Survival characteristics of *Salmonella enterica* serovar Newport in the dairy farm environment

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ABSTRACT

Multi-drug resistant (MDR) *Salmonella enterica* serovar Newport (*S. Newport*) has established a reservoir in dairy cattle. Infected herds suffer significant mortality in both adult and young animals, posing a considerable economic loss to producers. Land application of manure from infected animals may further spread the pathogen into the agroecosystem, causing public health concerns. Previous work by our group demonstrated that the organism persisted in manure and manured soil for 6 to 10 mo under laboratory conditions. In the present study, we determined the survival characteristics of MDR *S. Newport* in a dairy lagoon, compost pile, and soil of a grass field under natural conditions using environmental sentinel chambers with an initial concentration of *S. Newport* around $7 \log_{10}$ per gram. In the static compost pile at 64°C, *S. Newport* was eliminated within 18 h. In the dairy effluent lagoon, the pathogen survived for >137 d, whereas in the field soil, the organisms persisted for over 276 d. The survival of MDR *S. Newport* in both the lagoon and field soil followed a pattern of (1) an increase or plateau for a few days, (2) log-linear decline for 6 to 13 wk, and (3) a long tailing phase at low and variable concentration for 4 to 9 mo. Log reduction times (days required for 90% decrease in concentration) based on the log-linear decline phase were 7 d in the lagoon and 14 to 20 d in the soil. Conditions leading to faster inactivation during the initial phase do not necessarily translate into a quicker elimination of the pathogen. Regression models of the log-linear phase may be inaccurate for estimating complete pathogen elimination.

Key words: dairy environment, *Salmonella* Newport, pathogen survival, sentinel chamber

INTRODUCTION

Zoonotic pathogenic bacteria including *Salmonella* can move from animal production into food and water supply systems, causing human illnesses and suffering (Sanchez et al., 2002). Laboratory-confirmed cases of salmonellosis in the United States exceeded 40,000 in 2005 (CDC, 2006), with an estimated 15,000 hospitalizations and 400 deaths annually (Voetsch et al., 2004). Although proving point of entry of pathogens into the human food and water supply chains is difficult, use of animal manures as nutrient sources for crops and irrigation with waters contaminated by animal wastes have been implicated in several outbreaks (Doyle and Erickson, 2008).

Multi-drug resistant (MDR) *Salmonella enterica* serovar Newport (*S. Newport*) has undergone a rapid epidemic spread on dairy farms in recent years (Cobbold et al., 2006). Consequently, the American Association of Veterinary Laboratory Diagnosticians has named this *Salmonella* serotype an emerging disease (Clark, 2004). Both adult and young animals, when infected, suffer significant mortality, posing a considerable economic loss to dairy farmers. In both clinical and subclinical cases, animals infected with MDR *S. Newport* shed the bacteria in manure continuously or intermittently for weeks or even months. Feces excreted by infected animals contain the pathogen, with concentrations typically ranging from 10^2 to 10^7 cells per gram of fresh feces (You et al., 2006). Addressing the post-shedding fate of MDR *S. Newport*, our group conducted a laboratory incubation study and found that the organism could survive up to 26 wk in manure and 47 wk in manure-amended soils (You et al., 2006). The long survival of *S. Newport* under laboratory conditions suggests the possibility for environmental dissemination beyond farm boundaries when animal manures are used as nutrient sources for growing crops.

The survival characteristics of manure-borne pathogens may differ under naturally varying conditions (Kudva et al., 1998). It is imperative to enhance our understanding of MDR *S. Newport*'s behavior in the natural farm environment. Toward this end, the en-

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vironmental sentinel chamber devices developed by Jenkins and colleagues (1999, 2002) provide a suitable means for studying highly contagious pathogens such as MDR *S. Newport* in natural environmental settings. The sentinel chamber is a small cylinder with ends capped with semi-permeable porous membranes, allowing moisture and gas exchange, but preventing escape of potentially dangerous agents into the surrounding environment. In the present study, we used sentinel chambers to monitor the survival patterns of MDR *S. Newport* in the dairy farm environment, including a static manure compost pile, a dairy effluent lagoon, and a field soil. Our primary objective was to determine the dynamics and deactivation trends over time as well as the maximum survival time of MDR *S. Newport* in the agroecosystem. A secondary objective was to evaluate the integrity and performance of the sentinel chambers when used in the different farm settings.

