Title

Necrotizing hepatitis associated with Clostridium novyi in a pony in western Canada.

Journal

Canadian Veterinary Journal, 58(3)

ISSN

0008-5286

Authors

Davies, Jennifer L
Uzal, Francesco A
Whitehead, Ashley E

Publication Date

2017-03-01

Peer reviewed

Case Report Rapport de cas

Necrotizing hepatitis associated with *Clostridium novyi* in a pony in western Canada

Jennifer L. Davies, Francesco A. Uzal, Ashley E. Whitehead

Abstract — Severe icterus, peritoneal effusion, localized fibrinous peritonitis, and necrotizing hepatitis were found at necropsy of a 20-year-old female pony with a history of acute onset depression, inappetence, fever, and marked elevation in hepatic enzymes. Gross pathology, histopathology, and immunohistochemistry were compatible with a diagnosis of clostridial hepatitis caused by *Clostridium novyi*-group bacteria. This is believed to be the first reported case of clostridial hepatitis in an equid in Canada, and only the third report of this rare disease in North America.

Résumé — Hépatite nécrosante associée à *Clostridium novyi* chez un poney de l'Ouest canadien. Un ictère grave, une effusion péritonéale, une péritonite fibrineuse localisée et une hépatite nécrosante ont été constatées chez un poney femelle âgé de 20 ans avec une anamnèse d'apparition soudaine de dépression, d'inappétence, de fièvre et d'élévations marquées des enzymes hépatiques. La pathologie clinique, l'histopathologie et l'immunohistochimie étaient compatibles avec un diagnostic d'hépatite clostridiale causée par une bactérie du groupe *Clostridium novyi.* On croit qu'il s'agit du premier cas signalé d'hépatite clostridiale chez un équidé au Canada et seulement le troisième rapport de cette maladie rare en Amérique du Nord.

(Traduit par Isabelle Vallières)

Can Vet J 2017;58:285–288

Clostridial hepatitis is most commonly seen in small and large ruminants (1,2). In ruminants, there are 2 clinical syndromes caused by the *Clostridium novyi* group of bacteria; black disease (infectious necrotic hepatitis) caused by *C. novyi* type B and bacillary hemoglobinuria caused by *C. haemolyticum* (also known as *C. novyi* type D) (3,4). There is considerable overlap in the gross pathology, histopathology, and pathogenesis of these diseases. Both diseases are characterized by acute hepatic necrosis and systemic lesions associated with toxemia and generalized vascular damage (3). As the name suggests, bacillary hemoglobinuria is further characterized by intravascular hemolysis with anemia and hemoglobinuria attributed to the phospholipase activity of the beta toxin produced in large amounts by *C. haemolyticum* (3,4). Although gross changes in sheep and cattle with infectious necrotic hepatitis are similar to those of bacillary hemoglobinuria (BH), the lesions associated with hemolytic anemia, which are characteristic of BH, are usually not seen in ruminants with infectious necrotic hepatitis, probably because the beta toxin gene is far less expressed in *C. novyi* type B than in *C. haemolyticum.*

The pathogenesis of both diseases begins with the ingestion of environmental spores with seeding to histiocytes in the liver, spleen, bone marrow, and perhaps other organs. Spores lie dormant in the liver until the formation of a localized anaerobic environment allowing for germination of spores and the production of potent exotoxins by vegetative bacteria. In ruminants, migration of the common liver fluke, *Fasciola hepatica,* is thought to be the main initiating event (4).

Clostridial hepatitis is rare in equids with only 7 cases in the veterinary literature (1–3,5–7). Cases have been reported in Australia, New Zealand, the United Kingdom, and the United States. To our knowledge, this is the first reported case of equine clostridial hepatitis in Canada and only the third case in North America. Here we describe the clinicopathologic features of this rare entity including ultrasonographic findings that have not been thoroughly documented.

Diagnostic Services Unit (Davies) and Department of Veterinary Clinical and Diagnostic Sciences (Whitehead), University of Calgary Faculty of Veterinary Medicine, 11877 85th Street NW, Calgary, Alberta T3R 1J3; California Animal Health and Food Safety Laboratory, Faculty of Veterinary Medicine, University of California–Davis, 105 West Central Avenue, San Bernardino, California 92408, USA (Uzal).

