

Dose Determination for Acute *Salmonella* Infection in Pigs

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Pigs were exposed to various levels of *Salmonella enterica* subsp. *enterica* serovar Typhimurium by either intranasal inoculation or by subjecting them to a contaminated environment. More than 10³ salmonellae were required to induce acute *Salmonella* infection. These results indicate that intervention against acute *Salmonella* infection in lairage may be more readily achieved than previously thought.

Pigs frequently harbor *Salmonella* spp. subclinically, allowing the organism to be transmitted among pigs prior to slaughter (4, 6, 7, 10). Transmission of *Salmonella* from pigs with subclinical infections to naïve pigs during transportation and lairage has been proposed to be a major source of *Salmonella* introduction into the food chain (5, 10). Events immediately prior to slaughter have been shown to correlate with an increased rate of *Salmonella* isolation from pig carcasses (5, 6) and from pork products (2, 8, 12).

Previous work in our laboratory has shown that numerous *Salmonella* serovars are capable of acutely infecting both alimentary and nonalimentary tract tissues within 3 h after intranasal inoculation (9). Acute *Salmonella* infection has been shown to occur in market-weight pigs after they have been rooting in an environment contaminated at doses comparable to those reported in lairage (<10⁶ salmonellae) (4).

The objectives of this study were to determine the minimum dose required to induce acute *Salmonella* infection in pigs by intranasal inoculation with *Salmonella enterica* subsp. *enterica* serovar Typhimurium (trials 1 and 2) and to evaluate acute *Salmonella* infection in pigs exposed to a contaminated environment containing various levels of *Salmonella* (trials 3 and 4).

Isolation rooms. Prior to pig arrival, drag swab samples of the rooms were preenriched in buffered peptone water (Becton Dickinson, Difco), selectively enriched in Rappaport Vassiliadis broth (Becton Dickinson, Difco), and selectively plated on xylose lysine deoxycholate (XLD) agar (Becton Dickinson, Difco). *Salmonella* suspect colonies were then transferred to differential biochemical media as previously described (9).

Animals. Crossbred pigs, 10 to 14 days old, were randomly assigned to one of three principal groups (five animals per group) or to a negative control group. The pigs were acclimatized for 7 to 14 days in isolation rooms and given water and irradiated feed (Harlan Teklad, WI) ad lib. During acclimatization,

rectal swabs and pooled pen fecal samples (3) were obtained to verify the pigs to be free of detectable *Salmonella*.

Salmonella. The challenge strain, *S. enterica* subsp. *enterica* serovar Typhimurium strain HL 10969, was derived from nalidixic acid-resistant strain χ 4232, which was genetically modified to produce green fluorescent protein as previously described (1). The isolate was selected for increased acute infection virulence determinants by inoculating the organism into pigs and reisolating it from the ileocecal lymph node 3 h later.

Strain HL 10969 was grown to late log phase in Luria-Bertani Miller broth (Becton Dickinson, Difco) and centrifuged at 5,000 rpm for 15 min at 5°C. The cell pellet was washed in phosphate-buffered saline and centrifuged two additional times. Following the third centrifugation step, the cell pellet was resuspended in phosphate-buffered saline containing 20% glycerol and frozen at –80°C.

Challenge/necropsy. (i) Intranasal challenge (trials 1 and 2). Strain HL 10969 was removed from the freezer and serially diluted to 4.5 × 10⁵, 4.2 × 10³, and 4.8 × 10¹ (trial 1) or 2.8 × 10⁷, 2.8 × 10⁵, and 2.8 × 10³ (trial 2) organisms per ml, as determined by viable plate counts. Animals were intranasally inoculated as previously described (9).

(ii) Contaminated-environment challenge (trials 3 and 4). During acclimatization, feces were collected and stored at 4°C until the day of challenge. Five days prior to challenge, the pooled feces were verified to be free of *Salmonella* by preenrichment, selective enrichment, and selective plating techniques. Twelve hours before challenge, approximately 1 liter of physiological saline was added for every 1,500 g of feces. The feces were mixed using an electric mixer (Hamilton Beach/Proctor Silex Inc., NC) on setting 1 for 2 min. The feces were then placed into bowls for each *Salmonella* dilution, and the appropriate numbers of salmonellae were added to obtain 5.2 × 10⁴, 5 × 10², and 2.5 × 10¹ (trial 1) or 4 × 10⁶, 4.1 × 10⁴, and 9.1 × 10² (trial 2) organisms per g of feces as determined by direct plate counts. Twenty-five grams of the spiked feces was applied to each square foot of the challenge environment.

