Review Article

Clostridium Difficile Infection in Horses

Keshan Zhang[1,4], Serge Martinod[3] and Xingmin Sun[1,2]*

[1]Tufts University Cummings School of Veterinary Medicine, Department of Infectious Diseases and Global Health, North Grafton, MA 01536, USA
[2]Tufts University, Clinical and Translational Science Institute
[3]Jaguar Animal Health, 185 Berry Street Suite 1300, San Francisco, Ca 94107
[4]State Key Laboratory of Veterinary Etiological Biology, National Foot and Mouth Disease Reference Laboratory, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China

*Corresponding author: Xingmin Sun, Tufts University Cummings School of Veterinary Medicine, Department of Infectious Diseases and Global Health, North Grafton, MA 01536, USA

Abstract

Clostridium difficile (C. *difficile*) is the most common cause of antibiotic-associated diarrhoea and the etiologic agent of pseudomembranous colitis in humans. *C. Difficile Infection* (CDI) has also been associated with diarrhoea 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 difficilis* 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

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-ribosyltransferase) 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, tympanitic 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-octahedral 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 if 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

1. Songer JG. Clostridial enteric diseases of domestic animals. Clin Microbiol Rev. 1996; 9: 216-234.

2. Keel MK, Songer JG. The comparative pathology of Clostridium difficile-associated disease. Vet Pathol. 2006; 43: 225-240.

3. Båverud V. Clostridium difficile diarrhea: infection control in horses. Vet Clin North Am Equine Pract. 2004; 20: 615-630.

4. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol. 2009; 7: 526-536.

5. Kelly CP, LaMont JT. Clostridium difficile infection. Annu Rev Med. 1998; 49: 375-390.

6. Hurley BW, Nguyen CC. The spectrum of pseudomembranous enterocolitis and antibiotic-associated diarrhea. Arch Intern Med. 2002; 162: 2177-2184.

7. Diab SS, Rodriguez-Bertos A, Uzal FA. Pathology and diagnostic criteria of Clostridium difficile enteric infection in horses. Vet Pathol. 2013; 50: 1028-1036.

8. Ruby R, Magdesian KG, Kass PH. Comparison of clinical, microbiologic, and clinicopathologic findings in horses positive and negative for Clostridium difficile infection. J Am Vet Med Assoc. 2009; 234: 777-784.

9. Diab SS, Songer G, Uzal FA. Clostridium difficile infection in horses: a review. Vet Microbiol. 2013; 167: 42-49.

10. Ehrich M, Perry BD, Troutt HF, Dellers RW, Magnusson RA. Acute diarrhea in horses of the Potomac River area: examination for clostridial toxins. J Am Vet Med Assoc. 1984; 185: 433-435.

11. Donaldson MT, Palmer JE. Prevalence of Clostridium perfringens enterotoxin and Clostridium difficile toxin A in feces of horses with diarrhea and colic. J Am Vet Med Assoc. 1999; 215: 358-361.

12. Weese JS, Staempfli HR, Prescott JF. A prospective study of the roles of clostridium difficile and enterotoxigenic Clostridium perfringens in equine diarrhoea. Equine vet J. 2001; 33: 403-409.

13. Madewell BR, Tang YJ, Jang S, Madigan JE, Hirsh DC, Gumerlock PH, et al. Apparent outbreaks of Clostridium difficile-associated diarrhea in horses in a veterinary medical teaching hospital. J Vet Diagn Invest. 1995; 7: 343-346.

14. Larsen J. Acute colitis in adult horses. A review with emphasis on aetiology and pathogenesis. Vet Q. 1997; 19: 72-80.

15. Baverud V, Franklin A, Gunnarsson A, Gustafsson A, Hellander-Edman A. Clostridium difficile associated with acute colitis in mares when their foals are treated with erythromycin and rifampicin for Rhodococcus equi pneumonia. Equine Vet J. 1998; 30: 482-488.

16. Båverud V, Gustafsson A, Franklin A, Aspán A, Gunnarsson A. Clostridium difficile: prevalence in horses and environment, and antimicrobial susceptibility. Equine Vet J. 2003; 35: 465-471.

17. Medina-Torres CE, Weese JS, Staempfli HR. Prevalence of Clostridium difficile in horses. Vet Microbiol. 2011; 152: 212-215.

18. Ossiprandi MC, Buttrini M, Bottarelli E, Zerbini L. Preliminary molecular analysis of Clostridium difficile isolates from healthy horses in northern Italy. Comp Immunol Microbiol Infect Dis. 2010; 33: e25-29.

19. Britton RA, Young VB. Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance. Trends Microbiol. 2012; 20: 313-319.

20. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. Gastroenterology. 2014; 146: 1547-1553.

21. Janoir C, Pechine S, Grosdidier C, Collignon A. Cwp84, a surface-associated protein of Clostridium difficile, is a cysteine protease with degrading activity on extracellular matrix proteins. J Bacteriol. 2007; 189: 7174-7180.

