Clostridium Difficile Infection in Horses

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Abstract  
Clostridium difficile (C. difficile) is the most common cause of antibiotic-associated diarrhea and the etiologic agent of pseudomembranous colitis in humans. C. Difficile Infection (CDI) has also been associated with diarrhea and colitis in many animal species. CDI in horses usually cause acute colitis, rapid weight loss with high mortality unless timely and effectively treated. However, little is known about how and why C. difficile induces severe disease in horses compared to other animals. This review will focus on CDI in horses.

Keywords: Clostridium Difficile Infection (CDI); Horse; Epidemiology; Pathogenesis

Introduction

Clostridium difficile is a gram-positive, toxin-producing, spore-forming, anaerobic rod bacterium commonly associated with colitis and diarrhoea in humans and other mammals [1,2]. C. difficile was first isolated from healthy newborns in 1935, and was originally named Bacillus difficile because of the difficulties in cultivating it and its morphology [3]. For humans, C. Difficile Infection (CDI) is the leading cause of hospitalized Antibiotic-Associated Diarrhoea (AAD) in industrialized countries [4,5]. CDI has also been associated with diarrhoea and colitis in many animal species [1,6] including foals and adult horses [3,7-9]. C. difficile was first isolated from horses with diarrhoea in 1984 in the Potomac River area [10]. Acute colitis can cause marked rapid weight loss with high mortality in horses unless timely and intensively treated. C. difficile has been proven to be associated with acute colitis in horses [11,12], especially for the horses in large scale breeding farms after antibiotic administration [13-15]. Among the horses with AAD, 28% were positive for C. difficile based on the TcdB assay [16]. However, healthy horses may also carry C. difficile, and it can be isolated from feces of 0–17% of healthy adult horses [17,18]. This review will summarize etiology, pathogenesis, epidemiology, diagnosis, prevention and treatment of CDI in horses.

Etiology and Pathogenesis

Indigenous microbiota in the intestine acts in concert with the host to inhibit expansion and persistence of C. difficile. Antibiotic treatment disrupts microbiota-host homeostasis and creates an environment within the intestine that promotes C. difficile spore germination and subsequent vegetative cell growth [19,20], it is the known mechanism of pathogenesis of CDI in AAD, but others unknown factors appear to play a role. C. difficile can then adhere to the mucus layer carpeting the enterocytes and penetrate the mucus layer with the help of proteases and flagella. Virulent factors that play important roles in intestinal colonization and adherence include cysteine protease Cwp84 [21], S-layer P36, P47 [22], Cwp66 [23], GroEL [24], Flagellin, and flagellar cap protein [25]. Cwp84 is a dynamic molecule that not only has enzymatic activity against host proteins and may function as an exo-enzyme facilitating pathogenesis, but also functions at the cell surface to process proteins for incorporation into the cell wall and S-layer [26]. Immunization using GroEL decreases C. difficile intestinal colonization in the hamster model [27]. Following spore germination and vegetative cell colonization, the vegetative cells secrete toxins A and B (TcdA, TcdB), two major virulent factors of C. difficile [28-30]. These two toxins trigger the pathogenic host responses that are characteristic of CDI, including epithelial barrier disruption, inflammatory mediator release, immune cell infiltration and altered mucosal secretory responses [31,32]. TcdA and TcdB share similar structures that include the N-terminal catalytic Glucosyltransferase Domain (GTD), the autolytic Cysteine Proteinase Domain (CPD), the central Translocation Domain (TMD) and the C-terminal Receptor-Binding Domain (RBD) [33,34]. TcdB is usually 100-1000 times more potent than TcdA in in vivo cell cytotoxicity assay. The pathogenesis of CDI is thought to involve inflammation-associated tissue damage secondary to the intoxication of the intestinal epithelial cells. Following the breakdown of the intestinal epithelial barrier, immune cells within the mucosa (resident or recruited macrophages; resident mast cells) are exposed to TcdA and TcdB, triggering a “second-wave” of inflammation and tissue damage.

