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Natural Transmission of *Salmonella choleraesuis* in Swine

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This experiment was designed to study the natural transmission of *Salmonella choleraesuis* in swine. Forty pigs were divided into three groups. Group 1 ($n = 12$) was challenged with 10^8 CFU of *S. choleraesuis* per ml by intranasal inoculation. One day postinoculation (p.i.), group 2 ($n = 24$) was commingled with group 1. Group 3 ($n = 4$) served as uninoculated controls. Serum samples were collected weekly. Blastogenesis assays and necropsies were performed at 1, 2, 4, 6, 9, and 12 weeks p.i., and 16 tissue samples per pig were collected and cultured. Environmental (pooled feces from the pen floor) levels of *S. choleraesuis* were 2.61 $\log_{10}$ CFU/g of feces at 24 h p.i. (immediately prior to commingling). Severe clinical signs were observed in groups 1 and 2. The results indicated that at least 16% of group 2 pigs were shedding *S. choleraesuis* within 24 h of commingling. At 1 week p.i., 32 of 32 group 1 and 39 of 62 group 2 tissue samples were positive for *S. choleraesuis*. Only 3 of 12 group 2 pigs were positive at 6, 9, and 12 weeks (1 pig for each week), indicating that only a small proportion of infected swine become long-term carriers. At 12 weeks p.i., only the colon and colonic lymph node samples of one pig from group 2 were positive. Humoral, mucosal, and cellular immune responses were similar between groups 1 and 2. These data demonstrate that a few pigs shedding low levels of *Salmonella* organisms before slaughter can result in rapid transmission and subsequent shedding by many swine.

Salmonella choleraesuis is a host-adapted, facultative, intracellular pathogen that causes swine paratyphoid (29). It is the most frequent *Salmonella* serotype recovered from swine (10) and was isolated from >95% of swine salmonellosis outbreaks in Iowa in 1989 (21). The National Animal Health Monitoring Survey estimated that swine salmonellosis is responsible for 28 million dollars in annual production losses in Iowa and 100 million dollars in annual losses nationwide (21).

Although *S. choleraesuis* is the serotype most frequently isolated from swine, it is rarely isolated from swine feed or nonporcine *Salmonella* reservoirs. The source of *S. choleraesuis* seems to be limited to carrier pigs and facilities previously contaminated with this serotype (29). The carrier state of *Salmonella typhimurium* (28, 31), as well as that of *S. choleraesuis* (12), in swine after experimental infection has previously been described. Epidemiological studies have indicated that crowding and contact with infected feces increased infection rates (14, 18). However, these studies did not provide data involving the numbers of bacteria infecting or excreted by individual animals. Fedorka-Cray et al. (9) studied the natural transmission of *S. typhimurium* to swine and observed a short-term carrier state after exposure to low levels of *S. typhimurium* in the environment. Similar information involving *S. choleraesuis* infection is not available. While no data describing the carrier state after natural transmission of *S. choleraesuis* in swine are available, Hinton et al. (15) studied naturally occurring *Salmonella* infections in cattle and chickens. They concluded that naturally occurring infection patterns were different from those observed for experimentally infected animals and suggested that natural infection models are important for understanding salmonellosis.

The purpose of this experiment was to study the shedding patterns, tissue colonization, and immune response after natural transmission of *S. choleraesuis* in swine and to determine if a carrier state can develop after contact with experimentally infected pigs.

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MATERIALS AND METHODS

Bacterial strains and challenge cultures. Wild-type *S. choleraesuis* subsp. *kunzendorf* χ3246 (16), kindly provided by the laboratory of Roy Curtiss III, Washington University, St. Louis, Mo., was used to challenge a 12-week-old pig by intranasal inoculation and redesignated 3246pp, as previously described (12). This strain has a naturally acquired resistance to streptomycin. Challenge cultures were prepared by inoculating 10 ml of Luria-Bertani broth with 100 μl of a thawed (frozen at −70°C) stock culture of 3246pp. This culture was grown overnight at 37°C on an orbital shaker at 150 rpm. A 1% inoculum was transferred into fresh Luria-Bertani broth and grown for 3.5 h at 37°C with shaking at 220 rpm. This culture was centrifuged, and the pellet was resuspended in 0.5 volume of phosphate-buffered saline (PBS) and adjusted to a final concentration of 10^8 CFU/ml (optical density [OD] at 600 nm = 0.132) in PBS.

