Clostridial Colitis in Adult Horses and Foals: A Prospective Study

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Clostridium difficile and enterotoxigenic C. perfringens are significant causes of enterocolitis in horses. Based on detection of specific clostridial toxins in fecal samples, C. difficile was associated with 22% and 17% of cases of colitis in adult horses and foals, respectively. Enterotoxigenic C. perfringens was associated with 19% and 29% of cases in adult horses and foals. Incorporation of such testing can greatly increase the diagnosis rate in cases of enterocolitis. Author’s addresses: Department of Clinical Studies (Weese, Stämpfli) and Department of Pathobiology (Prescott), Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. N1G 2W1. © 2001 AAEP.

1. Introduction

Clostridium difficile is a strictly anaerobic bacterium that is emerging as a serious enteric pathogen of a number of species. This organism is a well-recognized cause of enteric disease in humans, where it is reported to be the primary cause of nosocomial and antibiotic-associated diarrhea. Clostridium difficile causes disease via growth of the organism in the gastrointestinal tract followed by the production of a variety of toxins. Strains of C. difficile may produce up to 5 toxins: however, only toxin A and toxin B are well understood. Clinical diagnosis is based on the detection of these toxins. The role of C. difficile in equine enteric disease was first reported by Jones et al 1987 where C. difficile was associated with diarrhea in 27 out of 43 foals in an outbreak of enterocolitis. Further reports have implicated this organism in cases of sporadic, antibiotic-induced, and nosocomial colitis in adult horses and foals, although the full extent of its role in equine disease is still unclear.

Clostridium perfringens is a relatively ubiquitous bacterium that has been associated with enteric disease in a number of diverse species. Various strains of C. perfringens have been implicated in equine enteric disease, based on a variety of methods. Unfortunately, the high prevalence of C. perfringens in healthy animals, the rapid overgrowth of C. perfringens during any disruption of the intestinal bacterial microflora, and the vast array of potential virulence factors possessed by this organism have made test interpretation difficult. Types of C. perfringens are differentiated based on the production of 4 major toxins; alpha, beta, epsilon, and iota. In addition, isolates may have the gene to produce another toxin designated as Clostridium perfringens enterotoxin (CPE). The production of CPE is most commonly associated with Type A strains, but can occur with other types. In humans, enterotoxigenic C. perfringens has been associated with sporadic diarrhea, food poisoning, and antibiotic-associated diarrhea. The role of C.
perfringens in equine colitis is less clear. Recently, Donaldson and Palmer reported a significant association of CPE with diarrhea in horses based on detection of CPE in feces via an ELISA.\textsuperscript{10} \textit{Clostridium perfringens}–associated enteric disease is difficult to diagnose on the basis of culture alone since the organism can be found in the feces of normal animals.\textsuperscript{17–20} Detection of specific clostridial toxins in fecal samples is the best method to diagnose disease caused by both \textit{C. difficile} and \textit{C. perfringens}.

This study was designed to prospectively evaluate the roles of \textit{C. difficile} and enterotoxigenic \textit{C. perfringens} in enterocolitis in adult horses and foals via selective culture and fecal toxin detection.

2. Materials and Methods

Fecal samples were obtained from diarrheic adult horses and foals and those with normal feces between 31 Mar 1998 and 30 Sept 1999. Anaerobic culture for \textit{C. difficile}\textsuperscript{a} was performed using standard techniques. \textit{Clostridium perfringens} spore quantitation was performed using alcohol spore selection followed by serial dilution, inoculation onto tryptose-sulphite-cycloserine (TSC) agar\textsuperscript{b} and anaerobic incubation. \textit{Clostridium difficile} toxins A and/or B\textsuperscript{c} and \textit{C. perfringens} enterotoxin\textsuperscript{d} were detected using ELISAs that were performed per the manufacturer’s instructions.

\textit{Clostridium difficile} isolates were tested for antimicrobial susceptibility using the Etest method.\textsuperscript{c}

Associations were evaluated using chi-square analysis. Linear regression was used to evaluate the association between \textit{C. perfringens} spore count and the presence of CPE. A \textit{p} value of \textless{} 0.05 was considered significant for all comparisons.

3. Results

\textit{Clostridium difficile} was isolated from the feces of 7 of 55 diarrheic horses (12.7\%) but only 1 out of 255 horses with normal feces (0.4\%, \textit{p} < 0.0001). \textit{Clostridium difficile} toxins were detected in the feces of 12 out of 55 diarrheic horses (21.8\%) but only 1 out of 83 horses with normal feces (1.2\%, \textit{p} < 0.0001). \textit{Clostridium difficile} was isolated from 11 of 31 diarrheic foals (35.5\%) and 0 of 47 foals with normal feces (\textit{p} < 0.001). Similarly, \textit{C. difficile} toxins were present in 5 of 30 diarrheic foals (16.7\%) and 0 of 21 foals with normal feces (\textit{p} < 0.05). Antibiotic administration preceded the onset of clinical signs in 42\% of \textit{C. difficile} toxin positive horses versus 37\% of \textit{C. difficile} toxin negative horses (\textit{p} = 0.78), and 60\% of toxin positive versus 48\% of toxin negative foals (\textit{p} = 0.62). The age range of affected horses ranged from < 24 h to 23 yr of age. Antimicrobial sensitivity testing was performed on 43 equine and environmental \textit{C. difficile} isolates. All \textit{C. difficile} isolates were susceptible to metronidazole and vancomycin while all but 2 were resistant to cefotaxime and bacitracin.

