An Australian Government Initiative
CRC AUSTRALIA
Pork CRC
CRC for High Integrity Australian Pork

2A-104: *Evaluation of diagnostic tests to detect Clostridium difficile in piglets*

Prof Thomas Riley, University of Western Australia

Project Participants – Mr Daniel R Knight and Ms Michele M Squire

Aims and Objectives

The project aim was to provide guidance to the pork industry, veterinarians and veterinary diagnostic laboratories about the suitability of currently available commercial assays to detect *C. difficile* in Australian piglets. To achieve this we evaluated the performance of four commercial assays to detect *C. difficile* in 157 specimens of piglet faeces obtained from neonatal piglets (49 scouring) aged <14 days during the period June 2012 to March 2013. Assays included two commercially available PCR methods for the detection of toxin A and B genes; (illumigene® *C. difficile* amplification assay (IG, Meridian Bioscience) and BD GeneOhm™ Cdiff Assay (GO, BD Diagnostics), an enzyme immunoassay for toxins A and B (QC, TechLab C. diff Quik Chek™ (Alere) and direct culture; *C. difficile* ChromID™ agar (CA, BioMérieux). Assays were compared against enrichment culture (EC) as a "gold standard".

Key Findings

- Overall, *C. difficile* was isolated by EC from 39.5% (n=62) of samples. PCR revealed 58.1% (n=36) of isolates were positive for at least one toxin gene (*tcdA*/*tcdB*). Five isolates (8.1%) had the uncommon genotype of tcdA-/tcdB-/CDT+ and the remainder (n=21, 33.9%) were negative for any toxin genes.
- PCR ribotyping of the isolates revealed heterogeneity of strain types, many of which are known to cause disease in humans.
- Compared with EC, the sensitivity, specificity, Positive Predictive Value, and Negative Predictive Value were, as follows: for CA; 100.0, 96.0, 88.9 and 100.0%; QC; 38.9, 92.6, 66.7 and 80.0%; GO; 42.9, 97.9, 88.2 and 82.3% and IG; 25.0, 95.8, 69.2 and 77.1%.
- CA performed the best of all the comparator assays with high sensitivity and specificity in recovery of *C. difficile* from piglet faeces irrespective of strain type.
- The performance of the molecular based assays (QC, GO and IG) in the detection of *C. difficile* in porcine faeces was unacceptably poor. Concordance with EC was low, due to a large number of false negative results, which could be attributable to a number of host and/or microbial factors including strain type, faecal composition and sample deterioration.

Application to Industry

This study highlights the high prevalence and unique strain types of C. difficile present in Australian neonatal piglet populations, and the need for further examination of existing assays and development of new rapid assays for detection of C. difficile in piglets. The results underscore the importance of developing porcine-specific assays with high sensitivities, PPVs and NPVs for the rapid reliable detection of C. difficile and its toxins in porcine faeces. However in the interim C. difficile ChromID™ agar provides all diagnostic laboratories with the ability to detect C. difficile in pigs in 24h.

There is an urgent need for better surveillance at national and local levels of the strain types circulating in Australian pig populations as there will most likely be temporal changes both in the strains of C. difficile found in the piglets and the risk factors contributing to their establishment and spread in piggeries. This data is also necessary for analyzing public health risks, if any.

Given the findings of this study and the increasing body of literature in this field, both global and domestic, every effort should be made by the pork industry to increase awareness among veterinarians, animal health groups and producers, of C. difficile as a pathogen of piglets and the challenges of detection and diagnosis.