Experimental design. After farrowing, source sows were tested and confirmed to be negative for *Salmonella* spp. by bacteriologic culture, as described below. At 2 weeks of age, 40 pigs were weaned and transported to isolation facilities, as previously described (7). Pigs were housed in a climate-controlled, fully enclosed building on concrete floors. Each group was housed in separate isolation facilities and allowed to acclimate for 1 week. Feces were physically removed, and each pen was washed with water once daily throughout the experiment. Tonsil, nasal, and rectal swabs were taken from pigs at 2, 3, 4, and 6 weeks of age and cultured for *Salmonella* spp. At 6 weeks of age, pigs were randomly divided into three groups. At 7 weeks of age (day 0), group 1 pigs ($n = 12$) were inoculated intranasally with 1 ml (0.5 ml in each nostril dropwise on inspiration, alternating nostrils) of *S. choleraesuis* 3246pp at 10^8 CFU/ml. One day postinoculation (p.i.), group 2 naive pigs ($n = 24$) were commingled with group 1 pigs. Group 3 ($n = 4$) served as uninoculated controls.

Swabs (tonsil, nasal, and rectal) were obtained from all pigs, and fecal samples (10 to 20 g) were collected from individual pigs (≥60% of the pigs in each group) in groups 1 and 2 on days 1 through 6, 8, 9, 11, 13, 16, 19, 23, 26, and 30 and

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weekly thereafter until week 12. Each swab and fecal sample was qualitatively cultured. Fecal pools for each group were obtained by combining approximately 2 g of feces from each pig per group. The *S. choleraesuis* environmental load was measured by combining all available feces, mixing well, and taking a sample (20 to 30 g). Fecal pools and environmental samples were quantitatively cultured.

Two pigs from group 1 and four pigs from group 2 were euthanized and necropsied at 1, 2, 4, 6, 9, and 12 weeks p.i. One pig from group 3 was necropsied at 1, 4, 9, and 12 weeks p.i. Tissue samples were collected aseptically for bacteriologic examination (sterile gloves and instruments were used for each tissue sample). Tissue samples collected included the turbinate (1 g [approximate weight]), tonsils (4 g), thymus (3 g), mandibular lymph nodes (3 g), trachea (1 g), esophagus (1 g), lungs (8 g), bronchial lymph nodes (2 g), stomach wall (2 g), spleen (5 g), liver (5 g), middle ileum (4 g), ileocolic junction (ICJ; 6 g), ileocolic lymph node (ICLN; 3 g), cecum (4 g), and colon (4 g). Stomach contents (20 g) and cecal contents (25 g) were also collected. In addition, the tonsils, lungs, ICLN, ICJ, and cecal contents from each pig were quantitatively cultured.

Clinical signs. Rectal temperatures and clinical signs were monitored twice daily until day 3 p.i. for pigs of all groups. Between days 4 and 14 p.i., pigs were monitored once daily; they were monitored once weekly after day 14 p.i.

Bacteriologic examinations. Tissue samples and fecal pools were processed as previously described (12). All tonsil, nasal, and rectal swabs; fecal pools; and tissue samples (collected at necropsy) were incubated at 37°C in GN-Hajna (Difco, Detroit, Mich.) broth for 18 to 24 h and then streaked on brilliant green agar with sulfadiazine (BGS; Microdiagnostics, Lombard, Ill.). Additionally, at 18 to 24 h 100 μl of GN-Hajna broth was transferred to Rappaport-Vassiliadis medium (25), incubated at 37°C for 18 h, and then streaked on BGS. All BGS plates were incubated for 24 h at 37°C. In order to facilitate recovery and identification of the challenge strain, streptomycin sulfate (200 μg/ml; Sigma, St. Louis, Mo.) was added to the media used for group 1 and 2 pigs.

Colonies with the typical appearance of *Salmonella* colonies were picked and inoculated into triple sugar iron and lysine iron agar slants. Positive isolates were confirmed as being serogroup C_1 by agglutination with *Salmonella* antiserum group C_1O (Difco). Representative isolates were serotyped at the National Veterinary Services Laboratories (Ames, Iowa). Quantitative bacteriology was conducted by using the five-tube most probable number method (32) with GN-Hajna, BGS, and Rappaport-Vassiliadis media, as described above, and reported data are the means for the respective groups on each necropsy day. Differences between groups were evaluated by the chi-square test (17).

Antigen preparation. *S. choleraesuis* 3246 lipopolysaccharide (LPS) antigen was prepared by the method of Westphal and Jann (26) and lyophilized for use in enzyme-linked immunosorbent assays (ELISA).

Endotoxin from *S. choleraesuis* 3246pp was prepared by the method of Morrison and Leive (19) and lyophilized for use in lymphocyte blastogenesis assays. Heat extract (HE) antigen from *S. choleraesuis* 3246pp was prepared as previously described (12).

Protein determination. Total protein was determined by the method of Bradford (2), with bovine serum albumin as the standard.

