Journal of Food Protection, Vol. 69, No. 7, 2006, Pages 1717–1719

Research Note

Quantity and Distribution of *Salmonella* Recovered from Three Swine Lairage Pens

A. M. O'CONNOR,[1]* J. GAILEY,[2]† J. D. McKEAN,[1] AND H. S. HURD[2]‡

[1]*Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50010; and* [2]*National Animal Disease Center, U.S. Department of Agriculture, Agricultural Research Service, Ames, Iowa 50010, USA*

MS 05-592: Received 30 November 2005/Accepted 20 February 2006

ABSTRACT

The quantity of *Salmonella* recoverable from three lairage pens in a swine abattoir was determined. Using dry four-ply cotton gauze pads measuring 10 by 10 cm, 100 fecal slurry samples were collected from each of the three pens. *Salmonella* recovery was expressed as the log CFU per milliliter of sample. Mean values were 2.5 log CFU/ml in pen A, 2.7 log CFU/ml in pen B, and 0.89 log CFU/ml in pen C. Median values were 2.6 log CFU/ml in pen A, 2.0 log CFU/ml in pen B, and below the detectable limit in pen C. In pen C, *Salmonella* was not recoverable from a high number of samples. Pen B results suggested spatial dependency, i.e., samples close together were more similar than samples farther apart. These results indicate that *Salmonella* concentrations vary within and between lairage pens. Because of the limited number of pens assessed, it was not possible to identify factors that were associated with the observed variation in *Salmonella* concentrations within and between pens. However, this variation suggests that numerous samples are required to adequately describe the concentration of *Salmonella* in a lairage pen.

Several studies have documented that recovery of *Salmonella* from swine gut contents and gut-associated lymph nodes is higher in penmates slaughtered at abattoirs than in penmates slaughtered on the farm *(5, 6).* Therefore, it was hypothesized that lairage pens expose swine to new nonfarm *Salmonella* serotypes. The consequence of this exposure is thought to be rapid infection of swine with these nonfarm serotypes of *Salmonella* and carriage of these serotypes into the abattoir, accounting for the postharvest pathogen recovery *(7).* Other studies have been focused on the prevalence of *Salmonella*-positive samples obtained from barn floors, but rarely has the concentration of *Salmonella* been reported *(1–3).*

In this study, the concentration of *Salmonella* recovered from the floor in three lairage pens from swine abattoirs was determined. The rationale for quantifying *Salmonella* on the abattoir lairage floor was to determine variability within and between pens. Our motivation for determining whether *Salmonella* populations were variable among pens was that if such variability exists then the probability of rapid swine infection may differ across pens. However, if pen levels of *Salmonella* are homogeneous, differences in *Salmonella* infection rates could not be attributable to differences in contamination levels among lairage pens. Although the issue of rapid infection with *Salmonella* provided the motivation for this study, factors associated with variations in *Salmonella* contamination among pens and the consequences of this variation were not addressed.

MATERIALS AND METHODS

Sample collection. Fecal slurry samples were collected from three lairage pens at the same abattoir. At each visit, a lairage pen was free of pigs between 7 a.m. and 10 a.m. Pigs had been in the pen prior to that time (slaughter begins at 5 a.m.), and the pens were hosed clean with water at 5 a.m. At each visit, a different pen was evaluated; therefore, the sampled pens differed in size, although all were in continuous use during the day. All the pens were designed to hold market groups of ~180 pigs. At each visit, 100 samples were collected on a sampling grid. For pens A and B, a large-scale grid with points 3.25 m apart was laid over the entire pen. A medium-scale grid with points 1 m apart was also placed in a randomly chosen location within the pens. Two small-scale grids with points 0.5 m apart were placed in areas considered likely to have higher concentrations of *Salmonella,* i.e., watering areas and entrances. For pen C, a simple systematic grid was used to identify the locations for sampling the floor. The rationale behind changing the sampling grid was to examine how an alternative sample grid would describe a pen.

A dry four-ply cotton gauze pad measuring 10 by 10 cm was placed at each intersection of the grid lines. After all 100 pads were laid on the grid, the pads were collected (in the order laid down) with sterilized forceps into Whirl-Pak bags marked to indicate their location in the lairage pen, and the bags were transported within 2 h to the laboratory for processing.

Sample processing. Upon arrival at the laboratory, the weight of liquid absorbed by the pad was determined, and 10 ml

* Author for correspondence. Tel: 515-294-5012; Fax: 515-294-1072; E-mail: oconnor@iastate.edu.

† Present address: Utah Army National Guard, 348 East Main Street, Lehi, UT 84043, USA.

‡ Present address: Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50010, USA.

