Long Term Survival and Infectivity of *Salmonella* Choleraesuis.

Jeffrey T. Gray and Paula J. Fedorka-Cray

USDA, ARS, Antimicrobial Resistance Research Unit. 950 College Station Road, Athens, GA 30605. Phone 706-546-3677, Fax: 706-546-3674. Jgray@saa.ars.usda.gov.

Abstract: It is believed that *Salmonella* choleraesuis, the host-adapted serotype of swine, does not survive well outside the host. Pigs were infected with *S*. Choleraesuis and feces was collected and pooled on days 2, 4, 7 and 10 post inoculation (PI). Feces was stored in a wet and a dry form and survival was measured over 13 months. *Salmonella* Choleraesuis was recovered from wet feces through 3 months of storage. In a desiccated (dry) form, *S*. Choleraesuis was recovered from at least 13 months. Direct PCR analysis did not detect *S*. Choleraesuis subsequent to culture. We also examined the infectivity of *S*. Choleraesuis resident in dry feces. Six or 13 week old pigs were inoculated with dry feces that had been stored either 2 months or 4 months, respectively. Pigs were inoculated either intranasally or by mixing dry feces with feed. Although clinical signs were mild, *S*. Choleraesuis was widely disseminated among the tissues of all the pigs inoculated. This study demonstrates that *S*. Choleraesuis remains viable and infective in the environment. Contaminated fecal matter can serve as a reservoir for *S*. Choleraesuis.

Keywords: Salmonella choleraesuis, Survival, Infectivity

Introduction: *Salmonella* Choleraesuis is a host adapted, facultative intracellular pathogen that causes swine paratyphoid (Wilcock 1992, Reed 1986). Although *S*. Choleraesuis is the serotype most frequently isolated from swine, it is rarely isolated from swine feed or non-porcine *Salmonella* reservoirs such as the environment. The purpose of this study was to evaluate the ability of *S*. Choleraesuis to survive in the environment and remain infective after being shed from infected swine.

Materials and Methods: Bacterial Strains and Fecal Survival Experiment. A group of six, 8 week old pigs were challenged with wild type *S*. Choleraesuis var. kunzendorf 3246pp (Gray 1995) as previously described (Gray 1996). On days 2, 4, 7 and 10 post inoculation, approximately 9 kg of feces shed over the previous 24 hours from all of the infected swine was collected and mixed thoroughly. One-half
of the feces was stored, each biologically secure, in a wet form, the other half in a dry form.

**Infectivity Experiments.** Feces from the long term survival experiment which had been stored dry for 2 months was either ground into a powder using a sterile mortar and pestle for intranasal inoculation or broken into small pieces for the feed inoculation. At 13 weeks of age (day 0) 8 pigs (two trials of 8 pigs were performed) were divided into two groups of four pigs for either intranasal or feed inoculation. Challenge doses ranged between $2.3 \times 10^4$ CFU and $1 \times 10^6$ CFU. All pigs were euthanized and necropsied on day 7 PI.

**DNA extraction and PCR analysis** *Salmonella typhimurium* chromosomal DNA was prepared by boiling bacteria in distilled water for 5 minutes. PCR primers 5' were used to amplify a 284 bp product of the of the invA gene from *Salmonella*

**PFGE Analysis.** DNA for PFGE analysis was prepared the method described by Thong et al. (Thong 1994). Restriction digestion of the plug imbedded DNA was performed using *XbaI*. PFGE was performed on the original *S. Choleraesuis* inoculum as well as the final *S. Choleraesuis* isolates.

**Results: Fecal Survival:** Qualitative bacteriologic results indicated *S. Choleraesuis* survived in wet swine feces for at least 3 months after being shed from infected animals. Feces shed on day 10 PI retained quantifiable levels $(1.2 \log_{10})$ of *S. Choleraesuis* for 2 months. The survival of *S. Choleraesuis* in feces shed from infected swine and allowed to desiccate was more prolonged when compared to the wet feces. Qualitative bacteriology indicated *S. Choleraesuis* survived for at least 13 months in the dry fecal environment.

**Postmortem bacteriologic examination.** Every animal which was challenged with the *S. Choleraesuis* infected feces has between 2 and 10 tissues positive for *S. Choleraesuis*. Regardless of the route of inoculation or the age of the animal infected, the tissues which were most often positive for *S. Choleraesuis* were the ICLN (15/16), Cec cont (14/16), ICJ (12/16) and II-mid (12/16).

**PCR and PFGE Analysis.** The analysis indicates that upon samples becoming culture negative there was also insufficient DNA available *S. Choleraesuis* DNA available for successful amplification. PFGE analysis of control *S. Choleraesuis* isolates as well as isolates recovered from stored feces produced identical banding patterns confirming the identity of the isolates recovered.

**Discussion:** We can conclude, from this and other studies, that *Salmonella spp.* have the ability to survive for long periods in the environment. The long term survival of *S. Choleraesuis* demonstrated here likely explains some of the new *S. Choleraesuis* outbreaks which have been observed in apparently healthy, uninfected animals. In addition, we have provided direct evidence that *S.*
Choleraesuis remains infective for susceptible animals for several months after desiccation in feces. Therefore, the control of all *Salmonella* spp. in the environment must include removal of all organic matter followed by thorough disinfection.

**References**


