Title
Clostridium difficile infection in horses: A review

Permalink
https://escholarship.org/uc/item/0sb8w139

Journal
Veterinary Microbiology, 167(1-2)

ISSN
0378-1135

Authors
Diab, SS
Songer, G
Uzal, FA

Publication Date
2013-11-29

DOI
10.1016/j.vetmic.2013.03.032

Peer reviewed
Clostridium difficile infection in horses: A review

S.S. Diab a, G. Songer b, F.A. Uzal a, *

a California Animal Health and Food Safety Laboratory, San Bernardino Branch, School of Veterinary Medicine, University of California, Davis, CA 92408, USA
b Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA

ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 5 March 2013
Accepted 27 March 2013

Keywords:
Clostridium difficile
Horse
Review

ABSTRACT

Clostridium difficile is considered one of the most important causes of diarrhea and enterocolitis in horses. Foals and adult horses are equally susceptible to the infection. The highly resistant spore of C. difficile is the infectious unit of transmission, which occurs primarily via the fecal-oral route, with sources of infection including equine feces, contaminated soil, animal hospitals, and feces of other animals. Two major risk factors for the development of C. difficile associated disease (CDAD) in adult horses are hospitalization and antimicrobial treatment, although sporadically, cases of CDAD can occur in horses that have not received antimicrobials or been hospitalized. The most common antibiotics associated with CDAD in horses are erythromycin, trimethoprim/sulfonamides, β-lactam antimicrobials, clindamycin, rifampicin, and gentamicin. Clinical signs and intestinal lesions of CDAD infection are not specific and they cannot be used to distinguish infections by C. difficile from infections by other agents, such as Clostridium perfringens or Salmonella sp. The distribution of lesions throughout the intestinal tract seems to be age-dependent. Small intestine is invariably affected, and colon and cecum may or may not have lesions in foals < 1-month old. Naturally acquired disease in older foals and adult horses has a more aboral distribution, affecting colon and sometimes cecum, but rarely the small intestine. Detection of toxin A, toxin B or both in intestinal contents or feces is considered the most reliable diagnostic criterion for CDAD in horses. Isolation of toxigenic strains of C. difficile from horses with intestinal disease is highly suggestive of CDAD. A better understanding of pathogenesis, reservoirs of infection, and vaccines and other methods of control is needed. Also further studies are recommended to investigate other possible predisposing factors and/or etiological agents of enteric diseases of horses.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Clostridium difficile is a gram-positive, anaerobic, spore-forming bacillus commonly associated with diarrhea and colitis in humans and other mammals (Keel and Songer, 2006; Songer, 1996). This microorganism was first isolated from feces and meconium of clinically healthy human babies, and was originally named Bacillus difficilis because of its morphology and the difficulties encountered in cultivating it (Hall and O’Toole, 1935; Keel and Songer, 2006). In the past 30–40 years, however, C. difficile has been implicated as the principal infectious cause of antibiotic-associated diarrhea in adult humans, and newborn and adult individuals of several animal species, including horses (Hurley and Nguyen, 2002; Keel and Songer, 2006; Kelly and LaMont, 1998; Songer, 1996). It is now recognized as one of the most important nosocomial pathogens of humans, although community acquired cases (non-nosocomial associated) have been occurring over the past years in increasing numbers (Khanna and Pardi, 2012).
C. difficile is amongst the most important agents of enteric disease of foals and adult horses (Diab et al., in press; Hurley and Nguyen, 2002; Jones et al., 1988a,b; Kelly and LaMont, 1998; Madewell et al., 1995; Magdesian et al., 2002; Songer, 1996; Weese et al., 1999, 2001). This review addresses the most important aspects of C. difficile—associated disease (CDAD) in horses.

