Infectious Dose Determination of Acute *Salmonella* Infection in Swine

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Summary and Implications

Minimal infectious doses have been determined for intranasal and *Salmonella* contaminated environmental models of acute *Salmonella* infection. Trials 1 and 2 were conducted in which *Salmonella enterica* subspecies *enterica* serovar Typhimurium were intranasally inoculated at levels of 1 X 10^1, 1 X 10^3, and 1 X 10^5 (trial 1) or 1 X 10^3, 1 X 10^5 and 1 X 10^7 (trial 2) colony forming units (CFU) per animal (5 animals per principle group). Trials 3 and 4 were conducted in which *S*. Typhimurium were used to contaminate holding pens to levels of 1 X 10^1, 1 X 10^3, and 1 X 10^5 (trial 3) or 1 X 10^3, 1 X 10^5 and 1 X 10^7 (trial 4) CFU per gram of feces (5 animals per principle group). Pigs were necropsied 3 hours following intranasal inoculation or introduction into a *Salmonella* contaminated environment. Blood, tonsil, mandibular lymph node, thymus, lung, liver, spleen, ileocecal lymph node, kidney, muscle, ileum, colon contents, and cecum contents were collected for *Salmonella* isolation and qualitatively recorded for the presence of *Salmonella*. These results indicate minimal levels in which *Salmonella* must be controlled during transportation and lairage in order to reduce acute *Salmonella* infection immediately prior to slaughter.

Introduction

Transportation and lairage of pigs immediately prior to slaughter remains to be a potential source of *Salmonella* introduction into the food chain.(3,4,8) At the abattoir, pigs from farms with moderate to high levels of *Salmonella* are frequently co-mingled with pigs with low to no levels of *Salmonella* in lairage. Though the time frame of holding in lairage commonly ranges from 2 to 8 hours this is sufficient time for *Salmonella* to acutely infect the gastrointestinal tract and non-alimentary tissues of previously *Salmonella*-free animals. Acute *Salmonella* infection during transport and lairage may allow both the introduction of more *Salmonella* and new *Salmonella* serovars to enter the food chain.(1,5,7) *Salmonella enterica* subspecies *enterica* serovar Typhimurium has been shown to acutely infect both alimentary and non-alimentary tissues in esophagostomized pigs within 3 hours of intranasal inoculation.(2) Additionally, it has been experimentally shown that the most prevalent *Salmonella* serovars found in swine and humans are capable of acutely infecting both alimentary and non-alimentary tissues of pigs within 3 hours after intranasal inoculation.(6) The objective of this study was to determine the minimal infective dose of *Salmonella* needed to acutely infect both alimentary and non-alimentary tissues in swine necropsied 3 hours following challenge.

Materials and Methods

In each trial, 10 to 14 day old cross-bred pigs were randomly assigned to 1 of 3 principle groups (5 animals per group) or to a negative control group. The pigs were acclimatized for 7 to 14 days on nursery decks in isolation rooms. During acclimatization, rectal swabs and pooled pen fecal samples were obtained to verify that the pigs to be free of *Salmonella*. Four separate trials were conducted in which pigs were challenged with various levels of *Salmonella* by intranasal (IN) methods (trials 1 and 2) or through a contaminated environment (CE) model (trials 3 and 4). Trials 1 and 2 were conducted in which *Salmonella* Typhimurium were inoculated at levels of approximately 1 X 10^1, 1 X 10^3, and 1 X 10^5 (trial 1), or 1 X 10^3, 1 X 10^5, and 1 X 10^7 (trial 2) organisms per animal . Trials 3 and 4 were conducted in which *Salmonella* Typhimurium were mixed with feces at levels of approximately 1 X 10^1, 1 X 10^3, and 1 X 10^5 (trial 1), or 1 X 10^3, 1 X 10^5, and 1 X 10^7 (trial 2) organisms per gram of feces. Twenty-five grams of spiked feces was then placed per square foot of the challenge pen (total of 500g). Three hours following either intranasal inoculation or introduction into the *Salmonella* contaminated environment animals were humanely euthanized and necropsied. During necropsy tonsil, ileum, colon contents, cecum contents, mandibular lymph nodes, thymus, lung, liver, spleen, ileocecal lymph nodes, muscle, and blood samples were aseptically collected for the isolation of *Salmonella*. Results were recorded as to the presence or absence of *Salmonella*.

