Isolation of *Campylobacter Fetus* Subspecies *Fetus* in a Two-Year-Old Quarterhorse with Chronic Diarrhea of an Undetermined Etiology
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Isolation of *Campylobacter fetus* subspecies *fetus* in a two-year-old Quarterhorse with chronic diarrhea of an undetermined etiology

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**Abstract.** A 2-year-old Quarterhorse was evaluated for chronic diarrhea and weight loss of 5 weeks duration after numerous diagnostic tests failed to identify an underlying cause. Historically, the horse was housed at pasture where human household waste vehicles were routinely cleaned and the effluent could run onto the field. Physical examination revealed poor body condition and frequent high-volume diarrhea. Diagnostic testing for *Salmonella* spp., endoparasites, *Cryptosporidium* spp., *Clostridium* spp., and diffuse infiltrative bowel disease were negative. Rectal tissue histopathology failed to identify *Mycobacterium* spp., spirochetal organisms, or submucosal infiltration with cells. Rectal tissue biopsy and a fresh fecal sample identified numerous *Campylobacter* organisms with microaerobic culture. Molecular testing revealed the species as *Campylobacter fetus* subsp. *fetus* as a possible organism associated with large bowel disease in this filly. The organism was sensitive to fluoroquinolone antimicrobials in vitro. The filly responded transiently to therapy, forming discrete fecal balls after 72 hr of treatment. At 5 months follow-up, the horse had gained weight, was alert and responsive, but reverted back to having soft “cow-pie” feces. Reculture of the feces at 9 months failed to identify any *Campylobacter* organisms. To the authors’ knowledge, this is the first report to identify *C. fetus* subsp. *fetus* from feces and tissue of a horse with the use of molecular methods. This organism could play a role in the etiology of chronic diarrhea in horses.

**Key words:** *Campylobacter;* diarrhea; horses.
A 2-year-old Quarterhorse filly was presented to The Ohio State University Veterinary Teaching Hospital (OSU; Columbus, OH) for evaluation of chronic diarrhea and weight loss of 5 weeks duration. Historically, the filly was 1 of 2 horses kept in a pasture that experienced wetary diarrhea; however, the other gelding was not losing weight and had not had diarrhea as long as the filly. The 2 horses had resided at the same property for at least the previous 1.5 years. Both horses were housed in a pasture where the run-off from the cleaning of human household waste disposal vehicles could potentially contaminate the field with general waste effluent, and no known pesticides or toxins had been used on the property. These animals are the only 2 on the property and no other animals (horses or livestock) have been on the pasture during the previous 5 years (duration of the owner’s occupancy).

The filly had been assessed before presentation by another specialty veterinary practice for chronic diarrhea and excessive water consumption that had developed 5 weeks before presentation. Extensive testing, including complete blood count (CBC), serum biochemistry, fecal flotation for endoparasites, fecal examination for Cryptosporidium spp., fecal culture and polymerase chain reaction (PCR) for Salmonella spp., and anaerobic fecal culture and enzyme-linked immunosorbent assay (ELISA) toxin testing for Clostridium spp., all failed to show any abnormalities or yield a diagnosis. Initial therapy by the referring veterinarian consisted of metronidazole, fenbendazole, flunixin meglumine, and Bio-Sponge® (ditrioctahedral smectite) to yield a diagnosis. Initial therapy by the referring veterinarian consisted of metronidazole, fenbendazole, flunixin meglumine, and Bio-Sponge® (ditrioctahedral smectite) to which no clinical improvement was seen.

Clinical examination parameters were unremarkable, except for subjective hypermotility of the gastrointestinal viscera on auscultation. Fecal staining had accumulated on the perineum and hind legs, resulting in dermal scalding. During a 45-min examination, the filly passed 6 watery, loose “cow-pie” feces. The horse’s appetite was normal but she was consuming an excessive volume of oral fluid, 40–60 l/day (120–150 ml/kg per day; normal: 40–60 ml/kg per day). In general, the filly appeared to have compensated for her fluid losses. Transabdominal ultrasonography and rectal palpation examination revealed a fluid-filled large colon and cecum with no other significant abnormalities. Areas of large colon wall measured up to 8 mm (normal: <5 mm) in thickness. No other abnormalities were visualized.