\textit{Clostridium perfringens} enterotoxin was detected in 9 of 47 adult horses with diarrhea (19\%) and 0 of 47 horses with normal feces (\textit{p} < 0.002). Eight of 28 diarrheic foals (28.6\%) had detectable levels of CPE in feces versus 0 of 8 foals with normal feces (\textit{p} = 0.09).

\textit{Clostridium perfringens} was present in 9 out of 9 CPE-positive fecal samples and 6 of 10 randomly selected CPE-negative fecal samples (\textit{p} = 0.033). There was no correlation between spore count and the presence of CPE (adjusted \textit{r} = −0.0164, \textit{p} = 0.41). The positive predictive value (PPV) of isolation of \textit{C. perfringens} with respect to the presence of CPE was only 60\%, while the negative predictive value (NPV) was 100\%. Results were similar in foals where \textit{C. perfringens} was isolated from 7 of 7 CPE positive samples and 4 of 9 CPE-negative samples (\textit{p} < 0.02). There was no correlation between spore count and the presence of CPE (adjusted \textit{r} = −0.021, \textit{p} = 0.42). The PPV was only 64\% while the NPV was 100\%.

Overall, 22\% of diarrheic adult horses and 6 of 33 (18\%) diarrheic foals died or were euthanized. \textit{Clostridium difficile} toxin positive horses were more likely to die than \textit{C. difficile} toxin negative horses (\textit{p} = 0.033). There was not a significantly increased mortality rate for CPE-positive adults, CPE-positive foals, or \textit{C. difficile}–positive foals (\textit{p} > 0.05).

4. Discussion

This study provides additional evidence that \textit{C. difficile} is a significant enteric pathogen in horses. Using fecal toxin detection as the standard for clinical diagnosis, \textit{C. difficile} was implicated in 21.8\% of cases of colitis in adult horses and 16.7\% of cases in foals. Culture-positive, toxin-negative samples were not considered \textit{C. difficile}–associated disease (CDAD) due to the possibility that non-toxin-producing strains of \textit{C. difficile} were isolated. Up to 25\% of strains of \textit{C. difficile} lack the ability to produce toxins, and are therefore believed to be clinically irrelevant.\textsuperscript{21,22} False-negative toxin assay results could be possible as well but were considered less likely. Toxin-positive, culture-negative cases were considered to be CDAD. \textit{Clostridium difficile} can be a difficult organism to isolate and is poorly aerotolerant.\textsuperscript{23} This poor aerotolerance can result in false-negative culture results, particularly when there is a delay from sample collection to processing.

Clinical presentation of CDAD was quite variable ranging from mild diarrhea to peracute, severe enterocolitis. The significantly higher mortality rate in adult horses with CDAD has not been previously reported. While a large percentage of cases with CDAD reported prior antimicrobial treatment, this was not significantly different from non-CDAD cases. In human medicine, CDAD is highly associated with antimicrobial therapy.\textsuperscript{2} This does not preclude a significant role of antibiotics in the induction of CDAD. The frequent association of antimicrobial administration with colitis of other etiologies may make it difficult to prove a statistical association between antimicrobial therapy and CDAD.
Metronidazole resistance was not detected in any isolated of *C. difficile*, however resistance has been reported in equine isolates in California.\(^{24,25}\)

This study confirms the finding by Donaldson et al\(^{10}\) that CPE is a cause of enterocolitis in adult horses. Enterotoxigenic strains of *C. perfringens* may also be an important cause of disease in foals, however the control group was too small to achieve statistical significance. This study also demonstrated that neither standard anaerobic culture nor quantitative culture are adequate for diagnosis of CPE-associated disease, as has been found in humans.\(^{16}\)

Diagnosis of this condition requires detection of fecal toxins, a requirement that hampers our understanding of this disease due to limited availability of such tests. This study only evaluated the role of one toxin, CPE, in equine disease. It is possible that CPE-negative but otherwise toxigenic (i.e., beta-2 toxigenic) strains accounted for a percentage of undiagnosed cases.

*Clostridium difficile* and enterotoxigenic strains of *C. perfringens* appear to be significant causes of diarrheic disease in adult horses and foals. Detection of specific bacterial toxins in fecal samples is required to diagnose clostridial enteritis. Fortunately, rapid and economical tests are becoming more readily available. Further study is required to clarify the roles of *C. difficile* and CPE in equine disease, and to determine the role of other strains of *C. perfringens*. In the meantime, CDAD and enterotoxigenic *C. perfringens*—associated disease should be considered to be differential diagnoses in cases of equine enterocolitis. Submission of fecal samples for detection of *C. difficile* toxins A and B, and *C. perfringens* enterotoxin are now routinely performed at the Ontario Veterinary College. Incorporation of this testing has increased the diagnosis rate for cases of equine enterocolitis from 9% to approximately 45% at the Ontario Veterinary College.

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References and Footnotes