Animals were euthanized 3 h following challenge. Approximately 5 g each of tonsil, mandibular lymph node, thymus, lung, liver, spleen, colon contents, ileocecal lymph node, dia-

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TABLE 1. Incidence of *Salmonella* infection following intranasal challenges of pigs with various levels of *Salmonella* serovar Typhimurium

Sample type	No. of positive tissue samples						Negative controls (n = 6)
	Trial 1 (n = 5)			Trial 2 (n = 5)			
	10 ^{1a}	10 ³	10 ⁵	10 ³	10 ⁵	10 ⁷	
Alimentary							
Tonsil	0	0	3	1	3	5	0
Ileum	0	0	2	0	3	5	0
Cecum contents	0	0	2	0	2	5	0
Colon contents	0	0	0	0	0	5	0
% Positive	0	0	35	5	40	100	0
Nonalimentary							
Mandibular lymph node	0	0	1	0	1	1	0
Thymus	0	0	0	0	0	1	0
Lung	0	0	0	0	0	0	0
Liver	0	0	0	0	0	0	0
Spleen	0	0	0	0	0	0	0
Ileocecal lymph node	0	0	0	0	0	0	0
Muscle	0	0	0	0	0	0	0
Blood	0	0	0	0	0	0	0
% Positive	0	0	3	0	3	5	0
% Positive (all tissues)	0	0	13	2	15	37	0

^a Intranasal challenge dose of salmonellae.

phragmatic muscle, and ileum; 20 g of cecum contents; and 20 ml of blood were aseptically collected for the isolation of *Salmonella*.

Salmonella isolation. Tissue samples were collected and processed as previously described (9), except for the selective plating techniques. For the selective plating process, samples

were plated onto XLD agar containing 50 µg of nalidixic acid per ml of agar. Tissues from the negative control animals were processed identically to those of the principal groups, except they were plated for isolation onto XLD agar without nalidixic acid. XLD plates were placed under UV light and observed for fluorescence typical of the green fluorescent protein-contain-

TABLE 2. Incidence of *Salmonella* infection following contaminated-environment challenges of pigs with various levels of *Salmonella* serovar Typhimurium

Sample type	No. of positive tissue samples						Negative controls (n = 4)
	Trial 3 (n = 5)			Trial 4 (n = 5)			
	10 ^{1a}	10 ³	10 ⁵	10 ³	10 ⁵	10 ⁷	
Alimentary							
Tonsil	1	0	2	0	2	5	0
Ileum	0	0	1	0	2	4	0
Cecum contents	0	0	1	0	1	3	0
Colon contents	0	0	0	0	0	2	0
% Positive	5	0	20	0	25	70	0
Nonalimentary							
Mandibular lymph node	0	0	1	0	1	4	0
Thymus	0	0	0	0	0	1	0
Lung	0	0	0	0	0	3	0
Liver	0	0	0	0	1	2	0
Spleen	0	0	0	0	1	3	0
Ileocecal lymph node	0	0	1	0	1	3	0
Muscle	0	0	0	0	1	2	0
Blood	0	0	0	0	0	2	0
% Positive	0	0	5	0	13	50	0
% Positive (all tissues)	2	0	10	0	17	57	0

^a Contaminated-environment challenge dose of salmonellae.

TABLE 3. Fifty percent infective dose calculations for acute *Salmonella* infections following intranasal challenge

Alimentary sample	Intranasal ID ₅₀ /ml ^a
Tonsil tissue.....	1.78 × 10 ⁵
Ileum tissue.....	1.48 × 10 ⁵
Cecum contents.....	6.76 × 10 ⁷
Colon contents.....	3.16 × 10 ⁷

^a ID₅₀, 50% infective dose.

ing challenge strain. The presence or absence of *Salmonella* in tissues was recorded.

Calculations. Fifty percent infectious dose calculations were done based on the Reed-Muench equation (<http://www.fao.org/DOCREP/005/AC802E/ac802e00.htm>).

Salmonellae were not isolated from the environmental drag swabs. All rectal swabs and pooled pen fecal samples taken during acclimatization were negative for *Salmonella*. All alimentary and nonalimentary tissues from the negative control animals (trials 1, 2, 3, and 4) were culture negative for *Salmonella* at necropsy. The results for intranasal and contaminated-environment challenge are presented in Tables 1 to 3.

These experiments establish a minimum dose of *Salmonella* needed for acute infection of both alimentary and nonalimentary tissues of swine. An intranasal challenge dose of greater than 10³ salmonellae is required to infect both alimentary and nonalimentary tissues. The ingestion of *Salmonella* from contaminated environments containing more than 10³ salmonellae per gram of feces induces acute infection of both the alimentary and nonalimentary tissues.

Regarding the movement of swine from farm to slaughter, a variety of conditions, such as the number of pigs shedding *Salmonella* into the environment, length of time in lairage, animal age, breed, concurrent disease status, and stress encountered during transportation and lairage, may influence the minimum dose of salmonellae required to induce acute infection in pigs. However, these results suggest that reduction of acute *Salmonella* infection may be readily achievable by simple environmental sanitation.

In Denmark, a *Salmonella* control program exists in which farms are categorized into groups of low, medium, or high *Salmonella* prevalence (13). This program mandates that pens are washed between group changes, and farms with low prevalence have transport, lairage, and slaughter separate from those with high and medium prevalence. These prophylactic

techniques have decreased acute infection, as evidenced by decreased *Salmonella* isolation from pig cecal contents and slaughter carcasses (2, 11), possibly by reducing the numbers of salmonellae below the minimal dose needed to cause acute *Salmonella* infection.

Thus, our results are consistent with the experience of the Danish *Salmonella* control program in that reducing the level of environmental *Salmonella* is adequate for minimizing acute infection by the organism.

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