Investigation of the Use of Pooled Fecal and Environmental Samples After an Enrichment Step for the Detection of *Salmonella* spp. by Real-Time Polymerase Chain Reaction

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Biosurveillance for *Salmonella* spp. in equine hospitals has become a recognized standard of care. The pooling strategy is able to reduce the overall costs of *Salmonella* spp. testing while maintaining the same detection accuracy as microbiological culture. Authors’ addresses: Department of Medicine and Epidemiology (Pusterla, Mapes, Akana, Wademan, Magdesian) and Department of Pathology, Microbiology and Immunology (Byrne), School of Veterinary Medicine, University of California, Davis, CA 95616; Loomis Basin Equine Medical Center, 3901 Sierra College Blvd., Loomis, CA 95650 (Fielding); Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY 40511 (Slovis, Elam); e-mail: npusterla@ucdavis.edu. *Corresponding and presenting author. © 2013 AAEP.

1. Introduction

Generally, microbiologic culture of feces, tissue, or body fluids is used to detect *Salmonella* spp. infection in horses. Reliability of isolation of the organism by culture is diminished by various factors, including method used to collect the sample, amount of sample submitted, temporal and seasonal variation in shedding of the organism, and method of bacteriologic culture. Clinical laboratories generally require at least 48 hours for presumptive detection of *Salmonella* spp. in feces with enrichment to detect small numbers of *Salmonella* spp. Several studies have evaluated the use of polymerase chain reaction (PCR) for the detection of *Salmonella* spp. in fecal and environmental samples. Although PCR has increased sensitivity and faster turn-around time, the costs associated with this testing platform are generally higher than microbiological culture. The purpose of this study was to evaluate the pooling of feces and environmental samples after a selective enrichment culture step for the detection of *Salmonella* spp. by real-time PCR.
2. Materials and Methods
For the purpose of this study, 677 equine fecal and 686 environmental samples were collected. Each sample was inoculated into selenite or tetrathionate broth and incubated for 18 to 24 hours. After incubation, the enrichment broth samples were subcultured onto xylose-lysine-tergitol-4 or hektoen agar plates. Suspected *Salmonella* spp. colonies were subcultured and further identified with the use of biochemical assays. Concurrently to the microbiological analysis, 1 mL of the enrichment broth was processed for DNA purification. The samples were analyzed individually (1363 samples) and in pools of up to 10 samples (139 pools) with the use of a *Salmonella* spp. real-time PCR assay targeting the *invasion A* gene.

3. Results and Discussion
The pooling strategy was able to detect all fecal and environmental samples dually positive by PCR and culture. Three environmental sample pools tested PCR-positive; each of these pools contained two to five individual culture and PCR-positive samples. Two additional PCR-positive and culture-negative environmental samples tested negative by PCR when pooled together. Eleven fecal samples cultured positive for *Salmonella* spp. All these fecal samples were also PCR-positive at the individual and pooled levels. Eight additional PCR-positive and culture-negative fecal samples gave rise to five positive and three negative pools.

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