Intestinal antibody. Local intestinal antibody secretions were measured in intestinal washes collected as previously described (6).

Antibody responses. Antibody responses in serum samples and intestinal washes were determined by ELISA, as previously described (12), except that serum samples were diluted 1/10 in diluent buffer. Data are ODs ± the standard errors of the means for the pigs in each group.

Lymphocyte blastogenesis assays. Blastogenesis assays were performed at 1, 2, 4, 6, 9, and 12 weeks p.i. on four pigs from group 1 (two pigs selected for necropsy plus two others, except at week 12 when only two pigs were available), four pigs from group 2 (pigs selected for necropsy), and all available group 3 pigs. Assays were performed as described previously (12), except that lymphocytes were incubated with the respective antigen for 48 h and 5-day incubations were not performed. Lymphocytes were incubated with either *S. choleraesuis* endotoxin (19) at 50.0 μg/ml or HE antigen (12) at 10.0 μg/ml. Positive control wells contained concanavalin A (Sigma) at 5.0 μg/ml. Negative control wells contained cells and media only. Data are the mean stimulation index (defined as experimental values divided by negative control values) ± the standard error of the mean for the pigs in each group.

RESULTS

Clinical signs. No clinical signs were observed on day 1 p.i. (day of commingling). Pigs in group 1 first elicited a febrile response on day 2 which peaked at 41.2°C 4 days p.i. and persisted until day 11 p.i. Group 2 pigs also developed a febrile response on day 3 p.i. (day 2 postcommingling) which peaked at 41.4°C on day 5 p.i. and persisted until day 8 p.i. All of the pigs in groups 1 and 2 elicited a febrile response on days 4 and 5, respectively. Severe clinical signs, including diarrhea, shivering, respiratory distress, and severe depression, were observed in both groups between days 2 and 10 p.i. Clinical signs in both groups were resolved by day 14 p.i.

TABLE 1. Magnitude of fecal shedding for group 1 pigs (intranasally challenged) and group 2 pigs (naturally exposed) and environmental load of *S. choleraesuis*

Time of sampling	$\log_{10}$ CFU/g		
	Group 1	Group 2	Environment
Day 1	2.61	NA[a]	2.61
Day 2	1.55	+[b]	2.55
Day 3	2.55	+	1.86
Day 4	ND[c]	0.64	2.29
Day 5	3.26	+	2.98
Day 6	3.35	0.46	1.60
Day 8	3.65	0.83	2.03
Day 9	2.97	1.51	2.25
Day 11	2.70	0.31	1.62
Day 13	1.73	0.43	1.00
Day 16	2.08	1.11	1.81
Day 19	1.72	0.49	1.35
Day 23	2.45	−[d]	1.08
Day 26	1.11	0.59	1.11
Day 30	2.25	−	2.10
Day 34	1.77	−	0.55
Week 6	−	−	−
Week 7	0.75	−	1.11
Week 8	−	0.63	−
Week 9	−	−	−

[a] NA, not applicable.
[b] +, positive by enrichment only.
[c] ND, not done.
[d] −, negative.

Antemortem bacteriologic results. All source sows were culture negative for *Salmonella* spp. All pigs were also culture negative for *Salmonella* spp. prior to challenge. After challenge, tonsil, nasal, and rectal swabs indicated that all group 1 pigs were shedding *S. choleraesuis* on day 1 p.i. A combination of rectal swabs and fecal cultures indicated that at least 16% (4 of 24) of group 2 pigs were shedding *S. choleraesuis* by day 2 p.i. or 24 h postcommingling. By day 4 p.i., at least 50% (12 of 24) of group 2 pigs were shedding *S. choleraesuis*, and 88% (21 of 24) of group 2 pigs were confirmed to be shedding *S. choleraesuis* (antemortem culture) by day 11 p.i.

The magnitude of fecal shedding for groups 1 and 2 and the environmental load of *S. choleraesuis* are presented in Table 1. At the time of commingling, the environmental load was 2.61 $\log_{10}$ CFU of *S. choleraesuis* per g of feces. Fecal shedding in group 1 peaked on day 8 p.i. (3.65 $\log_{10}$ CFU/g), while group 2 peaked on day 9 p.i. (day 8 postcommingling) (1.51 $\log_{10}$ CFU/g). With the exception of days 6, 8, and 9 p.i., the environmental fecal levels were close to the group 1 fecal levels. The last positive fecal culture was observed at 8 weeks p.i. for group 2.