2. Etiology, pathogenesis and virulence factors

As noted above, C. difficile is a Gram-positive, rod-shaped, obligate anaerobe, which was discovered in the 1930s in meconium of infants (McCollum and Rodriguez, 2012), but was only found to be a human pathogen in the late 1970s (Bartlett et al., 1977). Researchers eventually discovered that the organism’s toxic activity could be neutralized by serum prepared against toxins of C. sordellii, which suggested that the virulence of this organism was related to toxins (Willey and Bartlett, 1979). The primary virulence factors of C. difficile are the two major toxins, toxin A (TcdA) and toxin B (TcdB) (Vedantam et al., 2012). TcdA has a molecular weight of 308 kDa and toxin B is 269 kDa (Hussack et al., 2012). Furthermore, these two toxins share genetic characteristics, making it likely that one appeared first in evolutionary time and that the other has resulted from a gene duplication event (von Eichel-Streiber et al., 1992). Divergent evolution has led to some differences between TcdA and TcdB. The C-terminal portion of TcdA and TcdB mediates toxin binding to enterocytes (Artushin et al., 2012). Toxins gain access to the cytoplasm and their enzymatic portions, which have monoglucosyltransferase activity, and catalyze glucosylation, inactivate small regulatory proteins of the eukaryotic actin cytoskeleton. Loss of these proteins, the Rho-GTPases, leads to disorganization of the cytoskeleton and cell death. However, the specific roles of TcdA and TcdB in the context of C. difficile infection is unknown (Davies et al., 2011).

Some strains of C. difficile may also produce an ADP-ribosylating binary toxin (CDT) that is made up of two components; CDTa is an enzymatic component and CDTb mediates entry of CDTa into target cells. CDTA ADP-ribosylates G-actin in target cells, disrupting the F-actin:G-actin equilibrium and leading to cell death (Davies et al., 2011).

The role of adherence in pathogenesis remains somewhat of an open question. Spores may be more hydrophobic or more hydrophilic and, as a general rule, the former adhere more vigorously to enterocytes (Joshi et al., 2012). Electron microscopic examination of hydrophobic spores revealed an exosporium that may have some role in the attachment process (Joshi et al., 2012). There is good evidence that cell wall proteins (e.g., CWP66) are also involved in attachment (Pechine et al., 2005).

Clinical CDAD in horses varies in extent of clinical signs and in severity. In general, metronidazole-resistant strains of C. difficile are more virulent than those that are metronidazole-sensitive. The basic factor behind these differences in virulence is the extent of toxicogenicity (Keel and Songer, 2006).

3. Epidemiology

For the development of C. difficile enteric disease, exposure to a toxigenic strain of C. difficile is required (Baverud, 2002). Although the vegetative forms of C. difficile do not survive for long periods of time in an aerobic environment, C. difficile can survive for prolonged periods of time in sporid form (Buggy et al., 1983). C. difficile has been isolated from a wide variety of sources, including, but not limited to, fecal samples of healthy and diarrheic foals and adult horses (Frederick et al., 2009; Madewell et al., 1995; Medina-Torres et al., 2011; Schoster et al., 2012a,b; Thean et al., 2011; Weese et al., 2001), outdoor soil samples from stud farms and, less frequently, farms with mature horses (Baverud et al., 2003), small and large animal hospitals (Weese et al., 2000a), human hospitals (Mulligan et al., 1980), marine sediment (Matches and Liston, 1974), soil and sand (Hafiz and Oakley, 1976), feces of non-diarrheic humans (Finegold et al., 1983; Viscidi et al., 1981), camels, donkeys (Hafiz and Oakley, 1976), dogs and cats (up to 39% prevalence rate) (O’Neill et al., 1993; Riley et al., 1991; Weber et al., 1989), domestic birds, cattle, ducks, geese, seals, and snakes (Levett, 1986). An environmental survey of a veterinary teaching hospital reported isolation of C. difficile from the stalls, floors, medical equipment and footwear of medical personnel in the large animal clinic, the small animal clinic and other sites (Weese et al., 2000a). Spores are also relatively resistant to most common disinfectants (Worsley, 1998), making C. difficile a hardy environmental contaminant.

Horses are susceptible as adults or foals and may develop C. difficile enteric disease as early as the first few days of life (Keel and Songer, 2006; Uzal et al., 2012). The disease can present as outbreaks (Jones et al., 1987; Madewell et al., 1995) or sporadic cases (Jones et al., 1988a,b; Jones, 1988; Perrin et al., 1993; Songer et al., 2009; Uzal et al., 2012). Transmission of the organism occurs by the oral-fecal route (Chapman, 2009) by ingestion of vegetative cells of C. difficile or its spores from other infected horses, contaminated environment or possibly other animals and human beings. C. difficile present in low levels in the gastrointestinal tract of subclinical carriers may also proliferate when predisposing factors appear (Baverud, 2004).