In addition to TcdA and TcdB, a limited number of C. difficile isolates, including the epidemic NAP1/027 strain, produce a Binary Toxin (CDT) that exhibits ADP-ribosyltransferase activity [35,36], but its role in the development of human disease is not well understood [37]. Interestingly, although CDT is found in approximately 10% of clinical isolates [38], recent epidemiological
an analyses showed that patients infected with strains producing CDT had 60% higher fatality rates compared to those infected with CDT-deficient strains [39]. CDT is composed of two separate subunits, CDTa (49 kDa, ADP-riboseyltransferase) and CDTb (99 kDa, receptor binding/translocation domains) [40]. The CDTa-CDTb complex induces cell rounding and cell death in VERO cells, and the uptake of CDT into cells requires endosomal acidification [41]. In addition to these effects, CDT may increase adherence of C. difficile to target cells, by the formation of microtubule protrusions [42].

**Epidemiology**

Most common disinfectants don’t work on C. difficile spores [43], making C. difficile an inflexible environmental contaminant. Sources of infection include, but are not limited to horses or foals infected with C. difficile [9]. Transmission occurs by the oral-fecal route [44] by ingestion of C. difficile spores from infected horses, possibly other animals, human beings, or contaminated environment. Adult horses or foals are susceptible to CDI [2,45] and the horses develop symptoms of CDI either sporadically or as outbreaks [13,46]. The CDI in horses ranges from 5 to 63% [47,48] and this huge variability maybe partially caused by the study designs, diagnostic methods, animal age, and sample collection variation, etc.

**Clinical Presentation**

The clinical signs vary depending on several factors including the age of the animal and the region of the gastro-intestinal tract that is affected. For both foals and adult horses, acute and watery diarrhoea is the most common clinical signs. In adult horses, anorexia, hyperemic mucous membranes, prolonged capillary refill time, pyrexia, tachycardia, tachypnea, variable degrees of dehydration, tympanic abdominal distension and mild to moderate or to severe colic are often associated with diarrhoea [49]. In most cases, diarrhoea appears suddenly and dramatically. Occasionally sudden death may occur even before the onset of diarrhoea. Clinical progression may be rapid. Severe dehydration and profound electrolyte disturbances typically develop. Laminitis is a possible complication.

In foals, C. difficile often causes enterocolitis. The disease could begin shortly after birth with mild to moderate abdominal distension, followed by brown, watery and fetid diarrhoea. If not treated, the mortality rate is high. Lesions vary in severity and distribution. Cecum and colon are principally affected in adult horses but foals develop severe duodenal, ileal and jejunal lesions [2]. At necropsy, the colon and cecum of adult horses are often filled with large amount of light brown to dark red hemorrhagic watery or dense fluid [9]. Affected segments are often edematous with ulcers or erosions but in most severe cases hemorrhagic necrotizing typhlocolitis has been reported [50]. Histologically, there is multifocal to diffuse coagulation necrosis with submucosal edema and congestion. In addition to the intestinal lesions evidence of endotoxic shock such as disseminated intravascular coagulopathy and thrombosis can be present. The small intestine content of the foals may be hemorrhagic with mucosal erosion and ulcers [51,52]. Severe necrosis of the villus epithelium is observed microscopically.

**Diagnosis**

Irrespective of the cause, many clinical signs and lesions associated with acute diarrhoea are indistinguishable. Therefore, the diagnosis of CDI as a cause of diarrhoea based on clinical signs in horses is difficult. The differential diagnosis include salmonellosis, Potomac horse fever, cantharid toxicity, other antibiotic associated diarrhoeas, Clostridium perfringens, carbohydrate/grain overload, sand irritation and thromboembolic disease [53,54]. Additional differentials in foals include rotavirus, coronavirus, foal heat diarrhoea, cryptosporidiosis and secondary lactose intolerance.