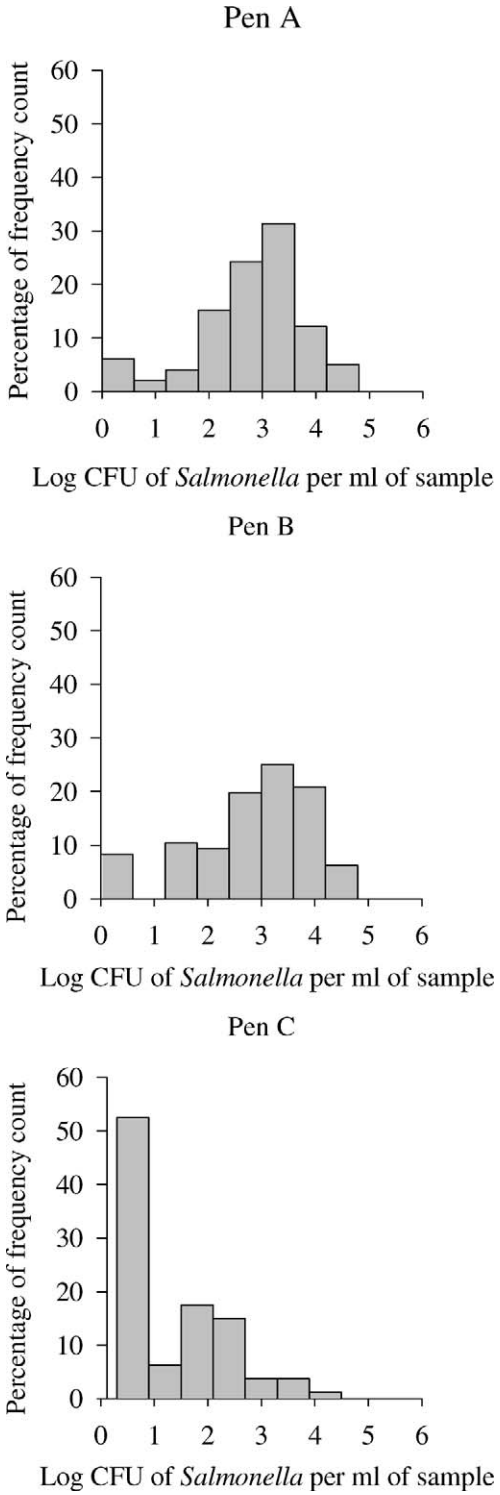

FIGURE 1. *Frequency distribution histograms of the log CFU of* Salmonella *per milliliter of fluid collected from the three study pens.*

of phosphate-buffered saline (PBS) was added to each bag. Samples not processed were immediately stored at 4°C until processing. All the samples were processed on the day of collection. After addition of PBS, the pads were homogenized with a stomacher (Seward Ltd., London, UK) at 260 rpm for 1 min. For enumeration of *Salmonella,* the most-probable-number (MPN) technique was used with five 1:10 serial dilutions of the each sample. Borosilicate test tubes (15 by 150 mm) containing 4.5 ml of buffered peptone water (Becton Dickinson, Sparks, Md.) were inoculated with 0.5 ml of sample. The tubes were covered and incubated at 37°C for 24 h, and then 0.1 ml of the culture was transferred to 10 ml of Rappaport-Vassiliadis medium (Becton Dickinson) and incubated at 42°C for 24 h. A 3-mm loopful of this culture was then streaked onto XLT agar (Becton Dickinson) and incubated at 37°C for 24 h. Colonies on each plate were then enumerated using the MPN technique *(8),* and *Salmonella* results are reported as the MPN of log CFU per milliliter of pen liquid collected.

TABLE 1. *Descriptive statistics of the most probable number of* Salmonella *collected from three lairage pens at a commercial swine abattoir*

Statistic	*Salmonella* concn (log MPN/ml)[a]		
	Pen A	Pen B	Pen C
Mean	2.53	2.78	0.89
Median	2.66	3.03	BDL
Standard deviation	0.98	1.16	1.05
Variance	0.96	1.34	1.10
Maximum	4.32	4.58	3.92
Minimum	BDL	BDL	BDL

[a] BDL, below detectable limit.

Statistical analysis. For each pen, mean, median, standard deviation, and range of the MPN of log CFU per milliliter of liquid was calculated. Because the formulae for the mean, median, and standard deviation assume sample independence, we tested this assumption by looking for spatial dependency within the pens.

PROC VARIOGRAM (version 8.2, SAS Institute, Cary, N.C.) and the spatial module in SPLUS 6 were used to compute a directional and nondirectional empirical and theoretical variogram for each pen. When there was no evidence of anisotrophy (directionality), then the variogram was calculated using the entire data set assuming isotrophy, i.e., no directionality in the data. Theoretical variograms were calculated using either the exponential or the linear function of the shape of the empirical variogram. The formulae and forms for these theoretical models were described by Cressie *(4).* Evidence of strata in pens A and B were examined by using median Polish kriging *(4).*

RESULTS

Figure 1 shows the frequency distribution histograms of the log CFU of *Salmonella* per milliliter of fluid collected for each of the three pens. Descriptive statistics for the three pens, assuming independence, are listed in Table 1.