Postmortem bacteriologic examination. The frequencies of *S. choleraesuis* recovery from tissue samples are presented in Tables 2 and 3 for groups 1 and 2, respectively. *S. choleraesuis* was recovered from nearly all of the tissue samples cultured from group 1 pigs at necropsies 1 (100%) and 2 (81%) weeks p.i. At the 4-, 6-, and 9-week necropsies, the numbers of tissue samples found to be infected declined to levels observed previously (31, 47, and 19%, respectively) (12). Similarly, at weeks 1 and 2 (when clinical disease was observed) *S. choleraesuis* could be found at high levels of frequency in many tissue samples for group 2 pigs; four of four pigs were positive for both the 1- and 2-week necropsies. The tissue samples in which *S. choleraesuis* was most frequently recovered from group 2 pigs were the ICJ (50%), ICLN (42%), cecal content (42%),

TABLE 2. Numbers of *S. choleraesuis*-positive tissue samples from 12 group 1 (intranasally challenged) pigs[a]

Type of tissue (n = 2)	No. of positive samples at necropsy of week:						% Positive
	1	2	4	6	9	12	
Turbinate	2	2	0	0	0	0	33
Tonsil	2	0	1	1	1	0	42
Thymus	2	0	0	0	0	0	17
Mandibular lymph node	2	2	1	1	0	0	50
Lung	2	2	0	0	0	0	33
Bronchial lymph node	2	2	0	0	0	0	33
Stomach wall	2	0	0	0	0	0	17
Spleen	2	2	0	0	0	0	33
Liver	2	2	1	1	0	0	50
Middle ileum	2	2	0	1	0	0	42
ICJ	2	2	2	2	1	0	75
ICLN	2	2	2	2	0	0	67
Cecum	2	2	0	1	1	0	50
Cecal content	2	2	2	2	1	0	75
Colon	2	2	1	2	1	0	67
CLN	2	2	0	2	1	0	58
% Positive	100	81	31	47	19	0	

[a] Data are results for two animals. The numbers of pigs positive at weeks 1 through 9 and 12 were two for each week and zero, respectively.

and cecum (33%) samples. During the subclinical carrier state (4 to 12 weeks), the tissue samples most frequently positive for *S. choleraesuis* in group 2 pigs were the ICJ (25%), ICLN (19%), cecal content, colon, and CLN (13%) samples.

Nearly all (10 of 12) group 1 pigs were tissue positive for *S. choleraesuis*; only the 2 pigs necropsied at 12 weeks were negative (Table 2). All group 2 pigs (eight of eight) were tissue positive at the 1- and 2-week necropsies, and three of four were positive at the 4-week necropsy (Table 3). One group 2 pig was tissue positive at each of the 6-, 9-, and 12-week necropsies.

The results of quantitative bacteriology for groups 1 and 2 are presented in Table 4. Relatively high-level populations of *S. choleraesuis* were found in the tonsil, lung, ICLN, ICJ, and cecal content samples at 1 week for both groups 1 and 2. The population in the lung samples of group 2 was the lowest (1.49 $\log_{10}$ CFU/g), and except for that in lung samples, the populations were similar between groups. High-level populations ($\geq$3.96 $\log_{10}$ CFU/g) were observed for group 1 tonsil samples through 9 weeks p.i. (excluding week 2). The ICLN, ICJ, and cecal content samples were also highly populated through 6 weeks p.i. For group 2, populations of $\geq$1.47 $\log_{10}$ CFU/g were observed in lung samples through 2 weeks p.i. Quantifiable levels of *S. choleraesuis* were observed in group 2 cecal content samples through 4 weeks p.i., ICJ samples through 9 weeks p.i. (excluding 6 weeks p.i.), and ICLN samples through 6 weeks p.i.

TABLE 3. Numbers of *S. choleraesuis*-positive tissue samples from 24 group 2 (naturally exposed) pigs[a]

Type of tissue (n = 4)	No. of positive samples at necropsy of week:						% Positive
	1	2	4	6	9	12	
Turbinate	0	0	0	0	0	0	0
Tonsil	3	1	0	0	0	0	17
Thymus	0	0	0	0	0	0	0
Mandibular lymph node	0	0	0	0	0	0	0
Lung	4	2	0	0	0	0	25
Bronchial lymph node	3	3	0	0	0	0	25
Stomach wall	0	1	0	0	0	0	4
Spleen	4	1	0	0	0	0	21
Liver	4	3	0	0	0	0	29
Middle ileum	0	2	0	0	0	0	8
ICJ	4	4	3	0	1	0	50
ICLN	3	4	2	1	0	0	42
Cecum	4	3	1	0	0	0	33
Cecal content	4	4	2	0	0	0	42
Colon	3	0	0	1	0	1	21
CLN	3	1	0	1	0	1	25
% Positive	61	44	13	5	2	3	

[a] Data are results for four animals. The numbers of pigs positive at weeks 1 and 2, 4, and 6 through 12 were four for both weeks, three, and one for each week, respectively.

