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Case report

Enteritis associated with *Clostridium difficile* and opportunistic candidiasis in a foal

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Abstract

A twelve-day-old foal was admitted to the Veterinary School of UFMG with profuse diarrhea. The animal was treated with several medications, including antibiotics. Despite the clinical care, the clinical condition did not improve. Due to poor prognosis, the foal was euthanized. Gross and histopathological examinations, showed pseudomembranous and/or membranous glossitis, esophagitis and gastritis with intense amount of pseudohyphae and blastoconidia compatible with *Candida* spp Also, fibrinous and necrotizing typhlocolitis was observed. *Clostridium difficile* was isolated from intestinal contents which were also positive for toxins A/B by ELISA. Using a multiplex-PCR assay, genes encoding toxin A (tcdA) and toxin B (tcdB) were detected. The severe lesions caused by *Candida* in this foal probably occurred due to changes in the microbiota induced by the treatment with antibiotics. However, the possibility of an acquired immunodeficiency cannot be excluded because information about quantity and quality of the colostrum ingested by this foal was not obtained.

Key Words: Foal, *Clostridium difficile*, enteritis, candidiasis, gastritis.

Introduction

Diarrhea is one of the more common conditions in foals that require veterinary intervention. Approximately 80% of the foals will have at least one episode of diarrhea during the first six months of life. Many infectious agents have been reported to cause diarrhea in foals, including *Clostridium (C.) difficile* and multiple *Salmonella* spp (10). *C. difficile* is a Gram-positive anaerobic bacterium that causes colitis in several species including horses worldwide (19). In many species, including humans, antibiotics therapy appears to be a major factor leading to infections by *C. difficile*, (22) but in foals, *C. difficile*-associated diarrhea can also occur in the absence of antimicrobial therapy (2). The small intestine and colon of diseased foals may be hemorrhagic and have patchy mucosal erosions or ulcers, with exudation of fibrin and adhered ingesta. Marked necrosis of the epithelium with adherent gram-positive, rod-shaped bacteria is observed on histology. Colitis has been reported, but such cases apparently are rare (15). Fungal infections are commonly opportunistic and arise from the microbiota (4). Members of the *Candida* genus are ubiquitous fungi found on many plants and are considered part of microbiota of the alimentary tract, upper respiratory tract, and genital mucosa of mammals (11). *Candida* spp are dimorphic fungi that are often identified as small ovoid yeasts that reproduce by budding. *Candida (C.) albicans* is the most common species isolated from healthy and sick humans and animals. Others common species include *C. tropicalis, C. glabrata, C. parapsilosis, C. famata* (7) and *C. guilliermondii* (17). Infection by *Candida*, particularly *C. albicans,* has been associated with gastric and/or esophageal ulceration in humans, foals, swine, kangaroos and dolphins (12). The oral candidiasis occurs most commonly in foals, swine, and dogs. It involves the

proliferation of yeasts and hyphae in the parakeratotic superficial layers of the oral epithelium. It appears grossly as patchy pale-gray pseudomembranous material on the oral mucosa and back of the tongue (5). Birds are affected more frequently with *Candida* than mammals and the lesions occur mainly in the oral cavity, esophagus, crop and ventriculus (1). This report describes a case of opportunistic fungal (candidiasis) infection in a foal with enteritis caused by *C. difficile*.

Case report

A twelve-day-old female Mangalarga foal was presented to a local veterinarian with a four days history of diarrhea, purulent conjunctivitis and hypopyon. The animal was treated with eye drops containing chondroitin sulfate "A" and ciprofloxacin. In addition to the ocular medication, florfenicol (Zelotril®), enrofloxacin (Nuflor®), flunixin meglumine (Niglumine®), butylscopolamine bromide (Buscopan®) were administrated. Five days later, the foal was admitted to the Veterinary Hospital. Clinically, the foal was weak, with prominent loss of body condition. The animal also had mild fever (40 Celsius degree), severe dehydration, prominent intestinal hypermotility, and greenish diarrhea. At this time, as the primary suspected disease was salmonellosis, the foal was treated with intravenous fluid therapy, and identical intramuscular antibiotics, anti-inflammatory and anti-spasmodic medication used by the local veterinarian. In addition, an adsorbent (activated charcoal), vitamin B1 (cobalamin®) and probiotics were administrated to the foal. Despite the intensive care, the clinical condition did not improve. Fibrinous pseudomembranes were recovered from the diarrheic feces. Eleven days after the onset of the disease, the foal was hypothermic. Due to an unfavorable prognosis, the foal was euthanized and a necropsy performed.