Abnormal laboratory findings on CBC included a decreased mean corpuscular volume of 39 fl (normal: 43–55 fl), increased red cell distribution width of 26.1% (normal: 19–25.4%), and mild segmented neutrophilia at 6.8 × 10⁹ neutrophils/l (normal: 2.4–6.4 × 10⁹ neutrophils/l). Plasma fibrinogen concentration was normal at 382 mg/dl (normal: 193–422 mg/dl). Biochemical abnormalities included decreased blood urea nitrogen of 11 mg/dl (normal: 13–27 mg/dl), increased alkaline phosphatase at 230 IU/l (normal: 80–187 IU/l), increased sorbitol dehydrogenase at 14 IU/l (normal: 4–13 IU/l), increased creatinine kinase at 425 IU/l (normal: 150–360 IU/l), decreased total protein of 6.2 g/dl (normal: 6.4–7.9 g/dl), and decreased globulin of 3.0 g/dl (normal: 3.6–4.3 g/dl). These findings were interpreted as indicative of a very mild hepatopathy and skeletal muscle trauma. Her urine-specific gravity was 1.015 and considered to be minimally concentrated, likely because of polydipsia.

Peritoneal fluid evaluation was normal with total protein less than 2.5 g/dl and a total nucleated cell count of 3,870 cells/µl (77% nondegenerate neutrophils and 23% large mononuclear cells). Although no physical examination or clinicopathologic findings were suggestive of small intestinal disease, an oral glucose absorption test was performed and showed no significant small intestinal malassimilation that might account for the weight loss.

Results of 5 fecal cultures for Salmonella spp. collected at 24-hr intervals were negative. A parasite fecal egg flotation also showed no endoparasite eggs. Several rectal mucosal biopsies were obtained for examination, including Salmonella culture, histopathology, and impression smear cytology. No Salmonella spp. were cultured from tissue specimens in enrichment media (selenite broth). Lawsoniasis was not considered likely in this filly because her albumin concentration was normal and she had no evidence of small intestinal thickening or significant protein-losing enteropathy. As such, specific diagnostic testing for Lawsonia intracellularis was not performed.

Histopathology revealed mucosal and submucosal changes consistent with mild to moderate, diffuse lymphohistiocytic and neutrophilic proctitis. Additional sections were stained with Ziehl–Nielsen, periodic acid–Schiff, and Warthin–Starry stains for acid-fast Mycobacterium spp., spirochetal organisms (e.g., Brachyspira pilosicoli), or intracellular bacteria (including Lawsonia spp.) in tissue, which were not evident. An impression smear from a fresh rectal mucosal sample rolled onto a glass slide and Gram stained showed the presence of numerous Gram-negative, “gull-shaped” organisms suggestive of a Campylobacter species. A rectal mucosa biopsy and fresh fecal sample were submitted for Campylobacter culture under microaerophilic conditions and yielded heavy growth of the organism. Samples were placed onto cefoperazone vancomycin agar (BD-CVA) and incubated in a microaerophilic culture system for 24 hr at 25°C and 42°C. Positive culture was observed in the samples incubated at 25°C and not 42°C. The isolate was sent in glycercol–brain heart infusion media to Massachusetts Institute of Technology (MIT) for speciation by molecular methods.

For PCR of genomic DNA, the isolate was grown on blood agar plates under microaerophilic conditions, harvested, and washed once with phosphate buffered solution, and a high pure PCR template preparation kit was used for DNA extraction according to the manufacturer’s specifications. Campylobacter genus-specific primers that amplify a 280–base pair product on the 16S ribosomal RNA (rRNA) gene were used as previously described. Amplification of the 16S rRNA cistrons, gene sequencing of 16S rRNA, and analysis of the 16S rRNA data were performed as described previously. For alignment, the 16S rRNA gene sequences were entered into a database that contains more than 600 sequences for Helicobacter, Wolinella, Arcobacter, and Campylobacter strains and more than 2,000 sequences for other bacteria. The entire 16S rRNA gene was sequenced, and all bases (>1,600 bases)
were aligned as previously reported. The Campylobacter species isolated from this filly was 100% identical by 16S rRNA analysis to Campylobacter fetus subsp. fetus.