There was no evidence of directionality in the spatial dependency for any of the pens. The predicted variograms for pens A and C suggest little or no correlation between *Salmonella* concentrations at various locations in the pens, i.e., within a pen, the *Salmonella* concentration at one location did not allow prediction of the concentration at another location. In pen B, gamma increased distinctly until becoming constant at 6 to 8 m, meaning that *Salmonella* concentrations were correlated with locations up to 6 to 8 m away, and the relationship was stronger for closer locations. The parameter estimates for the pen A theoretical variogram using a linear model were slope = 0.006 and nugget = 0.85. For pen B, the most appropriate model was an exponential model with range = 3.77, sill = 1.18, and

nugget = 0.69. For pen C, a linear model was most appropriate, with slope = 0.02 and nugget = 1.04. There was no evidence of strata in pens A and B as determined with median Polish kriging (results not shown).

DISCUSSION

We had anticipated that the movement of hundreds of pigs through barns over many days would create a high but steady concentration of *Salmonella* in pens. However, in the three lairage pens we examined the *Salmonella* concentrations were variable. This study was not designed to examine the numerous possible explanations for this variation, which include but are not limited to differences in the *Salmonella* status of the pigs previously in the pen, the humidity of the pens, use patterns, and differences in the clearing capability, which could be related to the slopes and drainage of the pens. Whatever the source of variation, the consequence of this variation is that in research studies examining factors associated with *Salmonella* in swine, a single or small number of pen samples is not likely a good descriptor of the pen.

Only *Salmonella* recoverable from the floor by our collection method, i.e., dry pads, were cultured. It is not clear whether this technique accurately reflects the bacterial load picked up by swine in lairage pens. Therefore, the concentrations of *Salmonella* we observed may be confounded by the amount of water present on the lairage pen floor. Differences in dampness were noted among pens. In particular, pen C was drier than the other pens, which may have made it more difficult to recover *Salmonella* from that pen. However, such limited sample size makes it inappropriate to attribute the variation in *Salmonella* to pen dampness.

Another potential complication in our study may be measurement error. Serial dilutions were used to quantify the recovered *Salmonella* cells. As with any enumeration method, measurement error is possible. However, in the absence of a ''gold standard'' for quantifying *Salmonella* in environmental samples, it is not possible to make an accurate determination of measurement error. Except for the exact measurement of the number of CFU, the quantification of *Salmonella* in pens should be compared within a laboratory using the same methodology. Comparisons of quantities across laboratories probably are not valid.

ACKNOWLEDGMENTS

This project was supported by funds from the U.S. Department of Agriculture Cooperative State Research Education and Extension Service, National Research Initiative grant no. 2001-35212-10864. The authors also acknowledge assistance from Dr. M. Kaiser (Department of Statistics, College of Agriculture, Iowa State University) for his assistance with statistical aspects of this study.

REFERENCES

1. Barber, D. A., R. M. Weigel, R. E. Issaacson, P. B. Bahnson, and C. J. Jones. 1999. Spatial and temporal patterns of the distribution of *Salmonella* on swine farms in Illinois, p. 97–100. *In* Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, Washington, D.C.
2. Beloeil, P. A., E. Eveno, P. Gerault, P. Fravalo, V. Rose, N. Rose, and F. Madec. 1999. An exploratory study about contamination of pens of finishing pigs by ubiquitous *Salmonella,* p. 101–105. *In* Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, Washington, D.C.
3. Carlson, A. R., and T. H. Blaha. 1999. Investigations into the infection-contamination-infection cycle of zoonotic *Salmonella* on swine farms—investigations into the occurrence of *Salmonella* in the environment of four selected Minnesota swine farms, p. 109–112. *In* Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, Washington, D.C.
4. Cressie, N. A. C. 1993. Statistics for spatial data. John Wiley & Sons, New York.
5. Hurd, H. S., J. K. Gailey, J. D. McKean, and M. H. Rostagno. 2001. Rapid infection in market-weight swine following exposure to a *Salmonella typhimurium*–contaminated environment. *Am. J. Vet. Res.* 62: 1194–1197.
6. Hurd, H. S., J. D. McKean, R. W. Griffith, I. V. Wesley, and M. H. Rostagno. 2002. *Salmonella enterica* infections in market swine with and without transport and holding. *Appl. Environ. Microbiol.* 68: 2376–2381.
7. Rostagno, M., S. Hurd, J. McKean, C. Ziemer, J. Gailey, and R. Leite. 2001. Abattoir holding pens as a source of *Salmonella* for swine, p. 298–300. *In* Proceedings of the 4th International Symposium on the Epidemiology and Control of *Salmonella* and Other Food-Borne Pathogens in Pork, Leipzig, Germany.
8. Wood, R. L., and R. Rose. 1992. Populations of *Salmonella typhimurium* in internal organs of experimentally infected carrier swine. *Am. J. Vet. Res.* 53:653–658.