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Comparing *Salmonella enterica* Detection in Feces using Aerobic Culture, Targeted Molecular Tests, and Shotgun Metagenomics

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Salmonella enterica is a common enteric pathogen of domestic animals, especially in livestock such as horses and cattle, and has important implications for public health. Diagnostic testing is an important tool in the management of this agent, but there is no ideal or perfect test available. Poor sensitivity (detection of *Salmonella* in truly infected animals) is perhaps the biggest problem with available diagnostic tests. Aerobic culture is the most common traditional test that is used, and enrichment is considered essential to improve sensitivity. While sensitivity of aerobic culture is variable, it is recognized as generally having fairly poor sensitivity. Polymerase chain reaction (PCR) is the other common method used for detection of *S. enterica*, but enrichment is still required when testing feces or other types of samples that contain PCR inhibitors or have quantities of related bacteria in the samples, as is true of feces. Another type of molecular test that is commercially available for targeted detection of *Salmonella* are lateral flow immunoassays (LFIA) that use monoclonal antibodies for specific detection of *Salmonella*-specific surface antigens; these tests are designed for use in food safety applications, but have been explored for use in detection of *S. enterica* in feces.[1,2] Recently, high-throughput genetic sequencing technologies have become highly affordable and widely available such that their use in research and clinical application is becoming increasingly common. Shotgun sequencing of metagenomic samples has the potential to open new avenues of research and provide improved understanding of the ecology of various microbes such as *S. enterica*, especially when our understanding is undoubtedly colored by imperfect diagnostic tools.

The objective of this study was to compare aerobic culture, PCR, LFIA, and shotgun metagenomic sequencing as tools for detection of *Salmonella enterica* in fecal samples obtained from animals that had a high probability of infection and shedding. This study was conducted as part of a trial evaluating the impact of metaphylactic antimicrobial treatment on the occurrence of antimicrobial resistance in fecal flora. Individual fecal samples were collected per rectum from 30 cattle upon arrival at a commercial feedlot, and again 11 days later. Each of the 60 samples was processed for testing in the 4 different tests. For aerobic culture, PCR, and LFIA testing, 4 grams of feces were enriched in tetrathionate broth at 42°C for 24 hrs before further processing. Samples of enrichment broth were plated to XLT4 agar for aerobic culture, and were tested in the LFIA. DNA was extracted from the broth for PCR testing using commercially available regents. For shotgun sequencing, metagenomic (whole microbial community) DNA was extracted from feces, and after library preparation was shotgun sequenced using the Illumina HiSeq 2000 platform. After sequencing, metagenomic reads were trimmed for increased quality and taxonomically classified with the Kraken software and the National Center for Biotechnology Information's (NCBI) RefSeq genome database.

Culture and PCR results showed 100% agreement, detecting *S. enterica* in only 0.05% (3/60) of samples. In contrast, there were positive LFIA tests in 22% (13/60) of samples, but none of these results agreed with culture or PCR results. Further testing of samples with the LFIA assay suggested that cattle eating high concentrate rations had a high prevalence of false-positive tests, which may explain these findings. In contrast with the results of culture and PCR which indicated a low prevalence of *S. enterica* shedding, initial analysis of shotgun metagenomic sequencing identified *S. enterica* in 60% (36/60) of samples. However, through further investigation it was recognized that the RefSeq genome database contains non-specific sequences found in individual isolates of one specific bacterial species that might be also found in isolates of other species. This is particularly true for mobile elements such as plasmids. As such, plasmid sequences are included within the reference genome files in the NCBI database, and as such plasmid sequences are considered to be specific to whichever bacterial species carried the plasmid when it was sequenced and submitted to NCBI. To investigate the impact of incorrectly classifying these plasmid sequences as being associated with a specific bacterial species, all of the plasmid sequences included with RefSeq genomes were identified and annotated to form one unique taxa representing all plasmids. Using this updated database, further analysis of the metagenomic sequencing data found that only 35% (21/60) of samples were classified as *S. enterica*, suggesting increased relative specificity. Not surprisingly, sequencing reads that were classified as belonging to plasmids in this subsequent analysis represented a small minority of all reads classified as bacterial. However, these plasmid reads were originally misclassified as representing sequences of bacteria belonging to 22 different phyla.

This study shows that the traditional techniques of aerobic culture and PCR provide similar results for *Salmonella enterica* identification in cattle feces, but that shotgun metagenomic sequencing provides new opportunities for novel investigations of microbial ecology of this important pathogen. However, classification of bacterial species must be considered cautiously as the results are largely influenced by the reference database employed in the bioinformatic analyses.

References

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