TABLE 4. Mean *S. choleraesuis* populations recovered from tissue samples of pigs challenged intranasally (group 1) or by natural exposure (group 2)

Group	Week	$\log_{10}$ CFU/g of tissue				
		Tonsils	Lungs	ICLN	ICJ	Cecal contents
1 (n = 2)	1	4.05	4.69	6.73	5.56	4.35
	2	−[a]	1.20	4.70	5.43	3.67
	4	4.47	−	1.32	3.79	2.17
	6	3.96	−	2.00	4.98	1.56
	9	5.35	−	−	0.62	0.06
	12	−	−	−	−	−
2 (n = 4)	1	4.68	1.49	4.33	5.13	2.98
	2	+[b]	1.47	3.99	4.26	1.50
	4	−	−	1.83	1.36	−0.28
	6	−	−	1.08	−	−
	9	−	−	−	0.50	−
	12	−	−	−	−	−

[a] −, negative.
[b] +, positive by qualitative bacteriology only.

Serum and intestinal antibody responses. The serum antibody responses to *S. choleraesuis* LPS for groups 1 and 2 are presented in Fig. 1a and b for immunoglobulin G (IgG) and IgM, respectively. High-level IgG responses to *S. choleraesuis* LPS in serum samples were observed beginning at 2 weeks p.i. for both groups and continued to rise until 5 and 6 weeks p.i. for groups 2 and 1, respectively. The group 2 response, although slightly lower, closely paralleled the group 1 response. The IgM response in group 1 serum samples was observed within 1 week p.i., while the group 2 IgM response was observed within 2 weeks p.i. The IgM response peaked for both groups at 2 weeks p.i. As observed for the IgG response, the group 2 response closely paralleled the group 1 response. IgA responses were not observed for serum samples from either group.

Intestinal IgG, IgM, and IgA responses to 3246pp LPS are presented in Fig. 2a, b, and c, respectively. A low-level mucosal IgG response was observed for group 1 at 1 week p.i. It increased markedly, peaked at 6 weeks p.i., and declined steadily until 12 weeks p.i. The intestinal IgG response for group 2 was not observed until 2 weeks p.i., and then it remained constant from 4 to 12 weeks p.i. An IgM response beginning at 1 week p.i. and continuing throughout the experiment was observed for both groups 1 and 2. The highest IgM response was observed at 6 weeks p.i. for group 1 and at 12 weeks p.i. for group 2.

Figure 2c indicates a low-level intestinal IgA response to *S. choleraesuis* LPS antigen for both groups at 1 week p.i. which

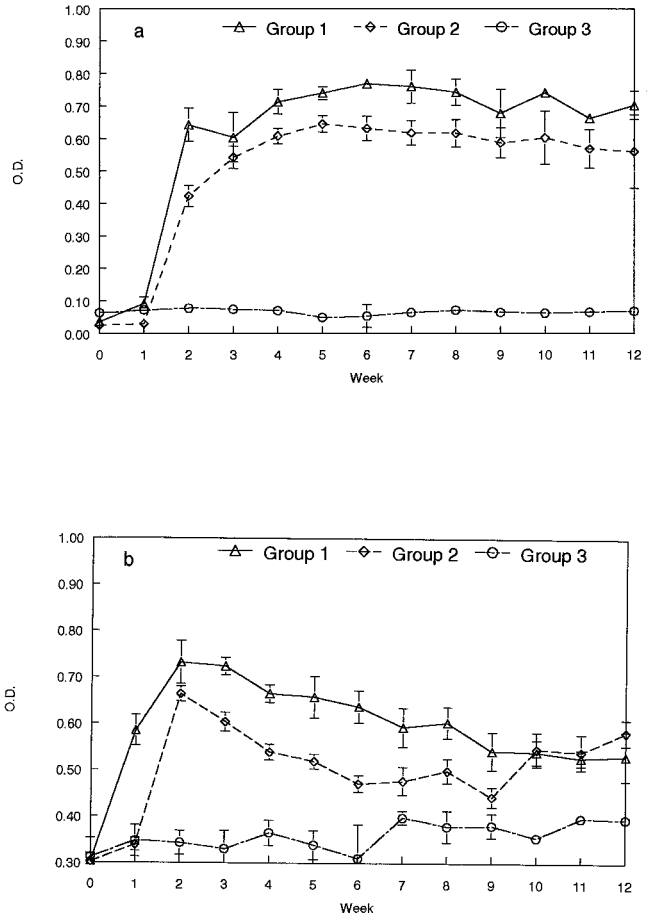


FIG. 1. Antibody response in serum samples of swine to *S. choleraesuis* LPS after challenge with *S. choleraesuis* (group 1) or natural exposure to a challenged group (group 2) or of uninoculated controls (group 3). Data are the mean ODs at 410 nm ± standard errors for either IgG (a) or IgM (b) isotype-specific ELISA.