MATERIALS AND METHODS

Site Description

The study was conducted at a research dairy facility with a 200-cow herd and 150-ha cropland, located in the Piedmont physiographic province in southeastern Pennsylvania. The area has a long history of agricultural production and is home to thousands of dairy farms with mostly family-operated herds of <200 cows. Regional annual precipitation averaged approximately 119 cm in the past 10 yr, and annual temperature means ranged from -0.7°C in January to 24.0°C in July (Pennsylvania State Climatologist, 2011).

Dairy manure, consisting of feces and urine from healthy, lactating Holstein cows, was collected from the barn floor immediately following excretion. The manure sample contained little bedding material. Manure was stored frozen at -20°C , and thawed before experimental use in the laboratory. Soil (0 to 20 cm depth) was obtained from a field that had been planted with a corn, alfalfa, and grass rotation for 11 yr. The soil was mapped as Conestoga silt loam, fine-loamy, mixed, active, mesic Typic Hapludalf (Penn State College of Agricultural Sciences, 2010). The soil pH was 6.5, OM was 2.2%, cation exchange capacity was 10.6 cmol of charge (cmol_c)/kg, and had available P, K, Ca, and Mg of 0.068, 0.112, 1.503, and 0.335 g/kg, respectively. The soil was partially air-dried and sieved to pass 2.36 mm. Random subsamples of soil and manure were tested for presence of *Salmonella*, and any manure or soil samples showing black colonies on xylose-lysine-deoxycholate agar (XLD; presumptive *Salmonella* or other bacteria with colony color and morphology similar to *Salmonella*) were discarded.

Sentinels, Bacteria, and Inoculation

Sentinel chambers were 3-cm length sections of 1.27-cm i.d. polyvinyl chloride (PVC) pipe. Ends were covered with 0.22- μm pore size nitrocellulose filter membranes sealed with nitrocellulose cement to retain inoculated manure or manure-soil materials while allowing air and moisture exchange. The PVC sections and membranes were gas-sterilized with ethylene oxide.

A pure culture of *S. Newport* (Salmonella Reference Center isolate 0007-33) was used. This is the same strain used in the previous study (You et al., 2006); the strain is resistant to ampicillin, chloramphenicol, streptomycin, spectinomycin, sulfamethoxazole, tetracycline, cephalothin, and ceftriaxone. To propagate, a single colony was transferred aseptically to 40 mL of sterile brain heart infusion broth (BHI) in centrifuge tubes and incubated 24 h at 37°C . After the first incubation, 1 mL of the culture was inoculated into BHI and incubated for an additional 24 h at 37°C . Following the second-stage incubation, the tubes were centrifuged (20 min, $2,700 \times g$), the supernatant decanted, and the pellet washed with physiological saline solution (PSS, 8.5 g of NaCl/L); then, the bacteria were resuspended with vortexing in 40 mL of PSS. *Salmonella* inoculum was stored at 4°C until use.

For inoculated manure-soil mixtures, 16 g of thawed manure was mixed with 1 kg of soil; the manure and soil ratio corresponds to an agronomic manure application rate to supply 180 kg of N/ha. The inoculum was added with thorough mixing, providing a target initial *Salmonella* population of 7 log₁₀ cfu per gram of soil-manure mixture. The moisture content was adjusted with sterile deionized water to bring it to 90% of the soil's field capacity (field capacity = 0.313 g/g of gravimetric moisture content), accounting for water in the soil, manure, and inoculum. Inoculated soil-manure mixtures were used in the field soil trials (see below). In the compost and lagoon trials, dairy manure alone without soil was inoculated and handled in a similar manner.

