movements among pens. Numbers within the boxes indicate the average number of animals in the pen. Numbers on the arrows indicate the total number of animals that were moved among pens during the follow-up period (12 weeks). Sick cows were housed separately in the hospital pen. Maternity housing was never used as a hospital area for sick cows. Cows with reproductive problems were housed in the "Problem cows pen." The "Problem cows pen" and the hospital pen received animals from all the other pens. Heifers were not included.

TABLE 1. SUMMARY OF *SALMONELLA* FECAL SHEDDING DURING THE FOLLOW-UP PERIOD (12 WEEKS) BY PEN AND SAMPLING DAY AT THE SAMPLE LEVEL

	No. of positive samples	No. of samples collected	By sampling day											
			13	18	25	31	39	46	52	60	75	81	88	95
Dry pens	1	31			0/6	0/8	1/4	0/3	0/4	0/6				
Fresh pen	21	580	10/62 ^a	1/10	4/72	0/8	2/72	0/8	1/79	1/75	1/89	1/10	0/87	0/8
Early lactation pen 1	0	383	0/7	0/63	0/6	0/66	0/8	0/70	0/8	0/5	0/5	0/70	0/5	0/70
Early lactation pen 2	0	92	0/8	0/10	0/10	0/8	0/8	0/7	0/8	0/6	0/5	0/7	0/6	0/9
Mid lactation pen	1	91	1/8	0/10	0/6	0/8	0/8	0/8	0/8	0/8	0/5	0/8	0/6	0/8
Late lactation pen	1	90	1/10	0/10	0/6	0/8	0/8	0/7	0/8	0/6	0/5	0/8	0/5	0/9
Problem cows pen	1	91	0/8	1/11	0/9	0/8	0/8	0/6	0/8	0/6	0/5	0/9	0/5	0/8
Hospital pen	2	78	1/8	0/9	0/6	1/8	0/5	0/7	0/5	0/6	0/4	0/7	0/5	0/8
	27	1436												

Blank cells indicate that no samples were collected.

^aPositive samples/collected samples.

the National Antimicrobial Resistant Monitoring System panel of 15 antimicrobial agents: amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfizoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Isolates stored on tryptic soy agar slants were recovered for antimicrobial susceptibility testing by an overnight subculture on tryptic soy agar with 5% sheep blood. The MIC of *Salmonella* isolates was determined using the Sensititre semiautomated antimicrobial susceptibility testing system following the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH). For each antimicrobial agent, the minimum dilution that inhibited growth of the *Salmonella* isolate was recorded as the MIC. Quality control was performed every week; antimicrobial susceptibility testing was conducted using the following four bacteria: *Escherichia coli* ATCC 25022, *Staphylococcus aureus* 29213, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853. Clinical and Laboratory Standards Institute (CLSI) ranges for quality control were used when available (CLSI, 2008). Quality control results were always within expected ranges.

CLSI interpretive criteria were used to classify *Salmonella* isolates as resistant or not resistant to individual antimicrobial agents based on MIC panel results (CLSI, 2008). CLSI resistant breakpoints were based on human data for Enterobacteriaceae. No interpretive criteria for Enterobacteriaceae were available for ceftiofur or streptomycin, so the resistant breakpoints presented in the National Antimicrobial Resistant Monitoring System 2000 Annual Report were used for these antimicrobial agents (USDA, 2000).

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed according to the Centers for Disease Control and Prevention (CDC) PulseNet protocol (Ribot, *et al.*, 2006), using the restriction enzyme *Xba*I (50 U/sample). PFGE was performed with the CHEF-Mapper (Bio-Rad Laboratories, Hercules, CA). Electrophoresis conditions included an initial switch time of 2.16 seconds, a final switch time of 63.8 seconds, and run time of 21 hours. The CDC *Salmonella* serovar Branderup strain H19812 was used as the reference strain and size marker. Images of PFGE gels were captured by Gel/ChemiDoc (Bio-Rad Laboratories, Milan, Italy). Gel analysis and pattern

comparisons were performed using BioNumerics Software package (Applied Maths, Ghent, Belgium). Similarity analysis was performed using the Dice similarity coefficient, and clustering was performed using the unweighted pair group method by arithmetic mean.

