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Case Report Rapport de cas

Isolation of *Campylobacter fetus* subspecies *fetus* from an abdominal abscess in an adult mare

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Abstract — A 12-year-old Warmblood mare was referred for evaluation of behavioral changes not explained by general physical examination or lameness evaluation. Transrectal ultrasound examination was performed to determine if the behavioral changes were related to ovarian abnormalities, and a large abscess was found near the base of the cecum. Laparoscopic-guided aspiration and lavage of the abscess cavity followed by injection of benzyl penicillin G was carried out. Culture of the lavage sample yielded *Campylobacter fetus* subspecies *fetus,* an organism not previously reported as an etiological agent in abdominal abscesses in horses. The mare was treated after surgery with an extended course of antibiotics (minocycline *per os* q12h for 10 days followed by enrofloxacin *per os* q24h for 42 days). The mare resumed work in competitive eventing 10 months after surgery, and the behavioral complaints had resolved according to the owner.

Key clinical message:
We describe the diagnosis and treatment of a mare with an abdominal abscess from which *Campylobacter fetus* subspecies *fetus* was cultured. This organism has not previously been reported as an etiological agent in abdominal abscesses in horses.

Résumé — Isolement de *Campylobacter fetus* sous-espèce *fetus* d'un abcès abdominal chez une jument adulte.
Une jument Warmblood âgée de 12 ans fut référée pour évaluation de changements de comportement non-expliqués par un examen physique général ou une évaluation de boiterie. Un examen échographique transrectal fut effectué afin de déterminer si les changements de comportement étaient reliées à des anomales ovariennes et un large abcès fut trouvé près de la base du caecum. Une aspiration guidée par laparoscopie et un lavage de la cavité de l'abcès suivis d'une injection de benzyle pénicilline G furent effectués. Une culture de l'échantillon de lavage permis d'identifier *Campylobacter fetus* sous-espèce *fetus*, un organisme n'ayant pas été rapporté antérieurement comme agent étiologique dans les abcès abdominaux chez les chevaux. La jument fut traitée après la chirurgie avec un régime prolongé d'antibiotiques (minocycline *per os* q12h pour 10 jours suivi d'enrofloxacine *per os* q24h pour 42 jours). La jument recommença à travailler dans des compétitions 10 mois après la chirurgie et les plaintes concernant le comportement étaient résolues selon le propriétaire.

Message clinique clé :
Nous décrivons le diagnostic et le traitement d'une jument avec un abcès abdominal à partir duquel on isola *C. fetus* sous-espèce *fetus*. Cet organisme n'a pas été rapporté antérieurement comme agent étiologique dans les abcès abdominaux chez les chevaux.

(Traduit par Dr Serge Messier)

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Abdominal abscesses are uncommonly diagnosed in mature horses and can be categorized as primary or secondary (1). The most common bacteria implicated in the formation of primary abdominal abscesses in adult horses are *Streptococcus equi* subsp. *equi* (2,3) *Streptococcus equi* subsp. *zooepidemicus* (4), and *Corynebacterium pseudotuberculosis* (5), although numerous other bacterial species have been reported (6,7).

Abdominal abscesses can be treated conservatively with long duration antibiotic therapy (6,8), although surgical intervention may be required (9). Surgical treatment options include excision, drainage and lavage, or marsupialization *via* a ventral midline laparotomy or standing flank laparotomy (10–12). The prognosis for horses diagnosed with abdominal abscesses is variable, with reported survival rates ranging from 24.6% to 71% (5,7,8).

To the authors' knowledge, this is the first report of isolation of *Campylobacter fetus* subsp. *fetus* from an abdominal abscess in a horse. *Campylobacter fetus* subsp. *fetus* should be considered among potential etiological agents associated with abdominal abscesses in horses.

Case description

A 12-year-old Warmblood mare was referred to the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California–Davis for evaluation of abnormal behavior including kicking out under saddle, reluctance to take the left canter lead, and unusual sensitivity when touched in the region of either flank. The mare was nulliparous with no history of abdominal surgery or penetrating wounds to the abdomen. The referring veterinarian's dental examination had not revealed any significant abnormalities. Physical examination parameters were unremarkable. Lameness and neurological examinations were performed; the mare displayed a mild right hind limb, and mild left forelimb lameness and no neurological deficits. The lameness identified was not considered significant enough to be the cause of the presenting complaints. Examination of the reproductive tract was subsequently performed in order to investigate the possibility of a urogenital condition such as an ovarian tumor, or less likely, nephro- or uretero-lithiasis resulting in thoracolumbar pain or discomfort.