Results and Discussion

These results suggest that greater than 1 X 10^3 *Salmonella* or 1 X 10^3 *Salmonella* per gram of feces are needed to acutely infect both alimentary and non-alimentary tissues when inoculated by intranasal or contaminated environment challenge models. Pathogenic processes caused by infectious disease agents require a minimal number of organisms to overwhelm the immune system and cause disease in a host. This work suggests that *Salmonella* may not need to be totally eliminated from transport and lairage, but only held below a minimal infectious level thus keeping *Salmonella* from acutely infecting the alimentary and non-alimentary tissues of previously uninfected animals.

Table 1. Intranasal Challenge Results for Trial 1

n=5	1×10^5	1×10^3	1×10^1
Alimentary			
Tonsil	3	0	0
Ileum	2	0	0
Colon Contents	0	0	0
Cecum Contents	2	0	0
% Alimentary	35	0	0
Non-alimentary			
Mandibular Ln.	1	0	0
Thymus	0	0	0
Lung	0	0	0
Liver	0	0	0
Spleen	0	0	0
Ileocecal Ln.	0	0	0
Muscle	0	0	0
Blood	0	0	0
% Non-alimentary	3	0	0
% Tissue Positive	13	0	0

Table 2. Intranasal Challenge Results for Trial 2

n=5	1×10^7	1×10^5	1×10^3
Alimentary			
Tonsil	5	3	1
Ileum	5	3	0
Colon Contents	5	0	0
Cecum Contents	5	2	0
% Alimentary	100	40	5
Non-alimentary			
Mandibular Ln.	1	1	0
Thymus	1	0	0
Lung	0	0	0
Liver	0	0	0
Spleen	0	0	0
Ileocecal Ln.	0	0	0
Muscle	0	0	0
Blood	0	0	0
% Non-alimentary	3	3	0
% Tissue Positive	37	15	2

Table 3. Floor Challenge Results for Trial 3

n=5	1×10^5	1×10^3	1×10^1
Alimentary			
Tonsil	2	0	1
Ileum	1	0	0
Colon Contents	0	0	0
Cecum Contents	1	0	0
% Alimentary	20	0	5
Non-alimentary			
Mandibular Ln.	1	0	0
Thymus	0	0	0
Lung	0	0	0
Liver	0	0	0
Spleen	0	0	0
Ileocecal Ln.	1	0	0
Muscle	0	0	0
Blood	0	0	0
% Non-alimentary	5	0	0
% Tissue Positive	10	0	2

Table 4. Floor Challenge Results for Trial 4

n=5	1×10^7	1×10^5	1×10^3
Alimentary			
Tonsil	5	2	0
Ileum	4	2	0
Colon Contents	2	0	0
Cecum Contents	3	1	0
% Alimentary	70	25	0
Non-alimentary			
Mandibular Ln.	4	1	0
Thymus	1	0	0
Lung	3	0	0
Liver	2	1	0
Spleen	3	1	0
Ileocecal Ln.	3	1	0
Muscle	2	1	0
Blood	2	0	0
% Non-alimentary	50	13	0
% Tissue Positive	57	17	0

Infectious dose 50 calculations for acute *Salmonella* infection

	Intranasal	Contaminated environment
	ID_{50} / mL	ID_{50} / gram of feces
Alimentary Tissues		
Tonsil	1.78×10^5	6.76×10^7
Ileum	1.48×10^5	5.62×10^7
Cecum Contents	6.76×10^7	1.78×10^7
Colon Contents	3.16×10^7	*
Non-Alimentary Tissues		
MLN	*	3.16×10^7
Lung	*	1.48×10^7
Spleen	*	1.78×10^7
ICLN	*	1.78×10^7

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