Crude susceptibilities to antimicrobials were achieved by plating the organism on sheep blood agar, incubating at 25°C for 24 hr with Kirby–Bauer antimicrobial impregnated disks. The organism was found to be minimally sensitive to metronidazole and moderately sensitive to enrofloxacin on the basis of zones of growth inhibition.

Treatment with enrofloxacin (7.5 mg/kg intravenously every 24 hr; enrofloxacin is not labeled for use in horses or for the treatment of diarrheal disease in any species) was started for 14 days, and the horse was given 1 subcutaneous injection of vitamin B12 (2 cc). Over the following 3 days, the horse was hospitalized and monitored. Within 48 hr, the volume and frequency of diarrhea had decreased. Discrete fecal balls were observed on occasion from the third day of treatment, and the filly’s daily voluntary water intake reduced to 60–80 ml/kg.

Initial follow-up consultation with the owner 2 weeks later was promising, as the filly was passing fecal balls with some soft, “cow-pie” feces at low frequency. The owner also thought the horse’s weight had stabilized and subjectively increased. After the cessation of enrofloxacin therapy, the filly reverted back to having soft, watery diarrhea and drinking excessively. In addition, the gelding was beginning to show moderate weight loss. Both horses were then started on oral enrofloxacin at the previously described dosage for 60 days. In addition, the filly was also given compounded iodochlorhydroxyquinoline (10 g total dose, orally every 24 hr for 60 days). The horse showed only temporary improvement for approximately 45 days. At this time, reculturing and possible surgical exploration were offered but declined by the owner. Follow-up 5 months after hospitalization via telephone revealed that both horses had gained weight, but continued to have increased voluntary water intake and semisolid feces. Follow-up fecal culture at 9 months failed to identify any Campylobacter spp.

Campylobacter fetus subsp. fetus is a Gram-negative, slender, spirally curved bacterial pathogen. In humans, C. fetus subsp. fetus causes both gastrointestinal and systemic infections, and infection relapse is common despite antibiotic treatment. Campylobacter fetus subsp. fetus has been associated with bacteremia, enteritis, abortion, cellulitis, endocarditis, graft infection, and meningitis of humans.

Campylobacter spp. have been isolated from the gastrointestinal tract previously in horses, and C. fetus subsp. venerealis is closely related organisms but induce different clinical disease; the latter being associated with venereal and abortigenic disease in both humans and animals, but it is rarely a cause of enteric disease. Molecular techniques can be used to accurately speciate them.

In the limited number of reports, all affected horses have been less than 1 year of age. To the authors’ knowledge, this is the first report of C. fetus subsp. fetus isolated as a possible causative pathogen for large bowel disease in a horse greater than 1 year of age and where molecular techniques were employed for accurate speciation. This was an unexpected finding. Campylobacter fetus subsp. fetus has been isolated from blood culture of a 10-month-old Standardbred with granulomatous enteritis restricted to the small intestine, as well as the gastrointestinal tract of a 7-month gestational age aborted Thoroughbred fetus.

In a 1980 report, Campylobacter jejuni was reported as a causative agent of hemorrhagic enteritis in young foals less than 6 months of age. Campylobacter coli from a foal with acute onset diarrhea, pyrexia, and colic was also isolated. In one study evaluating the presence of Campylobacter spp. in neonatal foals with diarrhea, the authors showed that both C. jejuni and C. fetus subsp. fetus could be isolated in the feces or tissue specimens; however, this was with rare frequency. In another study of healthy and diarrheic adult horses, no Campylobacter species could be identified. Campylobacter appears to be a bacterium predominantly found in young horses. Similar findings are observed in cats, wherein cats less than 1 year of age have a higher Campylobacter isolation rate (30%) than cats older than 1 year (3%).