Abdominal palpation per rectum followed by transrectal and transcutaneous abdominal ultrasound was performed. The left ovary was within normal limits in size and ultrasonographic appearance. The right ovary could not be palpated or visualized by transrectal ultrasound due to the presence of a large mass firmly adhered to the right dorsolateral body wall. The right kidney showed a normal ultrasonographic appearance; however, the renal pelvis and proximal ureter were intermittently moderately distended compared to the left kidney yet appeared actively motile. No evidence of intraluminal ureteral obstruction was found on transrectal ultrasound, and distention was felt to be secondary to partial functional obstruction by the caudally located mass. The mass could not be detected by transcutaneous ultrasound of the entire right caudal abdominal region. The mass was visualized *via* transrectal ultrasound, located cranial to the bladder, caudal to the right kidney and near the aorta and right ureter. It appeared well-encapsulated with hyperechoic intracapsular mineralizations (Figure 1A) and contained a large quantity of homogeneously echogenic fluid. The abscess measured 14 cm in diameter (Figure 1B) and capsule thickness measurements ranged from 1.1 cm to 1.6 cm. One enlarged periaortic lymph node was visible on the right side.

The following serological tests were submitted because *S. equi* subspecies *equi* and *C. pseudotuberculosis* are commonly isolated from internal abscesses in our geographic region (5,8). Serological testing for *S. equi* subspecies *equi* antibodies using a SeM-specific enzyme-linked immunosorbent assay (ELISA) showed a moderately positive titer (1:800). A *C. pseudotuberculosis* synergistic hemolysis inhibition (SHI) test produced a low positive antibody titer (1:16). A complete blood (cell) count (CBC) showed mild leukopenia [4.9×10^9 cells/L; reference range (RR): 5.3 to 14×10^9 cells/L] and marked lymphopenia (0.4×10^9 cells/L; RR: 1.6 to 5.8×10^9 cells/L). Fibrinogen was within normal limits at 2 g/L (RR: 1 to 4 g/L).

Standing flank laparoscopy was conducted to better evaluate the mass and determine whether surgical treatment would be possible. The mare was sedated with an IV constant rate infusion (CRI) of detomidine HCl (Zoetis, Parsippany, New Jersey, USA), 0.01 mg/kg body weight (BW) per hour. Broad-spectrum antimicrobials (gentamicin sulfate; VetOne, Boise, Idaho, USA), 6.6 mg/kg BW, IV, procaine penicillin G (Vet One), 22 000 IU/kg BW, IM, were administered 30 min before surgery because of the potential for contamination of the peritoneal cavity during procedures involving an abdominal abscess caused by an as yet unidentified microorganism. Flunixin meglumine (Merck Animal Health, Madison, New Jersey, USA), 1.1 mg/kg BW, IV, was also administered before surgery for pain relief.

The left and right flanks were clipped, aseptically prepared, and draped. Laparoscope and instrument portal sites were desensitized with 2% lidocaine HCl (Vet One). A laparoscopic portal was made in the left paralumbar fossa, dorsal to the internal abdominal oblique muscle and immediately caudal to the 18th rib. A threaded laparoscopic cannula (Endo TIP; Karl Storz America, Goleta, California, USA) was progressively advanced through the abdominal muscles and peritoneum and into the abdominal cavity. Visualization through a 0° angle-view 57-cm rigid laparoscope, partially inserted in the cannula during the process, aided in safe placement of the cannula within the abdominal cavity. Once there was access to the peritoneal cavity, abdominal exploration was performed as previously described (13). The pelvic flexure was observed to be displaced caudally and dorsally on the left side of the abdomen, potentially due to a mass effect exerted by the right dorsolateral body wall mass. No other abnormalities were detected.

A similar laparoscopic approach was made on the right side of the abdomen using the same anatomical landmarks. A large, round mass was noted at the level of the base of the cecum, caudal to the right kidney. The mass was adhered to the dorsolateral body wall by a large, thick base and did not appear to originate from the right ovary or the right uterine horn, although it had formed fibrous adhesions to the mesometrium and mesosalpinx. Adhesions between the mass and the mesoduodenum were also present.

