Ciência Rural, Santa Maria, v.42, n.3, p.498-500, mar, 2012
ISSN 0103-8478

First confirmed case of *Clostridium difficile*-associated diarrhea in foals in Brazil

Primeiro relato de diarreia associada à *Clostridium difficile* em potros no Brasil

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- NOTE -

ABSTRACT

*Despite of the substantial role of **Clostridium difficile** in causing diarrhea and colitis in foals, there have been no confirmed diagnoses of disease caused by this bacteria in Brazil. In this paper, we describe confirmed cases of colitis caused by **C. difficile** in two foals in Brazil. Two five-month-old foals with a five-day history of diarrhea after antibiotic treatment for a respiratory disease were treated at the Veterinary Hospital of the Universidade Federal de Minas Gerais. **C. difficile** A/B toxins were detected, and toxigenic strains of **C. difficile** were isolated from the foals' feces. The treatment was based on fluid therapy and antibiotics (metronidazole and ceftiofur), and the animals experienced a gradual recovery. The association between the medical history, clinical signs, laboratory exam results and therapeutic success confirmed the diagnosis of **C. difficile**-associated diarrhea. The present report raises the possibility that **C. difficile** is also a pathogen in equines in Brazil and highlights the need for up to date routine laboratory protocols for the diagnosis of this disease.*

Key words: *colitis, equine, nosocomial diarrhea.*

RESUMO

*Apesar da importância de **Clostridium difficile** como agente causador de diarreia e colite em potros, inexistem relatos confirmados de tal doença no Brasil. O objetivo deste trabalho foi descrever dois casos confirmados de diarreia causados por **C. difficile** em potros, ocorridos em Minas Gerais, Brasil. Os animais, com cinco meses de idade, foram encaminhados ao Hospital Veterinário da Universidade Federal de Minas Gerais (UFMG) com histórico de cinco dias de diarreia após antibioticoterapia com penicilina para uma possível pneumonia. Ambos os animais foram positivos para detecção das toxinas A/B de **C. difficile** e isolados toxigênicas de **C. difficile** foram isoladas de amostras de fezes. Os animais apresentaram melhora gradual com o tratamento baseado em metronidazol e fluidoterapia e receberam alta após sete dias. A associação do quadro clínico, exames laboratoriais e o sucesso terapêutico permitem confirmar o diagnóstico de colite por **C. difficile**. O presente trabalho chama a atenção para a possibilidade de diarreia causada por **C. difficile** em equinos no Brasil e reforça a necessidade do diagnóstico para tal infecção na rotina laboratorial.*

Palavras-chave: *colite, equinos, diarreia nosocomial.*

Clostridium difficile is a spore-forming, anaerobic, Gram-positive bacillus which has been recognized as an important bacterial pathogen in both humans and animals. It may be responsible for 95% of all pseudomembranous colitis cases and most cases of antibiotic-associated diarrhea in humans (SCHWAN et al., 2009). In adult horses, this pathogen can cause colitis often associated with antibiotic use but can also be nosocomial (SONGER et al., 2009). In foals, ***C. difficile*** causes diarrhea and colitis (BAVERUD et al., 2004). Despite the importance of ***C. difficile*** as a foal pathogen, there have been no confirmed diagnoses of diseases caused by this bacteria in Brazil, where to date all cases have been non confirmed cases. Therefore, the aim of this paper is to describe the first confirmed cases of colitis caused by ***Clostridium difficile*** in two foals in Brazil.

Two five-month-old Mangalarga Marchador foals with a five-day history of diarrhea

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Received 09.13.11 Approved 12.07.11 Returned by the author 02.01.12
CR-6014

were examined and treated at the Veterinary Hospital of the Universidade Federal de Minas Gerais (UFMG). The owner reported that three animals that shared the same stall had diarrhea on the fifth day of treatment with penicillin (Novapen®) for a suspected pneumonia. With the onset of diarrhea, the treatment with penicillin was discontinued, and the foals began to receive Nuflor® (florfenicol). Four days after the onset of diarrhea, one of the animals died. The other two animals were then sent to the Veterinary Hospital of the Federal University of Minas Gerais.

On the day of admission to the clinic, both foals were dehydrated, and green watery diarrhea was observed. There was no hyperthermia. The two primary suspected diseases were salmonellosis and ***Clostridium difficile***-associated diarrhea. Blood from both animals were collected for a complete blood count (CBC) and for serum biochemistry evaluation. Stool samples were collected for parasitological detection, routine bacteriologic culture, ***Lawsonia intracellularis*** detection by PCR (JONES et al., 1993), isolation of ***C. difficile*** (SILVA et al., 2011) and A/B toxin detection using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Ridascreen ***Clostridium difficile*** toxins A/B, R-Biopharm, Germany) and using African green monkey kidney cells (Vero–ATCC CCL 81) to analyze the cytotoxic effect. ***C. sordellii*** antitoxin (NIBSC - National Institute for Biological Standards and Control, England) was used to neutralize any A/B toxin present in the cytotoxicity assay. Initially, the treatment consisted in fluid therapy with ringer lactate, saline and glucose (3:2:1 - 130mL kg^{-1} day^{-1} with a total of 1.5g kg^{-1} day^{-1} of glucose), omeprazole (4mg kg^{-1} SID) and antibiotics (metronidazole: 20mg kg^{-1} BID, IV, and ceftiofur: 4.4mg kg^{-1} SID, IM).