At necropsy, the foal was in poor body condition. In the anterior chamber of the right ocular bulb, there was a small quantity of fibrin. On the dorsal and ventral surfaces of the tongue and on the mucosa of the esophagus, multifocal to coalescing friable creamy white plaques weakly adhered (pseudomembranous material) to the mucosa were observed. In addition, there were multifocal ulcerated areas (0.3 -1.0 cm of diameter) on the mucosa of the esophagus. The squamous stomach mucosa was diffusely thickened, with a grayish-white and friable membrane that was easily detached (Fig. 1). The mucosa of the small intestine, cecum and colon was hyperemic and edematous. Multiple small ulcerations (0.5 cm of diameter) were observed in the cecum and large colon (Fig. 2). In addition to the small ulcers, multiple fibrinous pseudomembranes were adhered to the mucosa.

Tissue samples were collected for histopathological and bacteriological examination. Contents of the large intestine, jejunum and ileum were collected for aerobic and anaerobic bacterial culture and isolation. For *C. difficile* toxins A/B detection from the intestinal contents, two assays were used: a commercial enzyme-linked immunosorbent assay kit (Ridascreen *C. difficile* toxins A/B, R-Biopharm, Darmstadt, Germany) and the detection of cytotoxic effect in Chinese-Ovary-Hamster (CHO) cells as previously described by Schleupner et al. (20).

Figure 1 – Increased thickness of nonglandular mucosa and a diffuse grayish-white pseudomembrane, which was easily detached.

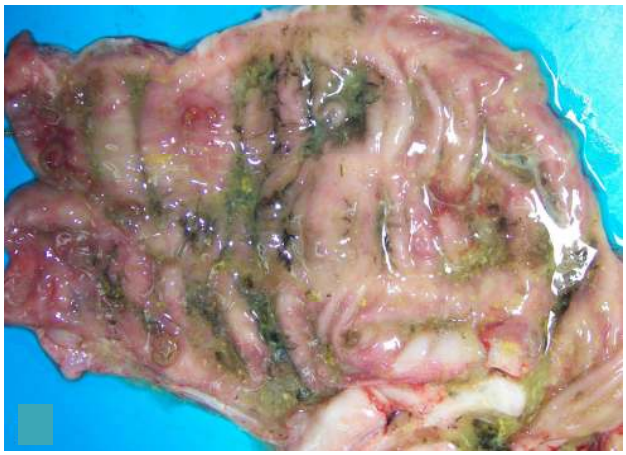

Figure 2 – Cecum, foal. A pseudomembrane of fibrin was removed and revealed the mucosa diffusely edematous with reddish areas associated with multifocal erosions.

The intestinal contents were positive for *C. difficile* toxins A/B by ELISA and a cytotoxic effect, neutralized by *C. sordellii* antiserum, was also detected in CHO cells. *C. difficile* was isolated in TCCFA agar (cycloserine-cefoxitin-fructose agar supplemented with 5% of horse blood and 0.1% of sodium taurocholate). Using a previously described multiplex-PCR assay (21), genes encoding toxin A (*tcdA*) and toxin B (*tcdB*) were detected, whereas the binary toxin gene (*cdtB*), an additional virulence factor of *C. difficile*, was not detected. The minimal inhibitory concentration (MIC) was determined by the agar dilution method, as recommended by the CLSI (8). The isolated strain was susceptible to metronidazole,

vancomicyn, oxytetracyclin and lincomycin, whereas showed an intermediate sensitivity to penicillin and resistance to erythromycin and enrofloxacin. Culture for *Salmonella* spp and *C. perfringens* were negative.