Sentinel chambers were filled with 3 to 4 g of inoculated manure or manure-soil mixture, and the upper nitrocellulose membrane cemented in place. Three sentinel chamber replications were done per treatment; sentinel chambers containing uninoculated materials served as controls. For ease of periodic sampling, 4 or 5 sentinel chambers were fixed in a 10-cm square block of foam rubber, covered with fine-mesh nylon cloth to prevent puncturing of the fragile end membranes, and then the foam sampler modules were placed in a 25-cell array constructed from PVC slats (Figure 1, A-C).

Figure 1. Environmental sentinel chambers: A. sentinel chambers, sampling module, nitrocellulose end membranes, and nitrocellulose cement; B. 25-cell sampler frame before installation in field; C. sentinel chambers installed under grass sod.

Experimental Settings, *Salmonella* Enumeration

Sentinel chambers were installed and the persistence of *S. Newport* was monitored in 3 dairy farm environmental settings: (1) in a static compost pile: manure solids from the dairy's separator system were filled to a depth of 60 cm in a 100 × 100 × 120 cm high, wire-mesh bin. An array of sentinel chambers containing inoculated manure was inserted and covered with 30 cm of the compost material. The temperature in the compost pile was monitored. Sentinel chamber samples were taken on d 0, 1, 5, 8, and 12 and the samples were processed in the laboratory for *Salmonella* enumeration; (2) in a dairy lagoon: the lagoon effluent consisted of milk parlor wash water and water from the twice-daily alleyway flushing, after removal of manure solids. Sentinel chambers containing inoculated manure were immersed at a depth of approximately 30 cm beneath the surface of the effluent and samples were taken periodically for *Salmonella* enumeration; and (3) in the grass border of an agricultural field 10 cm below the soil surface: 4 field soil trials were done, each initiated in a different season and with different durations: trial 1, November 2007 to February 2008; trial 2, April 2008 to May 2009; trial 3, June 2008 to September 2009; and trial 4, September 2009 to March 2010. Samples were collected periodically for *Salmonella* enumeration; soil and sentinel chamber contents moisture content, soil and air temperature, and precipitation were also recorded.

In the laboratory, after aseptically removing the membrane at one end, approximately 2 g of sample was weighed into a 50-mL centrifuge tube, 18 mL of PSS was added, and the contents were agitated thoroughly by hand, and then on a reciprocal shaker for 6 min at 180 oscillations/min. Serial 1:10 dilutions in PSS were made to bracket the expected pathogen concentrations. Aliquots of 100 μ L were plated in triplicate on xylose-lysine-deoxycholate agar, and incubated 24 h at 37°C. Black colonies with a narrow pale outer ring were

considered presumptive *Salmonella*. This direct plating procedure was used until *Salmonella* numbers dropped below the detection limit of approximately 2 log₁₀ cfu/g. After reaching the detection limit, an adaptation of the most probable number (MPN) technique (US FDA, 2010) was used to estimate *Salmonella* numbers.

Data Analysis

Colony-forming unit data were converted to log₁₀ cfu/g of soil-manure or manure mixtures. *Salmonella* die-off for the direct-count data was analyzed as a linear first-order deactivation using PROC GLM in SAS (SAS Institute, 2008). The time to extinction was calculated by multiplying the inverse of the deactivation rate per day by the initial *Salmonella* concentration in the inoculated manure or soil-manure mixtures. Differences in deactivation rates were tested for homogeneity of regression coefficients by covariance analysis in PROC GLM. Equilibration of sentinel chamber contents with bulk soil matrix moisture was compared using PROC TTEST in SAS.

RESULTS

Performance of Sentinel Chambers

The sentinel chambers weathered well in the field trials with natural soil wetting-drying and freeze-thaw cycles. The nitrocellulose membranes remained intact except for occasional cracked or broken membranes noted over the many months of the study. However, membranes were less durable in the effluent lagoon and static compost pile. Membranes were observed to soften and discolor rapidly in the compost. In the effluent lagoon, the experiment had to be terminated after d 137 because broken end membranes prevented valid sample collection thereafter.