22. Calabi E, Ward S, Wren B, Paxton T, Panico M, Morris H, et al. Molecular characterization of the surface layer proteins from Clostridium difficile. Mol Microbiol. 2001; 40: 1187-1199.

23. Waligora AJ, Hennequin C, Mullany P, Bourlioux P, Collignon A, Karjalainen T. Characterization of a cell surface protein of Clostridium difficile with adhesive properties. Infect Immun. 2001; 69: 2144-2153.

24. Hennequin C, Porcheray F, Waligora-Dupriet A, Collignon A, Barc M, Bourlioux P, et al. GroEL (Hsp60) of Clostridium difficile is involved in cell adherence. Microbiology. 2001; 147: 87-96.

25. Tasteyre A, Barc MC, Collignon A, Boureau H, Karjalainen T. Role of FliC and FliD flagellar proteins of Clostridium difficile in adherence and gut colonization. Infect Immun. 2001; 69: 7937-7940.

26. Vedantam G, Clark A, Chu M, McQuade R, Mallozzi M, Viswanathan VK.

Clostridium difficile infection: toxins and non-toxin virulence factors, and their contributions to disease establishment and host response. Gut Microbes. 2012; 3: 121-134.

27. Péchiné S, Hennequin C, Boursier C, Hoys S, Collignon A. Immunization using GroEL decreases Clostridium difficile intestinal colonization. PLoS One. 2013; 8: e81112.

28. Elliott B, Chang BJ, Golledge CL, Riley TV. Clostridium difficile-associated diarrhoea. Intern Med J. 2007; 37: 561-568.

29. Kelly CP, Pothoulakis C, LaMont JT. Clostridium difficile colitis. N Engl J Med. 1994; 330: 257-262.

30. Voth DE, Ballard JD. Clostridium difficile toxins: mechanism of action and role in disease. Clin Microbiol Rev. 2005; 18: 247-263.

31. Rineh A, Kelso MJ, Vatansever F, Tegos GP, Hamblin MR. Clostridium difficile infection: molecular pathogenesis and novel therapeutics. Expert Rev Anti Infect Ther. 2014; 12: 131-150.

32. Tonna I, Welsby PD. Pathogenesis and treatment of Clostridium difficile infection. Postgrad Med J. 2005; 81: 367-369.

33. Jank T, Aktories K. Structure and mode of action of clostridial glucosylating toxins: the ABCD model. Trends Microbiol. 2008; 16: 222-229.

34. Pruitt RN, Lacy DB. Toward a structural understanding of Clostridium difficile toxins A and B. Front Cell Infect Microbiol. 2012; 2: 28.

35. Carter GP, Lyras D, Allen DL, Mackin KE, Howarth PM, O'Connor JR, et al. Binary toxin production in Clostridium difficile is regulated by CdtR, a LytTR family response regulator. J Bacteriol. 2007; 189: 7290-7301.

36. Blossom DB, McDonald LC. The challenges posed by reemerging Clostridium difficile infection. Clin Infect Dis. 2007; 45: 222-227.

37. Stare BG, Delmée M, Rupnik M. Variant forms of the binary toxin CDT locus and tcdC gene in Clostridium difficile strains. J Med Microbiol. 2007; 56: 329-335.

38. Carroll KC, Bartlett JG. Biology of Clostridium difficile: implications for epidemiology and diagnosis. Annu Rev Microbiol. 2011; 65: 501-521.

39. Bacci S, Mølbak K, Kjeldsen MK, Olsen KE. Binary toxin and death after Clostridium difficile infection. Emerg Infect Dis. 2011; 17: 976-982.

40. Davies AH, Roberts AK, Shone CC, Acharya KR. Super toxins from a super bug: structure and function of Clostridium difficile toxins. Biochem J. 2011; 436: 517-526.

41. Perelle S, Gibert M, Bourlioux P, Corthier G, Popoff MR. Production of a complete binary toxin (actin-specific ADP-ribosyltransferase) by Clostridium difficile CD196. Infect Immun. 1997; 65: 1402-1407.

42. Schwan C, Stecher B, Tzivelekidis T, van Ham M, Rohde M, Hardt WD, et al. Clostridium difficile toxin CDT induces formation of microtubule-based protrusions and increases adherence of bacteria. PLoS Pathog. 2009; 5: e1000626.

43. Worsley MA. Infection control and prevention of Clostridium difficile infection. J Antimicrob Chemother. 1998; 41 Suppl C: 59-66.

44. Chapman AM. Acute diarrhea in hospitalized horses. Vet Clin North Am Equine Pract. 2009; 25: 363-380.

45. Uzal FA, Diab SS, Blanchard P, Moore J, Anthenill L, Shahriar F, et al. Clostridium perfringens type C and Clostridium difficile co-infection in foals. Vet Microbiol. 2012; 156: 395-402.

46. Songer JG, Trinh HT, Dial SM, Brazier JS, Glock RD. Equine colitis X associated with infection by Clostridium difficile NAP1/027. J Vet Diagn Invest. 2009; 21: 377-380.

47. Thean S, Elliott B, Riley TV. Clostridium difficile in horses in Australia--a preliminary study. J Med Microbiol. 2011; 60: 1188-1192.