The presumptive diagnosis of CDI can be based upon clinical signs (necrotizing enterocolitis) associated with a history of antibiotic use and / or hospitalization. Nonspecific clinicopathological abnormalities, which are consistent with dehydration and endotoxemia from diarrhoea and mucosal damage, include leukopenia, neutropenia, elevated packed cell volume, variable total protein, hypoproteinemia, and acid base and electrolyte abnormalities. Abdominal ultrasonography may reveal ileus or thickened intestinal wall and occasionally intramural gas. Confirmatory testing of CDI relies on culture of bacteria and C. difficile toxin detection from feces. It is necessary to obtain compatible clinical signs and microbiological evidence of toxigenic C. difficile. Toxigenic culture and cell culture cytotoxicity neutralization assay, which are gold standards for detection of C. difficile toxins, are not used routinely because of their long turnaround time and high cost. Toxigenic culture is very sensitive, but less specific because it also detects asymptomatic colonization; and cell culture cytotoxicity neutralization assay is specific but less sensitive than previously acknowledged [55]. Recently, the presence of Glutamate Dehydrogenase (GDH) has also been used for diagnosis of CDI. However, GDH is not specific for toxigenic C. difficile, and positive results must be confirmed by another method [56]. Isolation of C. difficile from intestinal content and/or feces is usually considered diagnostic in several species, but in horses isolation alone is not confirmatory [3,52]. Potential for toxin production by isolates can be determined by PCR, using primers specific for TcdA and TcdB genes [57,58]. Enzyme Immunoassays Assay (EIA) for TcdA and TcdB became the routinely used diagnostic assay in the past years, but false positive rate was unacceptable [59,60]. Validation results indicated that commercial EIA for detection of C. difficile toxins in feces of horses with acute diarrhea is a reliable, adequate, and practical tool for identification of C. difficile toxins in horse feces [61]. In the event of a positive molecular assay without toxin presence assessed by EIA or cytotoxicity assay, other causes of diarrhoea should be excluded before making a definitive diagnosis of CDI.

**Treatment**

The therapeutic goals can be divided in 3 categories: specific antimicrobial therapy, maintenance of physiological homeostasis of water and electrolyte balance and supportive care for diarrhoea. Metronidazole (15mg/kg orally every 8 hours) is generally considered the treatment of choice. Resistance to the drug has been identified but it is considered to be rare. Vancomycin has been used in cases with metronidazole resistance. However, because of the importance of vancomycin in the treatment of resistant bacteria in human medicine it should be used very carefully. When possible, all other antimicrobial treatment should be discontinued.

Fluid and electrolyte balance must be maintained. Fluid therapy should be aimed at intravascular and total body water volume replacement (colloid and crystalloid fluids). Aggressive intravenous
polyionic fluid therapy should be instituted immediately in a horse with CDI and the replacement fluid may be administered rapidly (up to 6 to 10 L/hour for a 500kg horse). Because of gastrointestinal losses and serum albumin catabolism, many horses with acute colitis are hypoproteinemic. Commercial colloids such as plasma, dextran 40, dextran 70 or hydroxyethyl starch (hetastarch up to 10 ml/kg/day) can make a big difference to the horses [62,63].

Oral intestinal protectants such as bismuth subsalicylate, activated charcoal or di-tri-ocathedral smectite (Biosponge*) can be used to reduce toxin uptake through the permeable bowel lining [64]. Biosponge* adsorbs clostridial toxin and endotoxin in vitro [65]. Antisecretory therapy using CFTR (cystic fibrosis transmembrane conductance regulator) and CaCC (calcium activated chloride channel) inhibitors have shown efficacy in different animal models and in humans and are being tested in horses.

Restoration of the microbial flora in the large intestine may be useful in the management of horses with CDI. *Saccharomyces boulardii* has been used in horses to reduce the severity and duration of acute enterocolitis [66]. *S. boulardii* has been also found to release a protease that can digest *C. difficile* toxin A and B [67]. Low doses of Non Steroidal Anti-Inflammatory Drugs (NSAIDs) such as flunixin meglumine, firocoxib or meloxicam can be used in patients that initially require analgesia and to prevent laminitis [68]. Other measures to prevent laminitis include proper hoof trimming and shoeing, deeply bedded stalls and cryotherapy of the feet [69,70].

**Prevention**

Currently, there is no approved vaccine for horses. Careful use of antimicrobials and caution in using orally administered antibiotics in high risk horses could be helpful in decreasing the incidence of CDI. *C. difficile* forms heat resistant spores that are often present in the environment. A product that contains chlorine bleach is an effective disinfectant to kill *C. difficile* spores [71]. Good management practices including regular cleaning and disinfection with hypochlorite of stalls and equipment should be performed at all times. Horses that are hospitalized with CDI should have a private room or share a room with someone who has the same illness [72].

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**Conflict of Interest Statement**

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

**References**


