Serologic and bacteriological responses of pigs infected with three serotypes of *Salmonella*

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**Introduction**

In 1993, food-borne infections due to pork contaminated with *Salmonella infantis* prompted the Danish government to institute a *Salmonella* Combat Program (1). Features of this program included the serologic testing of breeding and market swine (1). An indirect enzyme-linked immunosorbent assay (ELISA) was developed using lipopolysaccharide (LPS) from *S. typhimurium* and *S. choleraesuis* (mix-ELISA) (2). This test has been used to test either serum or meat juice (3).

The purposes of this study were to reproduce the work of Nielsen (Nielsen, Baggesen, et al. 1995) and to compare the serologic responses of pigs to *S. typhimurium*, *S. infantis*, and *S. choleraesuis*.

**Materials and Methods**

Forty, 17-day old pigs were obtained from a sow farm that had no history of clinical *Salmonella*. Each pig was individually identified by a numbered ear tag and randomly assigned to one of four treatment groups: controls (not inoculated), *S. typhimurium*, *S. choleraesuis*, or *S. infantis*. Each treatment group was randomly assigned to one of four isolation rooms for housing during the course of the study. For the first four weeks of housing, pigs were raised on elevated, woven wire decking within their respective rooms. Then, the decks were removed and pigs were placed onto solid flooring for the duration of the study. Each week following receipt, blood samples and rectal swabs were collected from all pigs. At the time that these samples were taken, pooled samples of feces were collected from each room’s floor. Five 5-gram (approximately) samples of feces were collected from each pen floor. Sera from the blood samples were tested for antibody to *Salmonella* using the mix-ELISA (2). Rectal swabs and pooled floor feces were cultured for the presence of *Salmonella*. On days -53, -46, -25, and -18, relative to date of challenge, one pig from each treatment group was randomly selected to be euthanized and necropsied. On day -4 relative to challenge, two pigs from each treatment group were randomly selected to be euthanized and necropsied. Pigs received 1.0 ml (10⁴ c.f.u. per ml) intranasal inoculum their respective serotype on day 0. Pigs that were inoculated with *S. infantis* were given a second inoculation of *S. infantis* at day 41. This second inoculation was given to provide a booster of antigen for the purpose of increased antibody in sera that would be used for reference sera. Following euthanasia, each pig was exsanguinated. Blood was collected for possible use as reference sera in the mix-ELISA. Samples from the tonsil, lung, liver, spleen, jejunum, ileum, ileocecal lymph node, cecum, and colon were submitted for culture of *Salmonella*. The pigs that were inoculated with *S. typhimurium* were euthanized at day 24 relative to challenge. This was done in order to have reference sera from a time when elevated OD₅₅₀ was detected. All organ samples, rectal swabs, and fecal samples were cultured for the presence of *Salmonella*. Samples were first incubated in buffered peptone water for pre-enrichment at 37°C for 24 h. After pre-enrichment BPW, tubes were mixed with a vortex mixer and 100 ml was transferred to 9.9 ml of Rapapport-Vassiliadis (RV) broth and incubated at 42°C for 24 h. After incubation in RV broth, RV tubes were mixed with a vortex mixer and an aliquot was transferred via sterile cotton swab to XLD agar plates, streaked for isolation, and incubated at 37°C for 24 h. Colonies suspected to be *Salmonella* sp. were picked and transferred to Kliger’s iron agar, SIM semi-solid agar, urea agar slants, and trypticase soy agar slants and incubated for 37°C for 24 h. Presumptive *Salmonella* colonies were tested for “O” antigens by agglutination with serogroup antisera. *Salmonella* suspensions were submitted to the National Veterinary Services Laboratory (NVSL, USDA, Ames, IA) for confirmation and speciation. Serum was tested for the presence of antibody to *Salmonella* by the mix-ELISA (2).

**Results**

There were no clinical signs observed in any of the groups of pigs except for loose stools on the floor of the pen that contained the *S. typhimurium*-challenged pigs.

All of the rectal swabs, pooled fecal samples, and post mortem organ culture samples that were collected from all pigs prior to challenge were culture-negative for *Salmonella*. *Salmonella* was isolated from all three of the groups that received *Salmonella* inoculations. All of the rectal swabs collected from the control pigs were culture-negative for *Salmonella*. The rectal swabs collected from all pigs that were inoculated with either *S. typhimurium* or *S. infantis*
were culture-positive for at least four days following inoculation. At least one pig that had been inoculated with *S. typhimurium* had a rectal swab that was positive for *S. typhimurium* for 23 days after challenge. Rectal swabs from two of the four pigs that were inoculated with *S. choleraesuis* were culture-positive for *S. choleraesuis*. The rectal swabs from all four pigs inoculated with *S. infantis* were positive for *S. infantis* for 4 days after inoculation. Then, rectal swabs were intermittently positive for *S. infantis* until days 41 and 42. Then the swabs from all four pigs were culture-positive for *S. infantis*. These two days corresponded to the time immediately following the second inoculation with *S. infantis*.

All organ culture results were negative for the control pigs during this study. All four pigs challenged with either *S. typhimurium* or *S. infantis* had at least one organ that was positive for the respective serotype.

Figure 1 is a summary of the time-course of antibody produced against *Salmonella* as measured by OD% with the mix-ELISA. Some pigs had detectable OD% at day –63 relative to challenge. This average OD% tended to fall during the course of the pre-inoculation period (days –63 to 0). The average OD% was detectable in all three challenge groups of pigs as early as 3 days following inoculation. The pigs inoculated with *S. typhimurium* developed the highest average OD%. The OD% increased in the *S. choleraesuis* and the *S. infantis*-inoculated pigs from day 0 to day 10, decreases until day 38, after which there is an increase in antibody. The OD% remained low in the control pigs (OD% less than 8) until day 45 when there is a slight increase in the average OD%.

![Antibody Production](image)

**Antibody Production**

**DAYS RELATIVE TO CHALLENGE**

- - - CONTROL ——— TYPHIMURIUM ——— CHOLERAESUIS ——— INFANTIS

**Discussion**

This study shows that the mix-ELISA detects pigs that have been infected with three serotypes of *Salmonella* and confirms the original report of the mix-ELISA (2). Pigs inoculated with *S. typhimurium* produced high concentrations of antibody in a very short period of time following inoculation. The mix-ELISA is also capable of detecting pigs that were infected with *S. choleraesuis*.

**References**