Measurements from the 4 field soil trials were combined to show sentinel chamber ability to equilibrate

Figure 2. Moisture content in soil-manure mixtures contained in sentinel chambers compared with moisture content of the field soil matrix in 2007 to 2008. Bars denote precipitation in centimeters. The gravimetric water content of the soil matrix at field capacity (0.313 g/g) is represented by the horizontal line.

with moisture conditions in the surrounding soil matrix. The manure-soil mixture contained in sentinel chambers generally equilibrated with moisture in the surrounding bulk soil (Figure 2). However, close examination revealed some seasonal effects (Figure 3): sentinel chamber moisture content was 78% of that of the soil matrix in spring and summer ($P > t < 0.001$ and $P > t = 0.028$, respectively, by season) but 104% in fall and winter ($P > t = 0.657$ and 0.958). Across all sampling times ($n = 95$), sentinel chamber contents were lower in moisture than the surrounding soil (pairwise comparison, $P > t = 0.003$).

Salmonella Deactivation in the Compost Pile

Salmonella Newport was not detected by either direct plating or MPN analysis in any of the samples collected, including the d-1 samples, which were obtained within 18 h of installation. The temperature in the compost pile was 64°C at the time when the d-1 sample was collected. Apparently, the high temperature had caused a rapid die-off of the inoculated pathogen, although a few other enteric bacteria colonies were found in some of the samples on d 1 and 3 of the trial.

Survival in the Effluent Lagoon

Samples obtained on d 1 and 3 showed an increase in *Salmonella* concentration from 6.50 to 6.78 log₁₀ cfu/g (or by 1.9-fold). This was followed by a steady decrease

to the detection limit (direct plating) of 2 log₁₀/g by d 35. The pathogen persisted at relatively low concentrations of 0.3 to 1.9 log₁₀/g (determined by MPN analysis) until d 137 (Figure 4). Maximum survival was >137 d but the experiment had to be terminated because at the d 161 sampling, the nitrocellulose membranes had deteriorated to the point where contamination of sen-

Figure 3. Seasonal differences in moisture equilibration of soil-manure mixture sentinel chamber contents compared with the surrounding bulk soil matrix.

Table 1. Survival of *Salmonella enterica* serovar Newport (*S. Newport*) in the dairy farm environment: in a static dairy manure solids compost pile (compost), a dairy effluent lagoon (lagoon), and in the soil of a grassed field (soil)

Environment	Log-linear regression ¹	R ²	Log reduction time ² (d)	Time to extinction ³ (d)	Time to reach detection limit ⁴ (d)	Maximum survival ⁵ (d)
Compost	—	—	—	—	—	<1
Lagoon	$Y = -0.1381X + 7.10$	0.95	7.2	47	35	>137
Soil trial						
1: Nov. 2007 to Feb. 2008	$Y = -0.0512X + 5.61$	0.91	19.5	122	43	—
2: Apr. 2008 to May 2009	$Y = -0.0612X + 5.86$	0.95	16.3	102	70	>219
3: June 2008 to Sep. 2009	$Y = -0.0697X + 6.57$	0.96	14.3	96	59	>276
4: Sep. 2009 to Mar. 2010	$Y = -0.0547X + 6.70$	0.96	18.3	126	92	—

¹Equation derived for the log-linear phase, excluding the initial increase in the first 3 d of the trials.

²Time to 90% reduction from the initial population, derived from the regression equation.

³Estimated time to extinction, derived from the regression equation.

⁴By direct plating approximately 100 cfu/g.

⁵Last detection of *S. Newport* by most-probable-number analysis before sentinel device deterioration (in lagoon), or *Salmonella* not detected for 2 consecutive samplings (field trials 2 and 3). The last detection was not determined in trials 1 and 4, which were terminated while *S. Newport* was still present.

tinel chamber contents by effluent could not be ruled out. Deactivation of *S. Newport* during the steady decline phase (d 3–35) could be described by a first-order kinetic model (i.e., log-linear reduction) with an R² of 0.95. Based on the regression equation (Table 1), the calculated log reduction time (i.e., 90% reduction) was 7.2 d and time to extinction 47 d.

