Long Term Survival and Infectivity of *Salmonella* Choleraesuis.

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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 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