The CBC of both animals indicated an initial anemia, with a packed cell volume of 24 and 20% and 9.2 and 8.8g DL^{-1} of hemoglobin, respectively. Both foals had also a decrease of total protein (4.7 and 4.0g DL^{-1}) and albumin (1.5 and 1.3g DL^{-1}). All those results are commonly found in cases of ***C. difficile***-associated diarrhea (BAVERUD, 2004). The parasitological exam was negative, and only few colonies of ***Escherichia coli*** were obtained in the routine bacteriologic culture of the stool samples. Ruling out the initial suspicion, ***Salmonella*** was no isolated. ***Lawsonia intracellularis*** was not detected by PCR. The two stool samples were positive for A/B toxins by EIAs and by the cytotoxicity assay using Vero cells. In addition, ***C. difficile*** was isolated from both foals, and the two strains were positive by PCR for the toxin A (*tdcA*) and B (*tcdB*) genes but negative for the binary toxin gene (*cdtB*). In an *in vitro* toxin production test performed according to the method of BRAZIER (1993), the isolated strains had a cytotoxic effect on Vero cells, which was neutralized by ***C. sordellii*** antitoxin (NIBSC, England), confirming that both strains were toxigenic.

On the second day after admission, the foals were hydrated and more active and experienced fewer episodes of diarrhea but still produced liquid feces. The consistency of the fecal material gradually changed from liquid to pasty between the third and fourth days of hospitalization, returning to solid with the characteristic odor on the fifth day. The animals were discharged from the Hospital seven days after admission.

The laboratory diagnosis of ***C. difficile*** infection is based on the detection of toxin A and/or toxin B by cell culture, the "gold-standard", or by ELISAs (DELMEÉ, 2001). In some cases, the association between isolation and *in vitro* toxin production may be useful. In the present case report, all of these assays were conducted: the A/B toxins were detected by a commercial EIA and by a cytotoxicity assay using Vero cells, ***C. difficile*** was isolated from both animals, and these strains were toxigenic in an *in vitro* test.

According to BAVERUD et al. (2003), ***C. difficile***-associated diarrhea in foals can occur in the absence of antimicrobial therapy, but treatment with some antibiotics also can lead to the excretion of ***C. difficile***. Under these circumstances, antibiotics would lead to a depletion of the indigenous microbiota, allowing colonization and toxin production by ***C. difficile*** (BAVERUD et al., 2002). The foals described in the present developed diarrhea after a five-day course of penicillin suggesting that this antibiotic treatment was associated with the pathogenesis of the ***C. difficile*** infection. A similar observation was described by BAVERUD (2004), which suggested that penicillin predisposes establishment of ***C. difficile*** in the equine intestine. It also important to note that in adult horses, ***C. difficile*** is the major cause of antibiotic-associated colitis and may even be a nosocomial agent, as reported for humans (SONGER et al., 2009). These findings emphasize the need to include ***C. difficile*** in the differential diagnosis of diarrhea in foals and of colitis after antibiotic therapy in horses, especially in cases in which the disease developed after admission to a veterinary hospital.

At the moment, there no commercial products available for immunoprophylaxis against ***C. difficile*** infections, but this is subject of ongoing research. For treatment, the first step is the withholding antibiotics followed by fluid therapy to maintain hydratation and acid base balance. In the case of severe disease, metronidazole is the preferred drug (BAVERUD, 2004). Antimicrobial susceptibility testing of ***C. difficile*** isolates from horses has not been performed routinely,

but previous studies shown that most isolates were susceptible to metronidazol. However, metronidazole-resistant isolates have been found in American horses (JANG et al., 1997) and in those cases, vancomycin is recommended (BAVERUD et al., 2004).

It is also interesting to note that predominant ribotypes in horses are also well-known in humans with colitis (AVBERSEK et al., 2009). In light of this, some recently reports raised the possibility of ***C. difficile*** as zoonotic agent (ARROYO et al., 2007). More studies are needed for confirmation as, until this moment, no evidence for transmission between humans and animals, including horses, was found (McNAMARA et al., 2011).

Although *antemortem* diagnosis is difficult due to the necessity to rule out a large number of other potential causes of diarrhea that may be discounted in *postmortem* examinations, the association of the clinical findings, laboratory results and result of treatment confirmed the diagnosis of ***C. difficile***-associated diarrhea. This report reveals the possibility of an underestimated incidence of diarrhea caused by this agent in foals in Brazil and suggests that the detection of the A/B toxins of ***C. difficile*** should be considered an important exam in the routine analysis of diarrhea cases.

ACKNOWLEDGMENTS

This work was supported by funds from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The authors clarify that the present study is a clinical case report, with no need of an ethical approval by an Animal Experiments Committee.

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