Enzyme-linked immunosorbent assay procedure

An enzyme-linked immunosorbent assay method was used to detect immunoglobulin (IgG) immunoglobulins to *Salmonella* Typhimurium lipopolysaccharide. The method used in this study has been previously described with slight modifications (Hoorfar and Bitsch, 1995; Hoorfar *et al.*, 1995; Hoorfar and Wedderkopp, 1995; Nielsen *et al.*, 2004). A cutoff optical density percentage of 25 was used to classify cattle as seropositive as recommended by the testing laboratory. We evaluated the association between fecal testing and seroconversion using the Fisher exact test (right-sided).

Duration of fecal shedding

Duration of fecal shedding was estimated by the Kaplan-Meier life-test method; animals without three consecutive negative samples after the last positive sample at the end of the observed period were right-censored. To account for the unobserved shedding time between sampling days, the following rules were used: (1) The unobserved shedding period before the first positive was calculated as the midpoint of time between the day of the first positive sample and the day of the index case. (2) The unobserved shedding period after the last negative was the midpoint between last positive and first negative. If the animal was not sampled after last positive, the time between last positive and the next day a sample would have been taken was used.

Transmission model

To assess the transmission processes underlying the observed dynamics, we developed the stochastic version of one of the models for *Salmonella* transmission in dairy cattle presented in Lanzas *et al.* (2008). Two infected (*I*) states were included in the model: clinically infected (*I_c*) and subclinically infected (*I_s*); a fraction of the infected animals was assumed to become *I_s* immediately after infection, and all *I_c* were assumed to become *I_s* before full recovery. The model was modified to include

movement of animals between pens to be able to compare the infection dynamics predicted by the model with the observed outbreak. The following assumptions were included: (1) direct contact transmission is the only route of new infections, and transmission was assumed to be density dependent (i.e., transmission is proportional to the number of infectious individuals), (2) animals had only contact with other animals within their pen, and therefore infection could only be spread from one pen to another by movement of infected animals, (3) animals with clinical infection were not moved from their pens, and (4) culling rate was assumed identical in all pens. The differential equations for the modified model for the pen i are as follow,

$$\begin{aligned}\frac{dS_i}{dt} &= \sum_{j=1}^j a_{ji} S_j + r R_i - (\beta_c I_{Ci} + \beta_s I_{Si} + \mu) S_i - \sum_{j=1}^j a_{ij} S_i \\ \frac{dI_{Ci}}{dt} &= f(\beta_c I_{Ci} + \beta_s I_{Si}) S_i - (e + m + \mu) I_{Ci} \\ \frac{dI_{Si}}{dt} &= \sum_{j=1}^j a_{ji} I_{Sj} + (1-f)(\beta_c I_{Ci} + \beta_s I_{Si}) S_i \\ &\quad + e I_{Ci} - (h + \mu) I_{Si} - \sum_{j=1}^j a_{ij} I_{Si} \\ \frac{dR_i}{dt} &= \sum_{j=1}^j a_{ji} R_j + h I_{Si} - (r + \mu) R_i - \sum_{j=1}^j a_{ij} R_i\end{aligned}$$

The transmission parameter definitions and values are as presented in Table 2 and are based on the transmission parameters described in Lanzas *et al.* (2008). The direct method of Gillespie (1976) was used to simulate the model stochastically. The method includes the following steps in each iteration: (1) the time to the next event is obtained by sampling an exponential distribution with the accumulative rates of all

possible events as a mean value, (2) the type of next event is randomly chosen by converting the event rates into probabilities, and (3) time and population variables are updated.