Most authors agree that the two major risk factors for the development of CDAD are antibiotic treatment and hospitalization (Baverud, 2002, 2004; Baverud et al., 1998, 2003, 1997; Diab et al., in press; Madewell et al., 1995). Antibiotics can have an adverse effect on the distribution and number of normal bacteria and protozoa in the cecum and colon by disrupting the balance of protective commensal organisms, which can permit the overgrowth of pathogenic species (Baverud, 2004; Papich, 2003; Vollaard and Clasener, 1994). In the hamster model of the disease, C. difficile cannot colonize the animal in the presence of an undisturbed microflora, yet it rapidly attains a large population size when introduced into the antibiotic-treated animal (Wilson et al., 1985). Essentially, all antibiotics are capable of causing diarrhea and enterocolitis in horses, especially in the presence of opportunistic enteropathogens or other risk factors.
However, the most frequent antimicrobials associated with *C. difficile* diarrhea in horses are erythromycin, trimethoprim/sulfonamides, β-lactam antibiotics, clindamycin, rifampicin, and gentamicin (Arroyo et al., 2004; Baverud et al., 2003, 1997; Divers, 2002; Gustafsson et al., 1997; Madewell et al., 1995; Magdesian et al., 2002, 1999). In addition to altering the normal intestinal microflora and its barrier, in vitro studies have shown that antibiotics such as ampicillin and clindamycin may be involved in CDAD by increasing the colonization factor (adhésins) gene expression (Deneve et al., 2008).

*C. difficile* associated with acute diarrhea and colitis in mares when their foals are treated with erythromycin and rifampicin for *Rhodococcus equi* pneumonia has been well documented (Baverud et al., 1998). The mares developed a sudden, profuse, often fatal watery diarrhea, in most cases 3 or 4 days after treatment of their foals was started. The only common factor in the affected mares was that their foals had been treated orally with erythromycin in combination with rifampicin for *R. equi* pneumonia. The hypothesis was that accidental ingestion of very small amounts of antibiotics by the mares from the treatment of their foals may have caused an imbalance of the intestinal flora and subsequently given rise to the fatal colitis. The foals treated with antibiotics were regarded as asymptomatic carriers and potential reservoirs, as *C. difficile* was found in 7 of 16 foals investigated (Baverud et al., 1998).

Hospitalization is the second major risk factor for the development of CDAD. It is important to note that colitis in hospitalized horses may be multifactorial and occur in conjunction with gastrointestinal and non-gastrointestinal diseases. In addition to potentially increased exposure to toxigenic *C. difficile* from contaminated hospital environment, changes in the diet, pre and post-surgical feed withdrawal, and antibiotic administration during hospitalization may all contribute to the nosocomial form of CDAD (Chapman, 2009).

Frequently in foals <7-day old (Arroyo et al., 2004; Diab et al., in press; Jones et al., 1987) and occasionally in older foals and adult horses (Baverud, 2004), the disease occurs without a history of antibiotic therapy or hospitalization. Interestingly, one study shows that antimicrobial therapy preceded the onset of diarrhea in only 26% of horses with CDAD, and that there was no difference in the incidence of antimicrobial therapy between CDAD and non-CDAD groups (Weese et al., 2006), suggesting that antibiotic therapy may not be a significant risk factor for CDAD. The predisposing conditions in these cases are not well established, but stress, change of diet, transportation, starvation, nasogastric intubation and surgical or medical treatment have been proposed as possible contributors (Baverud, 2004; Baverud et al., 1997). Five cases of foal enterocolitis associated with *C. difficile* and *C. perfringens* type C infection were recently reported (Uzal et al., 2012). In those cases, a possible synergism of *C. perfringens* type C and *C. difficile* was suggested, since none of the foals in the referred study had received antibiotic therapy or been hospitalized.