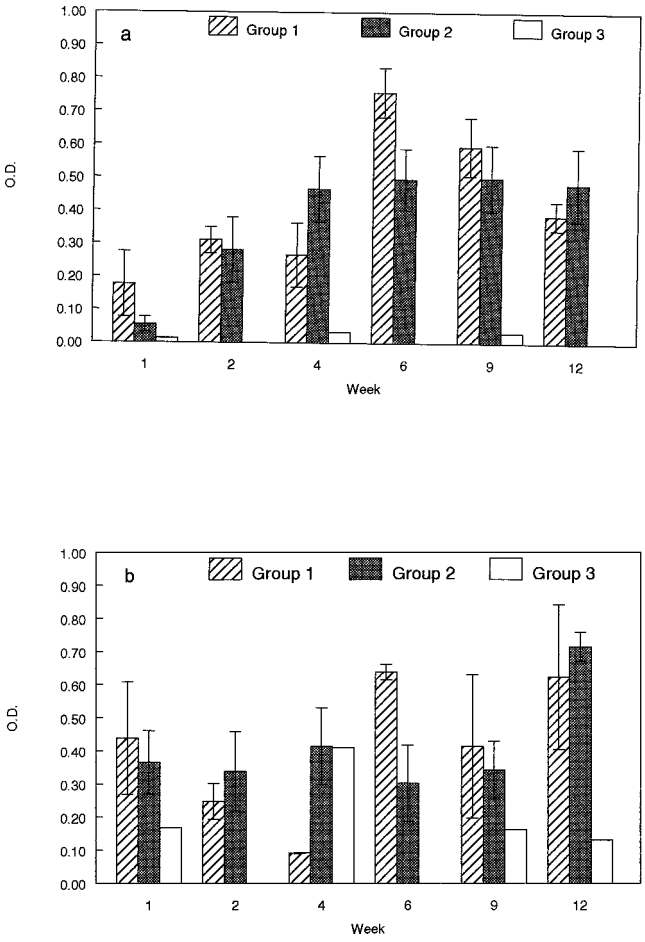
declined but continued until 12 weeks p.i. Although higher levels of response may have occurred at weeks 1 and 12 p.i., the large variations within groups make these results unclear.

Blastogenic responses. Lymphocytes from group 1 and group 2 pigs showed similar increases in proliferation in response to *S. choleraesuis* HE antigen beginning at 4 weeks p.i. compared with that of controls. Increased sensitivity to HE antigen persisted through 9 weeks p.i. before a return to normal levels at 12 weeks p.i. (Fig. 3). Similar trends were observed with *S. choleraesuis* endotoxin.

DISCUSSION

This experiment was conducted to study the natural transmission of *S. choleraesuis* between pigs, determine the effect of natural transmission on the carrier state, and compare the natural disease syndrome with that of experimentally infected pigs.

Pigs infected with *S. choleraesuis* usually exhibit clinical signs between 36 and 48 h after infection (12, 20). We designed this study so that pigs were commingled before clinical disease was evident. This represented a real-world scenario, mimicking field conditions encountered when new stock is purchased or when swine are moved and regrouped into new pens and exposed to a contaminated environment or swine which are not manifesting clinical signs.



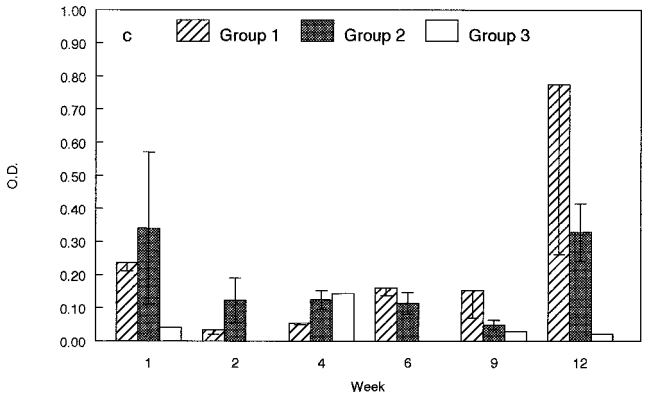


FIG. 2. Intestinal antibody response of swine to *S. choleraesuis* LPS after challenge with *S. choleraesuis* (group 1) or natural exposure to a challenged group (group 2) or of uninoculated controls (group 3). Data are the mean ODs at 410 nm ± standard errors for either IgG (a), IgM (b), or IgA (c) isotype-specific ELISA.