Babcock laparoscopic grasping forceps (Aesculap, Center Valley, Pennsylvania, USA) were inserted into the abdomen

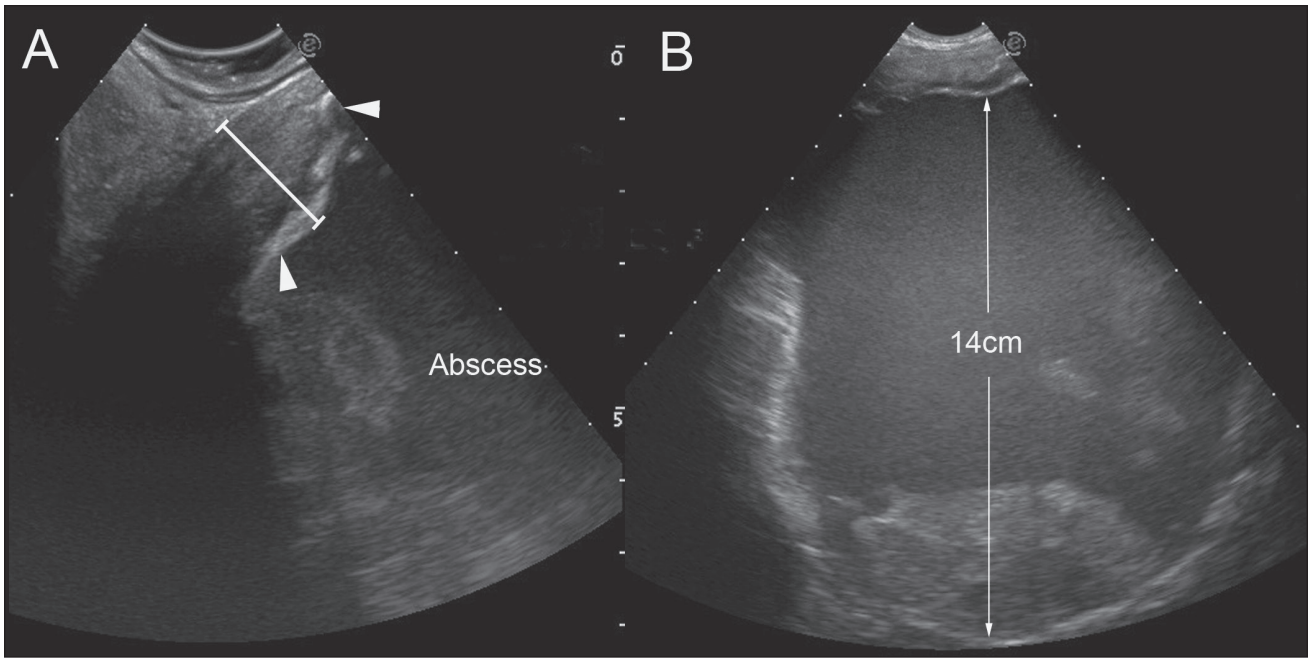

Figure 1. Transrectal ultrasonographic images of an abdominal abscess in a mare. A – The abscess was located cranial to the bladder and showed a thick capsule (brackets) containing hyperechoic mineral densities (arrowheads) within and on the capsular surface. B – The abscess contained homogeneously echogenic fluid and had a diameter of 14 cm. Hyperechoic capsular mineralization (arrowhead) partially obscured visibility of the abscess. Images were obtained with a 4–8 MHz microconvex transducer set at its maximum depth of 14 cm to visualize the entire abscess (B).

through a second portal created caudal to the laparoscopic portal on the right side of the abdomen. The forceps were used to deflect the right ovary axially, for better access to the mass. A 36-mm aspirating needle (Becton Dickinson, Franklin Lakes, New Jersey, USA) was inserted into the abdomen dorsal to the laparoscopic portal. The capsule surrounding the mass was punctured (Figure 2) and approximately 7 mL of thick yellow fluid was aspirated. Aspiration quickly became unproductive in that site, and therefore the mass was punctured in a more ventral location where a larger volume of fluid could be aspirated. Injection of approximately 60 mL of sterile saline followed by egress of purulent fluid and debris from the mass was repeated until the fluid aspirated became clear, instead of thick and yellow. The mass was then injected with 10 000 IU benzyl penicillin (Sandoz, Princeton, New Jersey, USA). All instruments were removed from the abdomen and laparoscopic portals were closed routinely.