Pathogenetic mechanisms for treatment evasion and infection relapse have been investigated extensively in humans. High-frequency phenotypic switching of surface layer proteins has been demonstrated to result in antigenic variation. Surface layer proteins are critical in virulence and are encoded by sap gene sequences. High-frequency genotypic switching within the sap island leads to antigenic variation. The mechanism by which C. fetus subsp. fetus causes diarrhea has been linked to liberation of cytolethal distending toxin (CLDT). The proposed actions of this toxin are reduction of cell growth and cell cycle arrest, resulting in the enterocytes becoming large, rounded, or polymorphic elongated cells. Cytolethal distending toxin has been found in high concentrations in feces of diarrheic humans and calves. In one study, all C. fetus isolates exhibited high activity of CLDT gene expression. Cytolethal distending toxin is thought to be an important virulence factor for this organism. Detection of CLDT is not commercially available and was not attempted from the isolate of this horse. Although this study does not prove that C. fetus subsp. fetus was the causative agent for the diarrhea in the filly herein, isolation of the organism and clinical response to targeted therapy, albeit short lived, suggest that this organism might have played a role in the enteric disease in this filly. The authors postulate that C. fetus subsp. fetus had a pathogenic role in the current case, in that other causes of chronic diarrhea were not identified. Another possible explanation for the transient success of treatment could have been that another unidentified organism was being treated, such as Salmonella spp., which are commonly susceptible to enrofloxacin therapy; however, repeated fecal and tissue cultures, as well as fecal PCR before the administration of antibiotics, failed to identify any Salmonella species. Another possibility was that the horse was merely shedding C. fetus subsp. fetus and that the bowel was not infected. The lack of identifying Campylobacter organisms on rectal mucosal histopathology might support this; however, large-colon biopsies would have been essential to support this.
Many cases of *C. fetus* subsp. *fetus* infection occur in immunocompromised humans, who often develop bacteremia. The contribution of immunocompromise to disease caused by this organism in horses has not been investigated, and tests of immune competency have not been performed on horses in the limited number of reports in the veterinary literature. Bacteremia with *C. fetus* subsp. *fetus* has been described in a young colt with granulomatous enteritis; however, the immune status of the animal was not reported, although the animal described was febrile (39.9°C) with an elevated fibrinogen concentration (900 mg/dl), suggesting significant inflammation and infection. Immunologic tests, aside from a CBC, blood fibrinogen concentration, and serum globulin concentration, were not performed in the filly in this report. As such, a possible transient, immunocompromised state could have contributed to the pathogenicity of *C. fetus* subsp. *fetus* in this filly.

The authors postulate that ingestion of the organism from contaminated pastures caused clinical disease because the source could have been the human household waste draining into the field. Although not substantiated by culture of environmental samples and identification of *C. fetus* subsp. *fetus*, it is reasonable to assume that with the cessation of effective antimicrobials, reinfection is plausible if the exposure potential existed. Environmental contamination has been reported as a source of infection in other animal species, but not specifically from human household waste. Ruminant effluent has been proposed as a major source of infection for sheep with *Campylobacter*-associated ovine abortion, in which *C. fetus* subsp. *fetus* is considered part of the normal biota of the ruminant gut and actively shed in the feces. The filly reported in this study had no history of exposure to ruminant feces. Other suitable accommodation for both horses was recommended; however, this did not occur. Finally, treatment failure could also have been related to the presence of other infectious agents or toxins that cause chronic diarrhea and were not identified.

*Campylobacter* spp. have fastidious growth requirements and are susceptible to adverse conditions during culture. Successful identification requires enrichment media (brain heart infusion), microaerophilic conditions, and fresh fecal or rectal tissue specimens, as well as molecular techniques like those described in this report. The identification of *C. fetus* subsp. *fetus* in this horse indicates that *Campylobacter* culture and species identification should be included on the list of possible diagnostic tests for potentially pathogenic bacteria that might be implicated in the development of chronic diarrhea in adult horses.

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Sources and manufacturers
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c. BD GasPak™ EZ Campy Container System, Becton Dickinson, Franklin Lakes, NJ.
d. DNA Isolation Kit for cells and tissue, Roche Diagnostics Corp, Indianapolis, IN.
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