It has been shown that experimentally infected swine shed between 10^3 and 10^6 CFU/g of feces during peak clinical dis-

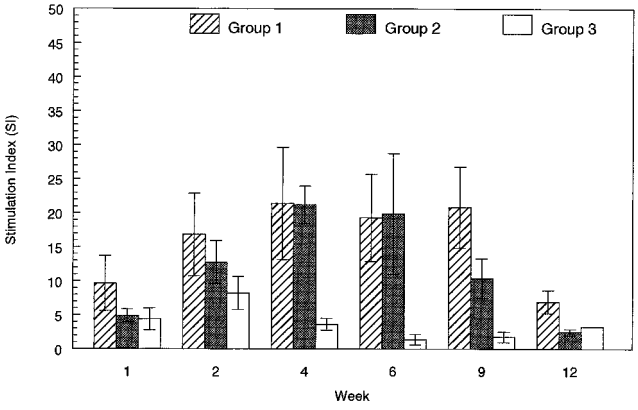


FIG. 3. Two-day in vitro blastogenic response to *S. choleraesuis* HE antigen of peripheral blood lymphocytes from swine after challenge with *S. choleraesuis* (group 1) or natural exposure to a challenged group (group 2) or from uninoculated controls (group 3).

ease (12, 22). It is unclear, however, how this level of shedding relates to that of natural infections and subsequently to the transmission of *S. choleraesuis* to naive swine. We have demonstrated that naive swine exposed to a population shedding as little as 2.61 $\log_{10}$ CFU/g (4.1×10^2 CFU/g) of feces begin to shed *S. choleraesuis* within 24 h of exposure. In addition, severe clinical signs were observed in group 2 pigs within 3 days of exposure to the experimentally infected population. This demonstrates that exposure to relatively low levels of *S. choleraesuis* may result in high morbidity and initiate a severe outbreak of swine paratyphoid within a relatively short period. This not only indicates the rapidity of *S. choleraesuis* transmission on the farm but also may translate to the transmission and spread of *Salmonella* spp. during transport and lairage before slaughter.

Nearly all (88%) of the naive pigs were confirmed by swabs or fecal cultures to be positive for *S. choleraesuis* by 11 days p.i., demonstrating that the majority of swine exposed to a population shedding between 2 and 3 log units of *S. choleraesuis* per g of feces will become short-term *S. choleraesuis* shedders.

Interestingly, although the clinical signs of group 2 were similar to those of group 1 in severity and duration, peak shedding was at least 2 log units lower (1.5 $\log_{10}$ CFU/g of feces for group 2 versus 3.6 $\log_{10}$ CFU/g of feces for group 1). This indicates that the high levels of shedding observed after experimental infection may not be indicative of shedding levels after natural infection.

The tissue distribution of *S. choleraesuis* in swine after experimental infection has previously been described for clinical infection (4, 20, 22, 27) and the carrier state (12). The tissue distribution observed for group 1 pigs was similar to that observed in other studies for both clinical and subclinical infection periods. However, in comparison to experimental infection, some differences were observed for naturally infected (group 2) pigs. High levels of *S. choleraesuis* were observed in the tonsil samples of both groups at 1 week p.i. This suggests that either the tonsils became colonized because of septicemia or they are a primary site of invasion for *S. choleraesuis*. Gray et al. demonstrated that tonsil bacterial populations declined to zero by 2 weeks p.i. and then increased during experiments after experimental *S. choleraesuis* challenge (12). This result was also observed at weeks 2, 4, 6, and 9 p.i. for group 1 pigs in this experiment (Table 4). The tonsils have been described as an important subclinical carrier state tissue for *S. choleraesuis* (12, 13) and *S. typhimurium* (31) after experimental infection. Interestingly, in this study after the 1-week necropsy, tonsil populations were not observed in group 2 pigs. It is unclear whether tonsil populations during subclinical disease (>2 weeks p.i.) are an artifact of high-dose experimental infection or are triggered by an unknown factor not present in the group 2 model. The results presented here suggest that positive tonsil cultures also indicate very recent exposure to *Salmonella* spp.

Pneumonia associated with *S. choleraesuis* infection has been previously described (1), and a recent increase in *S. choleraesuis*-associated pneumonia has been reported (24). It is unclear whether this predilection for lungs is due solely to the pathogen, poor ventilation in large confinement buildings, or some combination of these and other factors. Experimental infection models have not provided good answers because positive lung samples have been regarded as an artifact of intranasal or per os inoculation. The data presented here indicate that lungs are a site of initial *S. choleraesuis* infection in naturally exposed pigs. We recovered *S. choleraesuis* from six of eight lung samples of naturally infected (group 2) pigs during the first 2 weeks of infection.

