SWINE
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ARTICLES

Clostridium difficile associated disease in swine: guideline for a proper diagnosis

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Clostridioides difficile is a gram-positive, anaerobic, spore forming bacterium known to infect and cause clinical disease in swine. Very shortly after birth, the gastrointestinal tract is colonized by mixed bacterial populations present within the environment. The process of intestinal colonization is highly complex and influenced by numerous factors including, but not limited to, environmental bacterial load, sow's microbiota, antibiotic usage, sow diet, genetics and quantity and quality of colostrum (immunomodulation).

C. difficile is ubiquitous in the environment; despite the low survival rate of the vegetative form (strict anaerobe) in the environment and high susceptibility to a wide range of disinfectants, the chain of transmission is supported by the capacity of the bacteria to sporulate. Spores are resistant to oxygen and highly resistant to the most common disinfectants. Studies have shown that a majority of neonatal pigs within the commercial setting are colonized by C. difficile in the first few hours of life with nearly 100% of piglets being colonized within 48 hours of birth (Hopman N.E., et al 2011). The prevalence decreases dramatically and steadily as the pig ages; ranging from 3 to 9% in adult animals.

Clinical signs are typically observed in pigs within the first week of life. Clinical signs include lethargy and diarrhoea which can be followed by death. It is important to note that these clinical signs are non-specific and therefore needed to be interpreted alongside other diagnostic results prior to making a final diagnosis. Case fatality rate varies considerably depending on other risk factors, palliative treatment and concomitant infections.

The pathogenesis of *C. difficile* is directly associated with the cellular effects of the toxins A (TcdA) and B (TcdB) in the large intestine. Cytotoxicity neutralization assay on monolayers of hamster ovary cells is considered the gold standard for toxin detection (Bartlett J. G., 2006); however, this assay is time-consuming, relatively costly and requires access and expertise with cell culture methods. Alternatively, the presence and quantity of TcdA and TcdB can be measured by commercially available enzyme immunoassays (ELISA) that are commonly used in veterinary diagnostic laboratories. Although, majority of commercially available *C. difficile* ELISA assays were primarily developed and extensively evaluated for use in human medicine. To the authors knowledge, these assays have not been extensively evaluated for use in swine. The literature available indicates a much lower sensitivity and specificity when compared to the use in human samples (Keessen E. C. et al., 2011b; Anderson M. A., Songer, J. G 2008). Fecal material and colonic content are the recommended samples.

Samples should be refrigerated prior to testing.

PCR(s) designed to detect the TcdA and TcdB genes are becoming widely available; these assays typically offer a higher sensitivity and specificity when compared to conventional bacterial culture methods. Bacterial isolation is not routinely utilized to diagnose *C. difficile* disease in swine due to the need for special media and anaerobic nature of the bacteria. Bacterial isolation and/or PCR detection (toxin genes) are great tools in the investigation of clinical disease; however, given the endemic nature of this bacteria within young pigs, detection alone does not necessarily imply clinical disease.

Lesions are primarily localized in the large intestine; while edema of the mesocolon is commonly observed in pigs with *C. difficile* disease (Figure 1, A) this lesion is not pathognomonic nor a good predictor of the presence of TcdA and TcdB (Yaeger M. J. et al., 2007). Further confidence that *C. difficile* is associated with clinical disease can be achieved by histologic examination. Multifocal to locally extensive areas of ulceration within the large intestine accompanied by moderate numbers of neutrophils and moderate to large amounts of fibrin and cellular debris (volcano like lesions) (Figure 1, B) are typically observed in affected animals. Histologic lesions can be segmental and therefore examination of multiple sections is highly recommended. Longitudinal sections of the mesocolon containing multiple loops is recommended (Figure 1, A). Samples of approximately 1cm thick should be placed in 10% formalin fixed immediately after collection.

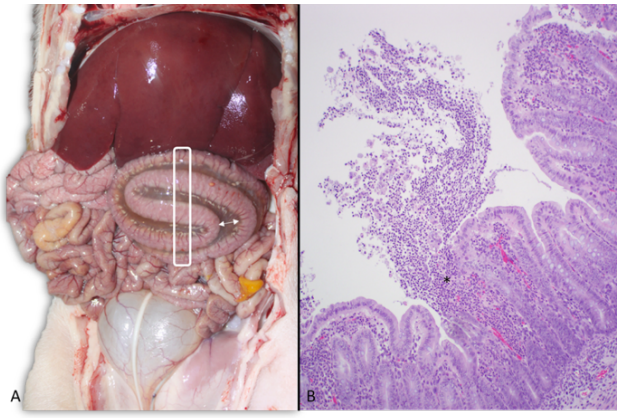

Figure 1. A. Mesocolonic edema observed on a 7-day-old pig diagnosed with *C. difficile* associated disease. Moderate to large amounts of edema (white double head arrows) separating colonic loops. The white rectangle illustrates a longitudinal section of the colon; formalin fixed longitudinal section of colon (1 cm in thickness) is recommended for histopathology examination. B. Histopathologic section of colon: * classic volcano-like lesion characterized by a focal ulceration and replacement by moderate to abundant amounts of cellular and karyorrhectic debris, degenerate neutrophils, and fibrin.

Issues	Solution
Inappropriate animal selection	Neonate pig with acutely watery diarrhea that accurately represents the herd problem
Submission of samples from only one pig	Submission of samples from at least 3 acutely affected pigs
Failure to identify macroscopic lesions	Macroscopic lesion identification training
Failure to collect appropriate tissues	Collection of fresh and fixed samples of mesocolon
Inappropriate preservation of fresh samples	Fecal material/colonic content should be refrigerated prior to testing
Inappropriate preservation of formalin fixed samples	Collection of a longitudinal section of mesocolon (1cm thickness) and immediately placement in 10% formalin solution
Evaluation of histologic lesion from one section of colon	Evaluation of multiple sections of colon as lesions can be segmental
Conclusions based on inadequate amount of evidence	Final diagnosis should be based on a combination of diagnostic results using multiple modalities.
Interpretation of diagnostic results and assign appropriate relevance to the herd	Evaluation diagnostic results in combination with clinical assessment from field veterinarian

Table 1. Common issues associated with diagnosing *C. difficile* associated disease in swine

The definitive diagnoses of *C. difficile* associated disease in pigs should be based on a combination of clinical information and diagnostic tools:

1. Appropriate age group (less than 2 weeks of age)
2. Presence of clinical signs (yellow watery diarrhoea)
3. Identification of macroscopic lesions (mesocolonic edema)
4. Detection of TcdA and TcdB (by PCR or ELISA methods)
5. Presence of histologic lesions (Ulcerative colitis).

For illustration of this process please refer to Figure 2. However, the diagnostic process is in a way a probability exercise in which not all lines of evidence are necessarily required at all times; the desired level of confidence in the final diagnosis will determine the amount of evidence needed in each investigation. Ultimately, the diagnostic process is not designed to achieve certainty, but rather to reduce the level of diagnostic uncertainty enough to allow the veterinarian to make optimal therapeutic and management decisions.

***The further you move on this direction the more confident you can be on your *C. difficile* associated disease diagnosis.**

Figure 2. Schematic of the stepwise process to diagnose *Clostridium difficile* associated disease in swine.

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