Numerous authors have studied the prevalence of *C. difficile* in healthy and diarrheic horses. Among healthy foals, the carrier rates usually range between 0 and 3% (Baverud et al., 2003; Jones et al., 1987; Weese et al., 2001). However, one research group reported a 29% culture-positive rate for asymptomatic foals <14 day old, a 0.6% rate in older foals and a 44% rate in non-diarrheic foals treated with erythromycin or gentamicin in combination with rifampicin (Baverud et al., 2003), suggesting that foals may occasionally be a significant source of infection. In healthy adults, the prevalence of *C. difficile* in the gastrointestinal tract is usually also low, ranging between 0 and 10% (Baverud et al., 2003; McNamara et al., 2011; Medina-Torres et al., 2011; Weese et al., 2001). A recent, one-year longitudinal prevalence study in adult healthy horses sampled monthly showed that 15 of 275 (5.45%) were positive for *C. difficile*. However, the cumulative prevalence was higher, as 10 of 25 horses (40%) were positive at least once throughout the year. This study suggests that *C. difficile* shedding is transient and dynamic, that horses may harbor a strain for short periods of time, that they appear to be exposed to a variety of strains, and that the exposure to *C. difficile* is a common event despite the low incidence of *C. difficile* infection in horses within a community (Schoster et al., 2012b).

A study designed to compare recovery of *C. difficile* from different intestinal segments showed that *C. difficile* was recovered from 8 of 15 horses from one or more of the following compartments: duodenum, jejunum, ileum, right dorsal colon, small colon and rectum. In only 5 of these 8 horses (63%), *C. difficile* was cultured from feces, which suggests that, although the correlation between presence of this microorganism in the gastrointestinal tract and fecal shedding is good, feces might not absolutely reflect the status of the proximal compartments (Schoster et al., 2012a).

*C. difficile* rates in foals and adult horses with gastrointestinal disease (diarrhea, enteritis or enterocolitis) vary considerably among authors, ranging from 5 to 63% (Baverud et al., 2003; Frederick et al., 2009; Jones et al., 1987; Thean et al., 2011; Weese et al., 2001). This variability may reflect differences in the study designs, sensitivity and specificity of diagnostic tests, regional or temporal prevalence, sample collection, animal age, predisposing factors, etc.

4. Clinics

The clinical presentation of CDAD in horses is highly variable in terms of clinical signs and severity of the disease. The main clinical sign of CDAD in foals and adult horses is diarrhea, which may be accompanied by hyperemic mucous membranes, prolonged capillary refill time, pyrexia, tachycardia, tachypnea, dehydration, abdominal distension and colic (Weese et al., 2006). However, adult horses with CDAD may have abdominal discomfort or fever as the primary presenting problem and in some cases ileus and gas distention of the small intestine may be the primary complaint (Jones, 2009). Foal spontaneous CDAD beginning immediately after birth is usually characterized by depression, watery or bloody diarrhea, dehydration and/or toxemia, often followed by death (Diab et al., in press). The clinical signs in
experimental *C. difficile* enterocolitis in newborn foals with normal transfer of passive immunity ranged from mild abdominal discomfort and pasty feces to colic and watery diarrhea within 24–72 h after challenge (Arroyo et al., 2004). The overall mortality rate, including foals and adults is variable between studies, ranging from 0 to 42% (Arroyo et al., 2004; Madewell et al., 1995; Weese et al., 2001, 2006). Although the clinical signs of CDAD infection can be characteristic, they are not specific. Therefore, there are no clinical features that specifically distinguish infections by *C. difficile* from infections by other agents of intestinal disease such as *C. perfringens*, *Salmonella* sp or *Neorickettsia risticii* (equine ehrlichial colitis) (Cordes et al., 1986; Diab et al., 2012, in press; Weese et al., 2006).

A syndrome known as duodenitis-proximal jejunitis, characterized clinically by large volumes of enterogastric reflux, has been known since the early 1980s. Over the past few years, an association has been suggested between *C. difficile* and duodenitis-proximal jejunitis (Arroyo et al., 2006; Jones, 2009). However, while the relationship between *C. difficile* and enteritis, colitis and enterocolitis has been proven, a cause-effect association between *C. difficile* and duodenitis-proximal jejunitis has not been proven. Neither this association has been ruled out.