Results

Longitudinal study

A total of five clinical cases were reported. Three salmonellosis cases were reported on October 20, 2005. One of the animals died on October 23. Two more clinical cases were reported the same day that the frequent sampling began (November 2, 2005). *Salmonella* Typhimurium was isolated from 18 animals. Thirteen of the *Salmonella*-positive animals were multiparous. *Salmonella* was cultured from a small percentage of the fecal samples (1.9%), with 37% of the positive fecal samples clustered in one time sampling in the fresh pen (Table 1). The isolates were represented by four different PFGE patterns (Fig. 2) and three antimicrobial resistance patterns (Table 3). One of the isolates was from a cow in the hospital pen, and was pan-susceptible and had a unique PFGE pattern (Fig. 2, pattern 1). PFGE patterns 2, 3, and 4 were similar. Nineteen isolates had PFGE pattern 2, and five isolates represented pattern 3. Seven isolates were from a single cow in the fresh pen. The isolates from this single animal represented PFGE patterns 2 and 3 and had antimicrobial profiles B and C, respectively. PFGE patterns 2 and 3 differed only in two bands. A low molecular weight band shifted from approximately 175 to 140 kb. A single isolate from one animal had pattern 4 (Fig. 2) and antimicrobial profile C. All the environmental samples taken from the pen floors and manure pit tested negative.

The median duration of shedding was 12 days (13.5 days if an estimation of the unobserved shedding period was added). The duration of shedding ranged from 1 to 68 days (or 8 to 78 days if an estimation of the unobserved shedding was added). A total of 535 blood samples were collected and analyzed for IgG response to *Salmonella* lipopolysaccharide. Seropositive samples (112 samples) were found in all the pens (Table 4), but the fresh pen and the early lactation pen 1 had more than 60% of the seropositive samples. No clear temporal pattern was observed on the IgG titers for all the tested samples or the tested samples from the fresh pen. No association was found between seroconversion and fecal testing ($p = 0.457$).

Transmission dynamics

We present the simulations for the fresh pen (Figs. 3 and 4), because the outbreak was mainly limited to the fresh pen. The number of fecal *Salmonella*-positive animals observed in the sampling days in which all the animals of the fresh pen were tested fell into the predicted distribution for subclinically infectious animals (Fig. 3A). Overall, the model predicted outbreaks with few infected animals; 50% of the outbreaks died out within days after the first infectious animal. The predicted number of recovered animals increased until approximately day 50 and then remained steady (Fig. 3B). The predicted number of recovered animals was lower than the number of estimated seroconverted animals (Fig. 3B). The extinction of the infection (i.e., when the pen had no infectious animals) was observed in the early phases of the outbreaks (Fig. 4). Twenty days after the onset of the outbreaks, the probability

TABLE 2. LIST OF PARAMETERS FOR THE *SALMONELLA* TRANSMISSION MODEL

Symbol	Parameter definition, units	Value
μ	Replacement and exit rate, d^{-1}	0.0011
β_c	Transmission coefficient for clinical animals, $\text{animal}^{-1} d^{-1}$	0.007 ^a
β_s	Transmission coefficient for subclinical animals, $\text{animal}^{-1} d^{-1}$	0.00026 ^a
f	Proportion of infected animals that develop clinical disease	0.50
e	Rate of clinical cases that become subclinical, d^{-1}	0.25
h	Recovery rate for subclinical cases, d^{-1}	0.041
m	Disease induced mortality rate, d^{-1}	0.011
r	Immunity loss rate, d^{-1}	0.01
a_{ji}	Animal movement rate from pen j to pen i , d^{-1}	^b
a_{ij}	Animal movement rate from pen i to pen j , d^{-1}	^b

^aTransmission parameters, β_c and β_s , were scaled to maintain the baseline basic reproduction number ($R_0 = 1.53$) from model presented in Lanzas *et al.* (2008).

^bValues presented in Fig. 1 were used to parameterize the animal movement rates between pens.

FIG. 2. *Xba*I pulsed-field gel electrophoresis (PFGE) patterns of *Salmonella* Typhimurium isolates.

of having at least one infectious animal was less than 50% for all scenarios, and the proportion of infected animals with clinical disease or pen size had little effect on these early extinctions (Fig. 4). On the other hand, increasing the proportion of infected animals with clinical disease and pen size significantly increased the persistence of those outbreaks that did not become extinct immediately after the outbreak onset (e.g., 20% of the outbreak lasted longer than 150 days when the proportion of infected animals with clinical disease was 0.75) (Fig. 4).