Based upon a moderately positive *S. equi* subspecies *equi* serological titer, and previous isolation of *A. equuli* from cases within our geographic area, polymerase chain reaction (PCR) and culture were performed (7,14). A sample of fluid aspirated from the abscess was submitted for aerobic and anaerobic culture. A qPCR for *S. equi* subspecies *equi* and *A. equuli* was conducted on the fluid aspirated from the abscess while culture results were pending, as it has a significantly shorter turnaround time. The qPCR was negative for both *S. equi* subspecies *equi* and *A. equuli*.

Aerobic culture was performed by inoculation on to 5% defibrinated sheep blood agar and MacConkey agar (Hardy Diagnostics, Santa Maria, California, USA) and incubation at

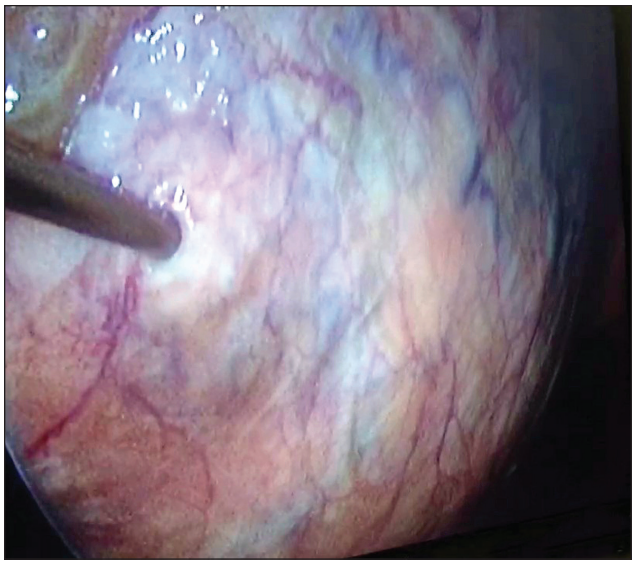

Figure 2. Intraoperative still image of a laparoscopic injection needle puncturing the capsule of the intraabdominal abscess. Cranial is to the left, and dorsal is at the top of the image. On the left side of the image (cranial), the right ovary and mesovarium are seen deflected axially using an atraumatic grasping instrument to improve visualization of the mass.

35°C in 5% CO_2. Anaerobic culture was performed by inoculation of the fluid sample on to PRAS Brucella agar (Anaerobe Systems, Morgan Hill, California, USA) and incubation under anaerobic conditions at 35°C. On day 2 of anaerobic culture, small numbers (2+) of small gray light brown colonies were apparent. An aerotolerance check revealed growth under

aerobic conditions; this isolate was not pursued further as an obligate anaerobe. No other anaerobic growth was noted following 6 d of incubation. The aerobic culture produced very small numbers of growth (1+) on day 6 of incubation. Gram staining of these colonies revealed small, curved, rod-shaped bacteria. Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometric (MS) analysis (Bruker Daltonics, Billerca, Massachusetts, USA) identified the aerobic and anaerobic colonies as *Campylobacter fetus* with a score of 2.4 (reliable identification to the species level). Polymerase chain reaction confirmation testing was performed using 3 separate PCR targets in order to account for genetic heterogeneity within *Campylobacter fetus* (15,16). The final identification was *Campylobacter fetus* subsp. *fetus.*

The mare was discharged from the hospital 4 d after surgery on oral minocycline (Aurobindo Pharma, Hyderabad, India), 4 mg/kg BW, PO, q12h, until culture results became available. Once the final identification of *C. fetus* subsp. *fetus* was made, antibiotic therapy was switched to oral enrofloxacin (Wedgewood Pharmacy, Swedesboro, New Jersey, USA) based on susceptibility patterns reported in the human literature. The mare received a total of 10 d of oral minocycline at 4 mg/kg BW, PO, q12h, followed by a course of enrofloxacin at 7.5 mg/kg BW, PO, q24h of 6 wk duration.

Repeat transrectal palpation and ultrasonography were performed 3 mo after surgery and the mass was found to be unchanged in size (Figure 1C). The mare's behavioral abnormalities had improved, although she was still sensitive to palpation of the left flank and would kick if the owner attempted to pick up her hind feet. A telephone interview conducted with the owner 13 mo after surgery established that the mare's behavioral abnormalities had resolved. The mare had returned to work several months earlier and was competing successfully in 3-day eventing at an introductory level.