48. Frederick J, Giguere S, Sanchez LC. Infectious agents detected in the feces of diarrheic foals: a retrospective study of 233 cases (2003-2008). J Vet Intern Med. 2009; 23: 1254-1260.

49. Weese JS, Toxopeus L, Arroyo L. Clostridium difficile associated diarrhoea in horses within the community: predictors, clinical presentation and outcome. Equine veterinary journal. 2006; 38: 185-188.

50. Pothoulakis C, Castagliuolo I, Kelly CP, Lamont JT. Clostridium difficile-associated diarrhea and colitis: pathogenesis and therapy. Int J Antimicrob Agents. 1993; 3: 17-32.

51. Jones RL, Adney WS, Alexander AF, Shideler RK, Traub-Dargatz JL. Hemorrhagic necrotizing enterocolitis associated with Clostridium difficile infection in four foals. J Am Vet Med Assoc. 1988; 193: 76-79.

52. Magdesian KG, Hirsh DC, Jang SS, Hansen LM, Madigan JE. Characterization of Clostridium difficile isolates from foals with diarrhea: 28 cases (1993-1997). J Am Vet Med Assoc. 2002; 220: 67-73.

53. Brewer BD, Koterba AM. Development of a scoring system for the early diagnosis of equine neonatal sepsis. Equine Vet J. 1988; 20: 18-22.

54. Magdesian KG. Neonatal foal diarrhea. Vet Clin North Am Equine Pract. 2005; 21: 295-312, vi.

55. Goldenberg SD, Cliff PR, French GL. Laboratory diagnosis of Clostridium difficile infection. Journal of clinical microbiology. 2010; 48: 3048-3049.

56. Le Guern R, Wallet F. [Laboratory diagnosis of Clostridium difficile infection]. Ann Biol Clin (Paris). 2013; 71: 395-400.

57. Kuhl SJ, Tang YJ, Navarro L, Gumerlock PH, Silva J, Jr. Diagnosis and monitoring of Clostridium difficile infections with the polymerase chain reaction. Clin Infect Dis. 1993; 16: S234-238.

58. Tang YJ, Gumerlock PH, Weiss JB, Silva J Jr. Specific detection of Clostridium difficile toxin A gene sequences in clinical isolates. Mol Cell Probes. 1994; 8: 463-467.

59. Sloan LM, Duresko BJ, Gustafson DR, Rosenblatt JE. Comparison of real-time PCR for detection of the tcdC gene with four toxin immunoassays and culture in diagnosis of Clostridium difficile infection. J Clin Microbiol. 2008; 46: 1996-2001.

60. Alcala L, Sanchez-Cambronero L, Catalan MP, Sanchez-Somolinos M, Pelaez MT, Marin M, et al. Comparison of three commercial methods for rapid detection of Clostridium difficile toxins A and B from fecal specimens. J Clin Microbiol. 2008; 46: 3833-3835.

61. Medina-Torres CE, Weese JS, Staempfli HR. Validation of a commercial enzyme immunoassay for detection of Clostridium difficile toxins in feces of horses with acute diarrhea. J Vet Intern Med. 2010; 24: 628-632.

62. Jones PA, Bain FT, Byars TD, David JB, Boston RC. Effect of hydroxyethyl starch infusion on colloid oncotic pressure in hypoproteinemic horses. J Am Vet Med Assoc. 2001; 218: 1130-1135.

63. Blikslager AT. Treatment of gastrointestinal ischemic injury. Vet Clin North Am Equine Pract. 2003; 19: 715-727.

64. Powell DG. Equine infectious respiratory disease. Vet Rec. 1975; 96: 30-34.

65. Weese JS, Cote NM, deGannes RV. Evaluation of in vitro properties of di-tri-octahedral smectite on clostridial toxins and growth. Equine Vet J. 2003; 35: 638-641.

66. Desrochers AM, Dolente BA, Roy MF, Boston R, Carlisle S. Efficacy of Saccharomyces boulardii for treatment of horses with acute enterocolitis. J Am Vet Med Assoc. 2005; 227: 954-959.

67. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. Infect Immun. 1999; 67: 302-307.

68. Boosman R, Németh F. [Pathogenesis and drug therapy of acute laminitis in horses: a literature review]. Tijdschr Diergeneeskd. 1988; 113: 1237-1246.

69. Wylie CE, Collins SN, Verheyen KL, Newton JR. Risk factors for equine laminitis: a case-control study conducted in veterinary-registered horses and ponies in Great Britain between 2009 and 2011. Vet J. 2013; 198: 57-69.

70. Slater MR, Hood DM, Carter GK. Descriptive epidemiological study of equine laminitis. Equine Vet J. 1995; 27: 364-367.

71. Hacek DM, Ogle AM, Fisher A, Robicsek A, Peterson LR. Significant impact of terminal room cleaning with bleach on reducing nosocomial Clostridium difficile. Am J Infect Control. 2010; 38: 350-353.

72. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013; 108: 478-498.

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