Discussion

The objective of this study was to improve our understanding of the dynamics of MDR *Salmonella* transmission within dairy cattle herds and the factors that influence the pathogen persistence at the farm level and, therefore, how long the herd represents a risk for *Salmonella* zoonotic transmission. The farm studied here had only a small cluster of

clinical cases. Small salmonellosis outbreaks are indeed the most common presentation of MDR *Salmonella* infection in dairy herds (Cummings *et al.*, 2009). The longitudinal and simulation studies indicated that the extinction of pathogen in the premises due to stochasticity during the early phase of the outbreak is the most likely cause of the short-lived outbreaks. Stochasticity increases the probability of breaking the chain of transmission (i.e., infectious animals fail to transmit to susceptible animals) and the probability that the pathogen goes extinct in the herd, with only a few infected animals as a final outbreak size. This phenomenon was illustrated on the simulations (Figs. 3 and 4). The model predicted that 80% of the outbreaks lasted less than 68 days (Fig. 4). The effect of stochasticity plays smaller role in larger populations, and thus larger pen size increased the duration of the larger outbreaks (Fig. 4). Larger pen size also increases the number of available susceptible animals (Keeling and Rohani, 2008). A convenience sample was taken for pens other than the fresh and early lactation pen 1 due to limitations on personnel and laboratory

TABLE 3. ANTIMICROBIAL RESISTANCE PATTERNS OF *SALMONELLA* ISOLATES OBTAINED DURING THE FOLLOW-UP PERIOD OF *SALMONELLA* TYPHIMURIUM OUTBREAK

Antimicrobial resistance pattern ID	Resistance pattern	Number of isolates	Number of animals
A	Pansusceptible	1	1
B	AUG, AMP, FOX, TIO, AXO, CHL, STR, FIS, TET	5	1
C	AUG, AMP, FOX, TIO, AXO, CHL, GEN, KAN, STR, FIS, TET	21	17

AUG, amoxicillin/clavulanic acid; AMP, ampicillin; FOX, cefoxitin; TIO, ceftiofur; AXO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline.

TABLE 4. SUMMARY OF *SALMONELLA* SEROLOGICAL STATUS DURING THE FOLLOW-UP PERIOD (12 WEEKS) BY PEN AND SAMPLING DAY AT THE SAMPLE LEVEL

	No. of positive samples	No. of samples collected	By sampling day											
			13	18	25	31	39	46	52	60	75	81	88	95
Dry pen	4	15			2/3	1/5	0/2	1/3	0/2					
Fresh pen	45	163	3/14 ^a	3/7	7/29	4/7	3/17	0/1	7/24	4/19	5/13	2/7	6/21	1/4
Early lactation pen 1	27	106	2/9	6/18	0/4	8/30	2/6	3/11	1/1	1/3		1/4	1/3	2/17
Early lactation pen 2	5	51	1/5	0/6	0/5	0/5	1/5	1/3	0/5	2/3	0/2	0/4	0/3	0/5
Mid lactation pen	9	56	0/6	0/6	0/4	2/6	1/5	2/6	0/4	0/5	0/3	1/3	0/3	3/5
Late lactation pen	3	42	1/8	1/6	0/4	0/4	0/5	0/4	0/4	0/1	0/1	1/3		0/2
Problem cows pen	11	60	2/6	0/8	0/5	0/6	1/4	0/4	1/5	1/4	1/5	1/5	2/3	2/5
Hospital pen	8	42	0/6	0/7	1/3	1/4	1/3	1/1	2/3	1/3		0/2	1/5	0/5
	112	535												

Blank cells indicate that no samples were collected.

^aPositive samples/collected samples. Samples with an optical density greater than 25 were considered seropositive.

capacity. The testing indicated that *Salmonella* infection was limited to the fresh pen. Although the use of convenience sampling may have caused a bias (i.e., underestimated the presence of *Salmonella* on those pens), model simulations agreed with the observation that outbreaks were likely to be limited to the fresh pen. Group structure (i.e., animals are subdivided in pens) makes major outbreaks less likely than would be the case in a homogenous population because of the reduced contact among animals of different pens (Xiao *et al.*, 2006).