Discussion

Campylobacter fetus subspecies *fetus* is generally considered a pathogen of ruminants and is associated with abortion (17). There is a limited number of studies describing *C. fetus* subsp. *fetus* infections in young horses (18–20). However, this is the first report of *C. fetus* subsp. *fetus* being cultured from an abdominal abscess in a mature horse. Ultrasound was important not only to further characterize the palpable mass as an abscess but also to evaluate involvement of regional structures including the right ureter, right kidney, and terminal aorta, and assist with surgical planning.

Isolation of this organism under culture conditions other than microaerophilic is unusual as this organism grows optimally under microaerophilic conditions. However, in the experience of one author (BAB), this organism will grow under aerobic and/or anaerobic conditions provided that incubation is prolonged (> 2 d). Use of MALDI TOF MS allowed rapid identification of this isolate to the genus level, but PCR testing was necessary to confirm the subspecies as *C. fetus* subsp. *fetus.*

The ultrasonographic appearance of abdominal abscessation is variable and can be related to the etiologic agent (5,8,9,21). Based on the appearance and periaortic location of the abscess, without ultrasonographic or laparoscopic evidence of right renal involvement, lymph node and/or mesenteric abscessation seems the most plausible origin of the abscess in this case. Internal abscessation due to *C. pseudotuberculosis* is regularly diagnosed at our hospital but typically involves the parenchyma of liver, kidneys, and/or spleen, infrequently shows encapsulation and is rarely mesenteric in origin (5). The well-encapsulated appearance seen in our horse has been reported in horses with abdominal abscesses due to *S. equi* subsp. *equi* (8,9). The presence of intracapsular mineralization is an unusual feature and likely represents long-standing abscessation.

Ideally, selection of antimicrobials in this case would have been based on susceptibility testing in accordance with the American Veterinary Medical Association policy for judicious therapeutic use of antimicrobials (22). However, sensitivity testing for *Campylobacter* spp. isolates was not available at our laboratory at the time of this patient's diagnosis and treatment. Therefore, antimicrobial selection was based on the reported susceptibility of *Campylobacter* isolates from other species, as well as pharmacokinetic and practical considerations specific to equine patients. Resistance to tetracyclines has been documented in *C. fetus* subsp. *fetus* isolates from livestock species (23–25). In contrast, resistance to fluoroquinolones and macrolides among *Campylobacter* spp. isolates is less prevalent (23,25). Intravenously administered ampicillin could have potential value in treating *Campylobacter* infections in hospitalized horses, but ability to penetrate an abscess should be considered because penicillins and carbapenem antibiotics have low lipid solubility (26). Similarly, aminoglycosides have poor ability to penetrate abscess capsules and may be inactivated by purulent material (27). Macrolides are highly lipophilic and conducive for use in the treatment of abscesses (26). However, the risk of severe colitis in adult horses precludes the common use of macrolides in equine practice (28). Fluoroquinolones are lipophilic, bacteriocidal, and have high oral bioavailability in horses (26,27). Enrofloxacin was therefore considered the most appropriate antibiotic for long-term therapy in the present case.

Surgical treatment options for large, mature abdominal abscesses are limited based on the location of the abscess and surgical access to it. In this case complete excision was not considered feasible; and incising the abscess to allow internal drainage would likely have resulted in peritonitis and was therefore avoided. Introduction of a Foley catheter into an internal abscess for repeat lavage and local antibiotic infusion has been described (9). This procedure may have been recommended in this case had the mare's clinical signs failed to improve.

The efficacy of the treatment regimen used in this case is uncertain. Although the behavioral issues were improved, there is insufficient evidence to determine if the treatment resolved the diagnosed condition or if it was self-limiting. Given the ultrasonographic appearance of the abscess 3 mo after surgery it is unlikely that the treatment caused complete resolution of the abdominal abscess, but it is possible that progression of the disease was halted.

This is the first reported case of isolation of *Campylobacter fetus* subsp. *fetus* from an abdominal abscess in a horse. The mare's clinical signs eventually improved despite a lack of

reduction in the size of the abscess. *Campylobacter*-specific culture conditions and species identification should be considered for abdominal abscesses in horses, particularly if routine aerobic culture does not yield a pathogen. CVJ

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