5. Gross and microscopic lesions

As with the clinical sings, *C. difficile* lesions may be characteristic but are not pathognomonic. Other infectious (*C. perfringens* type C, *Salmonella* sp. and *Neorickettsia risticii*) and non-infectious (nonsteroidal anti-inflammatory drugs) agents of intestinal disease can produce very similar gross and microscopic lesions (Cordes et al., 1986; Diab et al., 2012; Uzal et al., 2012). The distribution of the lesions throughout the intestinal tract seems to be dependent on the age of the horse. In foals < 1-month old, the small intestine is invariably affected, whereas colon and cecum may or may not have lesions. In older foals and adult horses, the naturally acquired disease has more aboral distribution, affecting the colon and sometimes the cecum and usually sparing the small intestine.
(Diab et al., in press; Jones et al., 1988a,b; Keel and Songer, 2006; Perrin et al., 1993; Uzal et al., 2012).

Externally, the small and large intestines are characterized by multifocally hemorrhagic or diffusely reddened or bluish serosal surface as the result of intense mucosal hyperemia or hemorrhage (Figs. 1 and 2). The intestinal contents in young foals are often hemorrhagic (Fig. 2) but may also present as yellow and pasty or green/brown and watery. In older foals and adult horses the colon and cecum are usually filled with large amount of green or light brown, watery contents (Fig. 3) and occasionally dark brown or red, hemorrhagic, watery or dense fluid. The wall of the small intestine can be normal or mildly thickened; the mucosa is often diffusely reddened and may have an overlying, multifocal, tan to orange pseudomembrane (Fig. 4). The wall of the colon and cecum is usually diffusely and markedly thickened by clear to hemorrhagic, gelatinous submucosal and mucosal edema; the mucosa is multifocally or diffusely dull green or red and may be multifocally covered by a tan or light green pseudomembrane (Fig. 5). When present, gross lesions other than those in the gastrointestinal tract are compatible with those seen in endotoxic shock and/or disseminated intravascular coagulation (DIC), including serous or serosanguineous pericardial effusion, pulmonary edema and congestion, and multifocal subendocardial and subserosal petechiae and ecchymoses (Diab et al., in press).

Histologically, there is multifocal to diffuse, often hemorrhagic, coagulative mucosal necrosis usually accompanied by submucosal edema and congestion (Figs. 6 and 7). Thrombosis of small to mid-size caliber blood vessels in the mucosa and/or submucosa is a very frequent and helpful finding, especially in those cases in which autolysis has partially masked the mucosal necrosis (Fig. 8). Inflammatory cells are not a prominent feature in many cases but some cases may present mild or moderate, mucosal and submucosal neutrophilic infiltration. The so-called "volcano" lesions, characterized by patchy focal erosions on the colonic mucosa through which fibrin and neutrophils exude, are rarely seen in horses. It is possible that at the time of necropsy and sample collection the lesions are too advanced to show the delicate classic volcano-like structure. In some cases, a few to numerous clusters of short and thick, Gram-positive rods can be observed on the surface or within the necrotic mucosa (Fig. 8) (Diab et al., in press).

Fig. 5. Large colon of an adult horse with C. difficile colitis showing diffuse mucosal necrosis and thickening of the mucosal folds (severe edema).

Fig. 6. Low magnification of a histological section of the small intestine of a neonatal foal with C. difficile enteritis depicting diffuse mucosal necrosis, submucosal edema and mucosal and submucosal congestion and hemorrhage. Hematoxylin and eosin stain, 20x.

Fig. 7. Low magnification of a histological section of the colon of an adult horse with C. difficile colitis showing diffuse coagulation necrosis of the mucosa and marked submucosal edema. Hematoxylin and eosin stain, 20x.

Fig. 8. High magnification of a histological section of the necrotic colonic mucosa of an adult horse with C. difficile colitis showing characteristic vascular thrombi (arrows) and clusters of intralesional bacteria (double arrows). Hematoxylin and eosin stain, 200x.
6. Diagnosis and diagnostic criteria

A presumptive diagnosis of equine CDAD can be based on the clinical history, clinical signs and gross and microscopic lesions. However, none of these findings is specific for C. difficile infection and a diagnosis of CDAD cannot be confirmed by these methods alone.