FIG. 3. (A) Number of subclinically infected animals and (B) number of recovered animals in the fresh pen predicted by the stochastic *Salmonella* transmission model. The solid line indicates the median, the dash-dot lines indicate the 25th and 75th percentiles, and the dash line indicates the maximum. The observed *Salmonella*-positive animals (fecal shedding) are indicated with a solid circle in (A). The number of seropositive animals estimated from a representative sample is indicated with a circle in (B).

Clinical cases may shed larger quantities of colony-forming unit than subclinical cases (Fossler *et al.*, 2005); as a result, a larger proportion of infected animals with clinical disease may contribute to longer outbreaks (Fig. 4). This may not apply for all *Salmonella* serotypes. Long persistence and high prevalence of fecal shedding in the absence or presence of a few clinically infected animals have been reported for some serotypes (e.g., *Salmonella* Cerro and *Salmonella* Kentucky) (Huston *et al.*, 2002; Van Kessel *et al.*, 2007). Nevertheless, long-term persistence of MDR *Salmonella* is often associated with the presence of only a few animals shedding *Salmonella* (Cobbold *et al.*, 2006). In this study, a single animal accounted for 7 out of 27 positive fecal samples, and had a shedding period considerably longer than the rest of the positive animals. From day 46 to 81 of the follow-up period, this animal was the only animal shedding *Salmonella*, and no new infections were observed during that period (Table 1). Isolates obtained from this long-term shedder animal

FIG. 4. Probability of infection persistence in the fresh pen for the baseline scenario (50% of the infectious animals developed clinical disease), low virulence (LV) (25% of the infectious animals developed clinical disease), high virulence (HV) (75% of the infectious animals developed clinical disease), smaller pen (SP, 50 animals), and larger pen (LP, 150 animals).

had two PFGE patterns that differed on a single band and two antimicrobial profiles because of the lost resistance to gentamycin and kanamycin. The lower weight band observed in pattern 3 may result from the loss of the genes encoding the resistance to gentamycin and kanamycin. When the role of long-term shedders was evaluated through mathematical modeling, the impact of long-term shedders on the transmission of the infection within a herd was found to be small (Lanzas *et al.*, 2008). The presence of one long-term shedder may not be enough to raise the challenge exposure above the minimum infectious dose.

Serologic response to *Salmonella* antigens provides an indirect indication of *Salmonella* exposure (House *et al.*, 2001). The short duration of the outbreak caused a limited serological response; no clear temporal pattern was observed in the serologic response to *Salmonella*. Few seropositive animals were found in pens other than the fresh and early lactation pens, suggesting that the exposure was limited to the fresh pen. Seropositive animals were detected in the early lactation pen 1 as they moved from the fresh pen. Seroprevalence was greater than the prevalence of fecal shedding. When the number of seropositive animals (estimated by a representative sample) was compared with the predicted number of recovered animals, more animals were seropositive than predicted by the model. This trend may be due to an earlier exposure to *Salmonella*, poor sensitivity of the isolation method, or a seroconversion without previous fecal shedding. Isolation methods using tetrathionate broth enrichment for isolating *Salmonella* from porcine fecal samples have a reported sensitivity ranging from 80% to 91% (Love and Rostagno, 2008). Seroconversion without fecal shedding may take place when animals are exposed to low levels of *Salmonella*.

Conclusions

Assessing *Salmonella* persistence within the herd has important public health implications, as subclinically infected animals can shed *Salmonella* intermittently for prolonged periods. In herds without a history of salmonellosis, a few clinically and subclinically infected animals, as the studied herd, are likely due to *Salmonella* extinction in the early phases of the outbreaks, and may represent a short risk for *Salmonella* zoonotic transmission. However, simulations indicated that a small percentage of the outbreaks can last for long periods.

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Disclosure Statement

No competing financial interests exist.

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