Persistence in Field Soil

A transient phase appeared to occur in the first few days of the field trials, in which *S. Newport* populations

showed little change or even small increases (except for trial 1, which showed substantial decreases starting on d 1). This was followed by a period of steady decrease in a log-linear fashion to the direct plating detection limit. This log-linear decline phase lasted from 43 to 92 d, depending on the time of the trials (Table 1). Subsequently, the pathogen persisted for a long time at low and variable concentrations ranging from 1.07 to 0.12 log₁₀ cfu/g (Figure 4 for trials 2 and 3). The maximum survival time (i.e., *Salmonella* no longer detected by MPN analysis over 2 consecutive sampling times) was

Figure 4. Survival dynamics of *Salmonella enterica* serovar Newport (*S. Newport*) in a dairy effluent lagoon and in 2 trials in field soil (trial 2: April 2008 to May 2009; trial 3: June 2008 to Sep. 2009), determined by direct plating until detection limit (horizontal line), then by most probable number analysis thereafter.

>219 d in trial 2 and >276 d in trial 3; trials 1 and 4 were conducted over shorter time periods; therefore, no maximum survival time was determined.

Despite the overall trends described above, trial 3 (the longest field soil trial, running from June 2008 to September 2009) exhibited a break in the log-linear phase between d 22 and 42 when *S. Newport* concentration remained almost constant before resuming the steady decrease. No dramatic or abrupt changes in soil moisture or air temperature occurred during this time, so we do not know what factors might have contributed to the hold-off.

There appeared to be a seasonal effect on *S. Newport* survival during the log-linear decline phase. As shown in Table 1, the value of the slope in the log-linear regression models was greater (i.e., faster deactivation) in trials started in warmer seasons than those in cooler seasons: 0.0697, 0.0612, 0.0547, and 0.0512, corresponding to trials that started in June, April, September, and November, respectively. Likewise, log reduction times were shorter in trials begun in spring and summer (14.3 and 16.3 d) than in fall and winter (18.3 and 19.5 d; Table 1), although differences were not significant by covariance analysis of homogeneity of regression coefficients.

DISCUSSION

In research investigations of pathogen behavior in the natural farm environment, the integrity of sentinel devices is important for preventing the inoculated pathogen from polluting the natural system. It appears that the durability of the sentinel chamber end membranes can be an issue for studying pathogens in compost and manure lagoons. As evidenced in the present study, membranes tended to disintegrate in such settings. High organic and solute contents in the lagoon as well as high temperatures in the compost likely contributed to the membrane deterioration. Different membrane materials may be tested in future studies. On the other hand, the sentinel device performed satisfactorily in the field soil trials. The small discrepancy in moisture contents between sentinel chamber contents and the surrounding soil matrix may be due to interruption of natural pathways of soil water movement because of the several-millimeter headspace between the manure-soil mixture and the membrane. Jenkins et al. (1999) reported equilibrium between sentinel chamber contents and field soil, but their comparison was based on only 3 replicated sentinels on 2 sampling dates. Nevertheless, moisture of the sentinel chamber contents fluctuated with seasonal and soil condition changes (Figure 2), and the relatively small moisture discrepancy should not hinder the usefulness of sentinel chambers as a device for studying pathogens in the natural setting.