CDAD should be suspected in any horse with diarrhea showing necrotizing enteritis and/or enterocolitis. A history of antibiotic use and/or hospitalization may help increase the suspicion of CDAD. However, CDAD has been documented in horses that have not received antibiotics or being hospitalized, and lack of a clinical history of antibiotic treatment and/or hospitalization does not preclude a diagnosis of CDAD. Ruling out other potential causes of diarrhea (e.g. Salmonella sp., C. perfringens, others) also helps to establish a presumptive diagnosis of CDAD.

Although by some authors isolation of C. difficile from intestinal content and/or feces is usually considered diagnostic in several species, in horses isolation alone is not confirmatory as a relatively reduced number of normal horses may harbor C. difficile in the gut (Baverud, 2002, 2004; Baverud et al., 2004, 1997; Gustafsson et al., 2004; Jones et al., 1987; Keel and Songer, 2006; Madewell et al., 1995; Magdesian et al., 2002; Weese et al., 2001). Another drawback of culture for diagnosis confirmation is the existence of non-toxigenic strains of C. difficile (Blake et al., 2004; Mathis et al., 1999). While in human medicine this is usually overcome by toxino typing the isolates, this is usually not done in veterinary medicine. Isolation of toxigenic strains of C. difficile from the gut of horses with clinical and pathologic findings compatible with CDAD is, however, highly suggestive of this disease. Potential for toxin production by isolates is determined by use of PCR, using primers specific for TcdA and TcdB genes (Gumerlock et al., 1993; Tang et al., 1994). Toxins in cultures can also be identified in culture filtrate by ELISA or cell culture assays (see below).

C. difficile is best isolated on selective media that are either commercially available or made in-house (Fedorko and Williams, 1997; George et al., 1979; Wilson et al., 1982). The intestinal contents or fecal samples should be submitted in an anaerobic transport medium unless they can be delivered to the bacteriology laboratory within 24 h (Weese et al., 2000b). Refrigeration of samples is preferred to avoid overgrowth by other bacteria.

It is generally assumed that final confirmation of CDAD requires detection of TcdA, TcdB or both in the intestinal content or feces (Baverud et al., 2003; Delmee, 2001; Keel and Songer, 2006). Toxin is detected by ELISA or by assessing the cytopathic effect of intestinal contents/fecal filtrates on cell lines. The ELISA is rapid, sensitive, and specific, and commercial kits are available (Post et al., 2002; Schue et al., 1994). The cytotoxin assay is specific and more sensitive, but requires cell culture facilities and takes considerably more time than the ELISA (Post et al., 2002; Sullivan et al., 1982).

Several studies showed discrepancies between culture of C. difficile and toxin assay (Baverud et al., 1997; Gustafsson et al., 1997; Weese et al., 2001). Negative C. difficile culture/toxin-positive cases should be considered as true positives because of the poor survival of C. difficile in aerobically stored fecal samples (Weese et al., 2000b, 2001). However, positive C. difficile culture/toxin-negative cases should be considered as suggestive of C. difficile enteric disease only, as some C. difficile isolates may be non-toxigenic and a small percentage of healthy carriers exist. The possibility of obtaining false-negative results in the toxin assay because of inactivation of the toxins during transport or storage highlights the importance of the bacterial culture to support the diagnosis of infection by C. difficile (Diab et al., in press).

7. Conclusions

C. difficile is amongst the most important causes of enteric disease in horses. Although our knowledge about the disease has increased substantially over the past few years, further studies are still needed to gain better understanding of pathogenesis, most likely reservoirs of infection, and vaccines and other methods of treatment and control. Also, although diagnostic criteria for CDAD have been refined over the past few years and we now have knowledge and tools to obtain a diagnosis of reasonable certainty in many cases, much has to be learned about diagnosing this disease, in particular defining the diagnostic criteria for CDAD. Overall, improving the diagnosis of enteric disease in horses by agents other than C. difficile is important as currently a still relatively large percentage of cases of enterocolitis in horses are written off as “undetermined etiology”. It might be worthwhile attempting metagenomics on some of these horses with enteric disease in order to determine which organisms are present in highest numbers.

References


Lewett, P.N., 1986. *Clostridium difficile* in habitats other than the human gastro-intestinal tract. J. Infect. 12, 253–263.