For histology, lungs, heart, tongue, esophagus, stomach, intestines, lymph nodes, spleen, liver and kidney samples were fixed in 10% neutral formalin and routinely processed. Histological sections were cut at 5 microns and stained with hematoxylin and eosin (HE). Periodic acid-Schiff (PAS) special staining was also performed on sections of the tongue, esophagus, stomach and intestines.

Histopathology examination of the tongue disclosed multifocal moderate hyperkeratosis, and numerous blastoconidia (1.5 - 5 x 1.5 to 5 um) and pseudohyphae (5-10 x 2.5-7.5 um) were adhered to the stratified epithelium and among the layers of keratin. Also, involving the glossal surface was a focally extensive area of ulceration associated with an intense infiltration of neutrophils, cellular debris, and numerous bacterial colonies. In the esophagus, there was marked multifocal to coalescing membranous and pseudomembranous esophagitis with myriads of fungal organisms morphologically similar to those previously described. The non-glandular stomach was diffusely thickened due to hyperplasia of the squamous epithelium, and parakeratosis. Neutrophilic infiltrates were present throughout the parakeratotic regions (Fig. 3). In some areas, circumferential and lamellar keratinization of the squamous epithelium could be seen. Numerous blastoconidia and pseudohyphae were observed attached to the epithelium or within the ulcerated areas. The fungal structures were also observed among layers of keratin, neutrophils and debris. In many sections detachment of the ulcerated or eroded epithelium with pseudomembranes composed of keratin, debris, neutrophils and yeasts could be seen. Blastoconidia and pseudohyphae stained positively with Periodic acid-Schiff (PAS) and were morphologically consistent with yeasts organisms of *Candida* spp (Fig. 4). Compatible lesions in the cecum and large intestine with *C. difficile* infection were characterized by moderate to marked necrosis of the superficial epithelium, mild neutrophilic infiltration and many gram-positive bacilli. These lesions were seen especially in the large colon and cecum.

Discussion

Gross and histological lesions observed in the cecum and colon of this foal were suggestive of an infection with *C. difficile.* The laboratory diagnosis of *C. difficile* infection is based on the detection of toxin A and/or toxin B (9). In some cases, the association between bacterial isolation and toxin detection may be useful. In the present report, all of the common assays were conducted: the A/B toxins were detected by a commercial ELISA and by a cytotoxicity assay and *C. difficile* was isolated. Thus, compatible gross and histopathological changes in combination with intralesional bacilli (confirmed by isolation) and toxin detection allowed the diagnosis of diffuse typhlocolitis caused by *C. difficile* in the foal. Fibrinous colitis with hemorrhage, edema and mild neutrophilic infiltration has been reported in foals (15, 16). In addition, deep ulcerative lesions, transmural venous infarction and colonic torsion were also described (16).

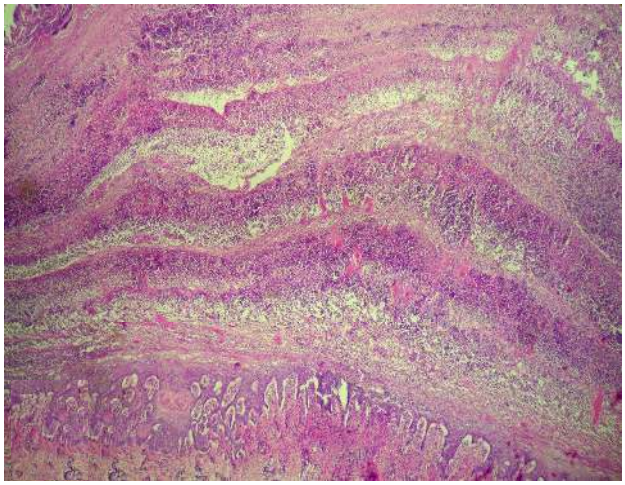

Figure 3 – Non-glandular stomach, foal. Marked parakeratotic hyperplasia of the epithelium in a lamellar pattern interposed by neutrophils and debris. An extensive focal area with circumferential and lamellar keratinization of the squamous epithelium could be seen. Hematoxylin and eosin staining. 200 x.