Composting is generally viewed as a way to decrease pathogen concentrations, thereby rendering animal manures or biosolids safer for land application (Zaleski et al., 2005; Albiñán and Vinnerås, 2007). However, most studies of pathogen dynamics in compost did not report the rapid and complete elimination of *Salmonella* that we found here. For example, Nicholson et al. (2005) reported that *Salmonella* inoculated into solid dairy manure piles survived 4 d, over which time temperature inside the piles increased from approximately 30 to 50°C. In composted biosolids, Ahmed and Sorensen (1995) found a log reduction time for *Salmonella* Typhimurium to be 1.13 d at 50°C and 2.5 d at 38°C with initial inoculated concentrations of 3.3 to 8.2 log₁₀/g. Vinnerås (2007) noted decreases in *S. Typhimurium* from 7.4 log₁₀/g to 5 log₁₀/g over 5 d; the temperature in their 90-L compost reactor containing fecal matter mixed with food waste only gradually increased and it took 12 d to reach 65°C. One study even noted growth of pathogens in composted sludge when the temperature was relatively low (28 to 36°C) but pathogen concentration eventually fell below the detection limit when the compost temperature was 44°C (Russ and Yanko, 1981). Apparently, how far and how fast the temperature rises in compost is the key factor determining the deactivation of pathogens. Burge et al. (1982) and Zaleski et al. (2005) considered a temperature of 55°C for 2 to 3 d to be sufficient to destroy pathogens potentially present in biosolids composts. In the present study, the temperature had reached 64°C within 18 h in the compost trial conducted in July, which caused swift and complete deactivation of the inoculated *Salmonella* contained in the sentinel chambers.

Comparing the survival characteristics of *S. Newport* in the dairy effluent lagoon (Figure 4; Table 1) with that of the earlier study (You et al., 2006), a similar pattern emerged: the initial increase, the steady log-linear decline, and a long tailing phase at low concentrations. However, the kinetics were different: the die-off was more rapid in the lagoon under natural conditions (present study) than in the dairy feces under constant temperature and moisture in the laboratory (previous study), signified by the slopes of the regression equations (0.138 vs. 0.0515, respectively), log reduction times (7.2 vs. 19.4 d), and times to reach the detection limit of direct plating (35 vs. 49 d). Direct comparison of maximum survival (184 d in the previous study) was not possible because of the premature termination of the experiment in the present study due to deterioration of sentinel chamber end membranes.

Several other studies have examined the survival of *Salmonella* serovars other than Newport in dairy wastes. These studies were conducted for shorter periods of time in general, and typically under controlled

conditions. For example, in a 60-d laboratory study at 20°C, Himathongkham et al. (1999) reported a log reduction time of 12.7 d for *S. Typhimurium* in supernatant from settled dairy manure slurry. Using 35,000-L vessels containing milking parlor wash water ("dirty water"), Hutchison et al. (2005) found *Salmonella* log reduction times to be 9 d in summer and 11.6 d in winter; they also noted *Salmonella* declined more rapidly in the dirty water than in other manure-handling types such as swine slurry and beef and dairy slurries at high and low moisture contents. In another study, Nicholson et al. (2005) inoculated dirty water stored in 20-m³ tanks outdoors with *S. Typhimurium* at 3.2 to 4.5 log₁₀/mL and found *Salmonella* survived for up to 32 d. Differences in physical, chemical, and other conditions (e.g., nutrients and competition with other microorganisms present in the waste) may interact and affect pathogen persistence. Results from the present as well as our previous study (You et al., 2006) indicate the long survivability of MDR *S. Newport*. Because manure is typically stored for up to a few months in the region (Dou et al., 2001), the dissemination of the pathogen beyond the farmstead through land application of manure is highly likely.

In the field soil, survival of MDR *S. Newport* in the present study also exhibited a pattern similar to the earlier study (You et al., 2006): little change in the first few days, followed by a steady log-linear decline, and subsequently a tailing phase at low concentrations (Figure 4). However, the deactivation kinetics differed and the die-off was more rapid in the natural field setting (present study) than under controlled laboratory conditions (previous study): slopes of the regression equations were 0.0512 to 0.0697 in the present study versus 0.0426 in the previous study, log reduction times were 14.3 to 19.5 versus 23.5 d, time to reach the detection limit (direct plating) 43 to 92 versus 107 d, and maximum survival 219 to 276 versus 332 d. Of various factors that might have contributed to the kinetic differences between the 2 studies (such as physical, chemical, and other conditions), perhaps the availability of nutrients played a major role. Presumably, limited nutrient transport occurred between the sentinel contents and its surrounding matrix, thus restricting the pathogens to the small amount of nutrients contained in the 3- to 4-g manure-soil mixture in the sentinels. The previous study, however, had 500 g of material in the incubation container and, therefore, a much greater supply of nutrients.