Swine have a large number of alveolar macrophages in their lungs (30). Fedorka-Cray et al. (8) hypothesized that swine alveolar macrophages have an impaired ability to contain *Salmonella* spp. within the first hours of infection. In addition, alveolar macrophages may act as a vehicle for *Salmonella* dissemination. Our data demonstrate that after natural infection, there is a *Salmonella* population which resides in lungs for at least 2 weeks after exposure, possibly residing in alveolar macrophages and acting as a continual source of infection.

Overall, the tissues in which *S. choleraesuis* can most frequently be found in naturally infected pigs are the ICJ, ICLN, and cecum, as well as cecal contents. During clinical disease (≤2 weeks p.i.), *S. choleraesuis* was isolated from many of the same tissues of group 2 pigs as have previously been isolated during field outbreaks and experimental infections (29). During the subclinical carrier state, the tissues of predilection are the ICJ, ICLN, colon, colonic lymph nodes (CLN), and cecum, as well as cecal contents, in naturally infected (group 2) swine. This is similar to the tissue distribution previously described to occur after experimental infection of swine with either *S. typhimurium* (28, 31) or *S. choleraesuis* (12). The sole exception is that tonsil samples from subclinical group 2 pigs were not positive.

The data presented here indicate that only one of four group 2 pigs was positive at each of the 6-, 9-, and 12-week necropsies, suggesting two things. First, even after a severe clinical outbreak of swine paratyphoid, the *S. choleraesuis* levels in deep tissues and the environment were markedly reduced for group 2 pigs. A significant ($P < 0.01$) number of naive pigs were able to clear *S. choleraesuis* between 9 and 12 weeks p.i., indicating that some pigs will not become long-term carrier animals. Although no management protocols were tested here, these data suggest that good management practices can control and significantly reduce *S. choleraesuis* levels in swine herds.

Secondly, these data suggest that a small fraction of carrier pigs are responsible for the maintenance of this pathogen in the swine population. It has been shown that *S. choleraesuis* is a serotype rarely isolated in feeds or the environment (27), implicating subclinical carrier swine in new outbreaks. However, the factors which induce carrier swine to shed the levels necessary to initiate a new outbreak are unknown.

Experimental infection models have used doses ranging from 10^6 to 10^{11} CFU of *S. choleraesuis* (29). Gray et al. (13) and others (5) have shown that experimental infection with a

dose of 10^6 CFU caused no mortality and few clinical signs of disease. In contrast, a dose of 10^3 CFU of *S. choleraesuis* is cleared with no clinical signs and no apparent shedding (13). In this study, group 2 swine were exposed to a population shedding 3.6 $\log_{10}$ CFU/g (4.0×10^3 CFU/g) of feces, with an environmental load equal to 3.0 $\log_{10}$ CFU/g (10^3 CFU/g) of feces. In comparison to experimental models, the group 2 swine would have needed to ingest between 250 (10^6-CFU dose) and 25,000 (10^8-CFU dose) g of feces each to manifest the severe clinical signs observed in group 2, which is unlikely.

Previous studies have shown that swine have a dose-dependent immune response to *S. choleraesuis* (13). In this study, the humoral and cellular immune responses of group 1 and group 2 pigs were similar, suggesting that they received nearly the same dose or that this organism was able to replicate to nearly the same levels in the host. Taken as a whole, these data indicate that during natural transmission, the infectious dose of *S. choleraesuis* may be much lower than experimental models have previously described. We (8) have previously hypothesized that routes other than the fecal-oral route are important in the pathogenesis of swine salmonellosis.

Another hypothesis is that factors involved in the natural transmission of *S. choleraesuis* are not reproduced in experimental infection. These factors may include increased virulence of *S. choleraesuis* after passage through the host, which may permit lower infectious doses and allow this organism to replicate to high levels in the host. In vitro studies have demonstrated the importance of the expression of induced antigens for intracellular survival (3, 11, 23). Other factors involved in the transmission of *S. choleraesuis* may include the unknown effect of multiple inoculations with smaller doses and inoculation mechanisms not reproduced during experimental infections. Some combination of these and other factors is likely.

As discussed above, the humoral, mucosal, and cellular immune responses of group 2 swine closely paralleled those of group 1. This indicates that in terms of immune parameters, experimental infection may be an appropriate model to study this response. In addition, serum samples collected from experimentally infected swine provide a useful tool for the development of serologically based *Salmonella* detection tests.

In conclusion, this model of *S. choleraesuis* transmission provides for evaluation of the development of this disease in a more natural way than that afforded by experimental inoculation. Additionally, this method of exposure may be applicable to further experiments on the effect of repeated exposure and the evaluation of vaccines.

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