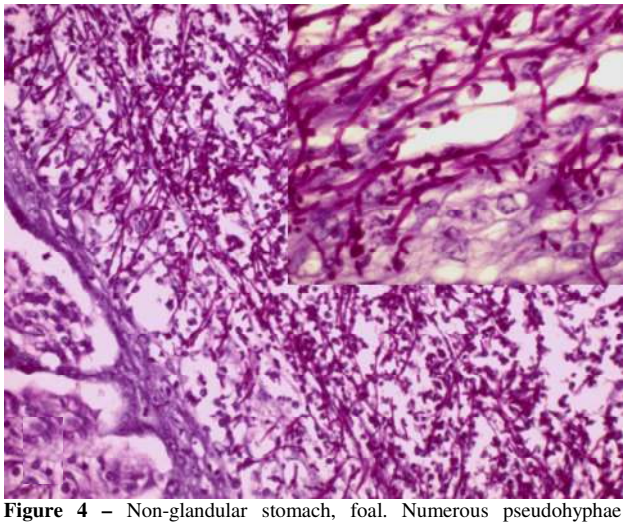

Figure 4 – Non-glandular stomach, foal. Numerous pseudohyphae stained in red are seen adhered to squamous cells. 200 x. Inset: Magnification of figure 4. Numerous blastoconidia stained in magenta. Periodic Acid-Schiff staining. 400 x.

In the present report, the *C. difficile* isolated strain was susceptible to metronidazole, vancomicyn, oxytetracyclin and lincomycin. These results corroborate previous reports in foals (2, 13). Metronidazole and vancomicyn are the first and second choice, respectively, for the treatment of *C. difficile* associated-diarrhea in foals.

Metronidazole-resistant strains of *C. difficile* are rare, but have been reported in American horses (13). The isolated strain also showed an intermediate sensitivity to penicillin and, according to Baverud et al. (2), previous use of beta-lactamic antibiotics are commonly associated with the onset of *C.difficile*-associated diarrhea in foals, mares and also in humans. *C. difficile* strains resistant to erythromycin or enrofloxacin are also common (2, 13). The resistance to erythromycin, likely occurs due to the presence of the *erm* gene, encoding for an erythromycin-resistant methylase (3). It is interesting to note that enrofloxacin was one of the antibiotics used during the treatment of the foal of this present report, what can explain at least partially the failure of the treatment.

The essential virulence factors of *C. difficile* are toxin A (*tcdA* gen) and toxin B (*tcdB* gen) which act directly on the mucosal epithelial cells. The toxins disrupt cell adhesion molecules and initiate an inflammatory cascade that can result in increasing damage to host tissues and exudation of fluid. A neurogenic effect of toxin A involving mast cell degranulation and excitation of submucosal plexuses also may contribute to diarrhea (15).

Infections caused by *Candida* spp are generally related to predisposing factors as immunodeficiency and/or therapy with antibiotics and glucocorticoids (18). The acquired form of immunodeficiency in foals can occur when there is a failure on passive transfer of colostral immunoglobulins. This cannot be excluded as a possible cause for the *Candida* spp infection in this foal since information regarding the quantity and quality of the colostrum ingested was not available. Multiple antibiotics can suppress or change the normal microbiota favoring the proliferation of *Candida* spp resident yeasts (6). Extensive therapy using antibiotic-corticosteroid combination can contribute significantly to the development of candidiasis (18). In a healthy individual, the presence of a normal intestinal microbiota keeps *Candida* species under control by competing for available glucose (5, 6). Virulence factors of *Candida* species include, among others, production of different hydrolytic enzymes and adhesins which allow the microorganism accession to the surface of the squamous epithelium (14). A few species of *Candida*, chiefly *C. albicans* can digest keratin *in vitro* in the presence of glucose. Electron microscopic studies have implicated mechanical as well as enzymatic factors on the invasion of the epithelium and traversing the stratum corneum (12).

Conclusion

Foals with bacterial infections in the respiratory tract and/or candidiasis in the digestive tract need further attention for passive or acquired immunodeficiency. The opportunistic fungal infection described can alert veterinarians and farm personnel toward management issues concerning the amount and quality of colostrum ingested and the use of multiple antibiotics. Reports regarding prevalence or occurrence of *C. difficile*-associated diarrhea in foals in Brazil were not found.

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