Several studies of *Salmonella* (mostly of *S. Typhimurium*) survival in soil have reported results generally comparable to the present study, with log reduction times of 2 to 3 wk (Chandler and Craven, 1980) and total survival time of 200 to 300 d (Jones, 1986; Islam

et al., 2004a,b). Field studies also exist that report a more rapid die-off of *Salmonella*. For example, Boes et al. (2005) found *S. Typhimurium* concentrations in soil receiving swine slurry decreased from an initial 4 log₁₀/g to 0 log₁₀/g in 10 d, whereas no *Salmonella* was detected if the soil was plowed and harrowed following manure application. Hutchison et al. (2004), with inoculated manure applied to field plots, reported log reduction times to be 0.9 and 4.0 d for *S. Typhimurium* in soil mixed with dairy manure and dairy slurry, respectively; and a maximum survival of 120 d. Soil moisture and temperature are among the important factors that influence the survival of manure pathogens in soils. Generally speaking, moist soils favor the survival of manure pathogens (Crane and Moore, 1986; Gagliardi and Karns, 2000); warm temperatures along with dry conditions tend to decrease their survival (Entry et al., 2000). Also, soil type, weather conditions, and manure application timing and method may interact and affect pathogen survival and, consequently, their fate in the agroecosystem.

It is worth noting that model-derived time to extinction was 47 d in the dairy lagoon; however, *S. Newport* was still present on d 137. Similarly, in the field soil trials, model-derived times to extinction were 102 and 96 d for trials 2 and 3, whereas the measured maximum survival times were 219 and 276 d (Table 1). The implication is that estimation of complete pathogen elimination based on regression models of relatively short-term experiments can be inaccurate. In addition, the persistence of a small cadre of *S. Newport* through the long tailing phase (Figure 4) raises questions regarding factors that may contribute to their prolonged survivability. Hutchison et al. (2005), in a study of survival of *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter jejuni*, and *Listeria monocytogenes* in swine, dairy and beef slurries, and dirty water stored in tanks outdoors, observed a similar tailing phenomenon in some, but not all, of their bacterial species and livestock waste types. The authors suggested several possible explanations including inhomogeneity of bacteria distribution in the sample medium or bacterial aggregation, and adaptation of a small proportion of the inoculated *Salmonella* to variable environmental conditions. Furthermore, the potential risks these super-surviving organisms may present deserve attention. Although a small fraction of the initial concentration, they still amount to tens of cells per gram of sample, which could be infective if ingested. They may amplify rapidly given nutrients and favorable growth conditions as well.

CONCLUSIONS

Multi-drug resistant *S. Newport* can survive in the dairy farm environment under natural conditions for

extended periods: over 137 d in the effluent lagoon and 276 d in the grassed field soil in the present study. This long-term persistence indicates the difficulty of eradication once a herd is infected, as the possibility of re-infection through contaminated manure can be considerable. The long survivability also suggests the potential risks of this pathogen's environmental spreading and subsequent transmission to humans. Nevertheless, manure composting can be an effective way to deactivate MDR *S. Newport* quickly before applying the manure to agricultural land. From a research perspective, the small super-survival fraction (i.e., those in the tailing phase in Figure 4), in the magnitude of tens of colony-forming units per gram of sample, is of interest. The nature of the prolonged viability of these organisms and the implications for food safety, as well as the health of the ecosystem, deserves further study. The sentinel chamber is a useful device for studying pathogens in natural environmental settings such as in field soils, although different membrane materials need to be tested to guarantee the integrity and durability of the sentinel chamber for its use in potentially corrosive conditions such as the dairy lagoon.

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