EPIDEMIOLOGY OF CLOSTRIDIUM DIFFICILE IN A VEAL FARM: PREVALENCE AND MOLECULAR CHARACTERIZATION

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Introduction: Clostridium difficile is a gram positive, anaerobic spore-forming bacterium associated with gastrointestinal disease in humans and animals. It is the most important cause of antibiotic associated diarrhea in humans, and a considerable increase in the incidence of nosocomial C. difficile infection (CDI) has been reported in North America and Europe. More recently, special attention has been given to increasing community-associated cases of CDI (Mulvey et al 2010).

Although the zoonotic potential of the bacterium has not been demonstrated, the presence of spores in food suggests that food borne transmission may be possible. In addition, undistinguishable C. difficile strains had been recovered from community-associated CDI in human and from the feces of pigs and calves.

Veal calves are considered a population susceptible to diseases since there is a high incidence of failure of passive transfer among these animals. Currently, there are no epidemiological studies investigating the prevalence of C. difficile in veal calves. The objective of this study was to evaluate C. difficile colonization in calves in one veal farm from admission until slaughter in a longitudinal manner and characterize the isolates recovered using PCR ribotyping.

Material and Methods: One veal farm in Southern Ontario, fattening male Holstein-Friesian calves obtained from multiple dairy farms, was enrolled for the study. Calves were 2 to 10 days of age at the time of arrival and were either housed individually in small pens or in groups. Grouped calves were housed in two different rooms with about 50 calves each (rooms 1 and 2). Calves that were in individual pens were kept in two separated rooms of 45 calves each (rooms 3 and 4). Pens were separated by cross bars and calves had direct contact with their neighbors. Calves were managed in an all-in-all-out management system and were fed a complete milk replacer diet prepared on the farm. Treatment with oral oxytetracycline (22.2 mg/kg) was given for 5 consecutive days after arrival in order to control respiratory diseases.

Rectal swabs were collected within 48 h after arrival (174 calves). Sampling was repeated after one week (172 calves), 17 weeks (183 calves) and 21 weeks (156 calves) after arrival. The last sampling was performed 5 days prior to slaughter. Individually housed calves were moved into groups of eight calves prior to the 3rd sampling.

Rectal swabs were inoculated into of C. difficile moxalactam norfloxacin (CDMN) selective-enrichment broth and incubated for 7 days and then onto CDMN agar for 48 hours. Subculture, isolation and identification of C. difficile were based on the characteristic morphology, odor and gram stain of the colonies and the presence of the L-proline aminopeptidase activity. Isolates were stored at -80°C and re-cultured prior to molecular analysis.

DNA from isolates was obtained by the use of a commercial DNA extractor kit. PCR-ribotyping was performed as has been described by Bidet et al. (1999). Ribotype patterns were evaluated visually and compared to an internal library of ribotypes. For bacterial strains recognized as a known international ribotypes listed with the Public Health Leadership Society, Anaerobe Reference Unit, the appropriate numerical designation (e.g., ribotype 078) was used. A multiplex PCR was used for detection of genes encoding toxin A (tcdA) and toxin B (tcdB) as described by Lemee et al (2004). A second PCR was performed for detection of toxin A gene constitutive difference between A-/B+ strains and A+/B+ strains, and thus, identification of toxin A negative strains was performed according to Kato et al (1998). Detection of CDT (cdaA) gene was performed according to Stubbs et al. (2000). An agarose gel was used to separate the PCR products, before the images were obtained using a computer software.

Descriptive statistics included the prevalence of the bacterium at the different sampling times. Using generalized estimating equations to account autocorrelated/ clustered data, multivariable logistic regression models were built to determine the impact of date of sampling, and room on the following outcomes: C. difficile (positive or negative), presence of ribotype E (ribotype E vs other), and presence of ribotype 078 (ribotype 078 vs other). Since individually housed calves were in direct contact with the consecutive calf, calves in the same room were considered clustered in one group. The computer software STATA version 10.0 was used for the statistical analyses.

Results and discussion: Clostridium difficile was isolated from 56 out of 174 (32%) calves at arrival, as well as from 88 out of 172 (51%) during the second sampling. Four out 183 (2%) and 4 out of 156 (2%) calves were positive during the third and fourth samplings, respectively. Overall, C. difficile was isolated from one or more samples from 122 out of 200 calves (61%). There was no difference in the prevalence between group- and individually-housed calves. The high prevalence of calves shedding the bacterium at arrival was not surprising, as high prevalences (15% to 45%) have been reported in calves (Rodriguez-Palacios et al 2006, Piris et al 2008). At the second sampling time, calves were twice as likely to be positive. Several factors may have contributed to this increase, including the use of antimicrobials, which are well known in disrupting the intestinal flora allowing an overgrowth of C. difficile. Contributing factors for the overgrowth of C. difficile might include stress of transportation, a new environment and overpopulation. In addition, dairy bull calves frequently receive poor or no colostrum in the dairy farm of origin leading to a high incidence of failure of passive transfer.

Although not yet fully established, the concern about the transmission of C. difficile from animals to humans via direct transmission and through food continuously grows. The similarity of the strains occurring in humans and food animals and the presence of spores in retail ground meat samples are suggestive that cattle may act as reservoirs of C. difficile for humans. Interestingly, in the present study, the prevalence decreased dramatically to 2% during the third and fourth sampling periods. This could be related with
adaptation of the gastrointestinal microflora and with the development of immunity and may be of importance when evaluating food borne risks, as the prevalence of colonization at the time of slaughter is presumably more relevant than colonization at earlier ages.

Molecular analysis of the recovered isolates revealed that 150 of the 152 isolates found in this study were toxigenic, of which, 148 have been identified in humans. Ribotype 078 was the most commonly isolated strain (103/152), something that is consistent with other studies involving food animals. (Hammitt et al 2008). The data shown in our study are important, since ribotype 078 has been linked with CDI in humans with increasing frequency and has also been associated with community acquired CDI (Mulvey et al 2010). In addition, ribotype 078 was recovered from 73% of the positive retail meat samples (Songer et al 2009), and from 86% of the positive ground beef samples (Weese et al 2009). These results suggest that the role of cattle in the contamination of food and ultimately human contamination deserves special attention. Ribotype E was present in 31 calves (20.4%) and other strains were isolated from 19 samples (12.5%).

There were differences in ribotype distribution between rooms, with calves housed in room 2 (grouped) significantly less likely to shed C. difficile ribotype 078 and more likely to test positive for ribotype E when compared to calves in room one. Overall, calves were almost 18 times more likely to shed ribotype E six days after arrival when compared to the initial samples. The reasons for the difference found in room 2 and the increase in shedding of ribotype E during second sampling remain uncertain, however, selection of resistant strains after the use of oxytetracycline should be considered.

Although calves were originated from multiple locations, these results are based on a longitudinal case study of one veal farm and, consequently, further studies using multiple farms are needed to determine variation in farm level on the prevalence of C. difficile in veal calves.

**Conclusions:** In conclusion, the high prevalence of pathogenic strains of C. difficile in veal calves is an important result that deserves attention since this species may serve as reservoir of the bacterium for humans. However, the low prevalence of the bacteria just before slaughter may reduce public health concerns. The dominance of ribotype 078 among the isolates is valuable epidemiologic information, as the strain has been increasingly found from clinical cases in humans. Based on the pattern of distribution among rooms where calves were housed, the use of group or individual housing was not a significant factor for the intestinal colonization by C. difficile in this facility.

**Key words:** epidemiology, clostridium difficile, veal farm, prevalence, molecular characterization.

**References:**