Address all correspondence to Dr. Ashley Whitehead; e-mail: ae.whitehead@ucalgary.ca

Case description

A 20-year-old, female pony was presented with a history of acute onset of depression, decreased appetite, and separation from herdmates. On presentation, the pony was tachycardic, tachypneic, and mildly febrile (38.6°C). Initial blood analysis consisting of a complete blood (cell) count (CBC) and serum biochemical profile, including liver parameters, were unremarkable [aspartate aminotransferase (AST); 491 IU/L, reference

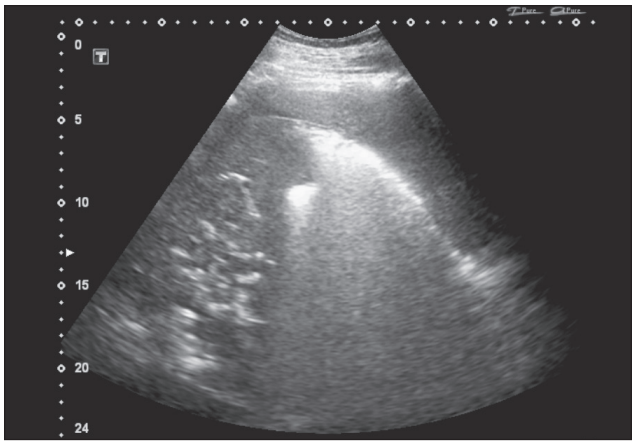

Figure 1. Ultrasound image of the liver of the pony obtained in the left 9th intercostal space. This image shows an increased echogenicity of the hepatic tissue with multifocal hyperechoic foci. Subcapsular and intraparenchymal gas are present. The spleen is overlying the liver. The left side of the image is dorsal and the right side is ventral.

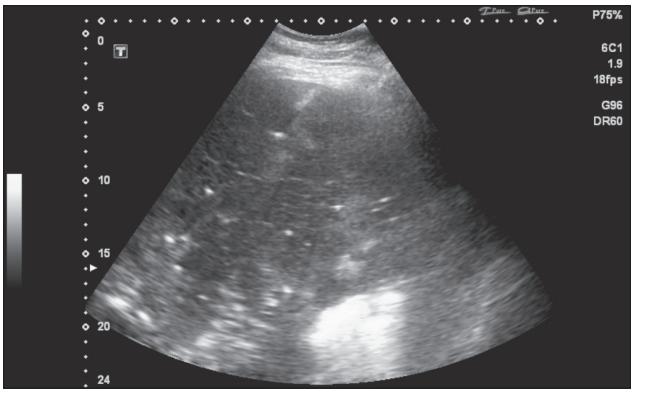

Figure 2. Ultrasound image of the liver of the pony obtained in the right 12th intercostal space. Note the increased number of multifocal hyperechoic foci deep within the hepatic parenchyma. The left side of the image is dorsal and the right side is ventral.

interval (RI): 180 to 570 IU/L], total bilirubin (35 μmol/L, RI: 33 to 63 μmol/L), alkaline phosphatase (ALP; 226 IU/L, RI: 73 to 327 IU/L), and gamma glutamyl transferase (GGT; 28 IU/L, RI: 5 to 30 IU/L). The mare was initially treated with oral electrolytes and water via nasogastric intubation. Flunixin meglumine (Flunixin injection; Zoetis Canada, Kirkland, Quebec), 0.5 mg/kg body weight (BW), IV, was administered to aid in controlling the mild pyrexia. An impending colitis was initially suspected by the admitting veterinarian.

Over a 2-day period, the pony deteriorated with development of a high fever (39.6°C) and marked icterus. A CBC performed at this time revealed a mild monocytosis (0.73×10^9/L, RI: 0.20 to 0.60×10^9/L) and mild thrombocytopenia (83×10^3/μL, RI: 100 to 250×10^3/μL). Marked increases in sorbitol dehydrogenase (SDH) (> 150.0 IU/L, RI: 1.9 to 5.8 IU/L), aspartate aminotransferase (AST; 1817 IU/L, RI: 180 to 570 IU/L), total bilirubin (286.3 μmol/L, RI: 33 to 63 μmol/L) and moderate increases in alkaline phosphatase (ALP) (415 IU/L, RI: 73 to 327 IU/L), alanine aminotransferase (ALT) (62 IU/L, RI: 5 to 30 IU/L), and gamma glutamyl transferase (GGT) (55 IU/L, RI: 5 to 30 IU/L) were noted on serum biochemical profile. Bile acids were within normal limits (10.2 μmol/L, RI: 0 to 19 μmol/L). Red-colored urine was collected which was 4+ positive for hemoglobin on urinalysis dip stick and no intact red blood cells were noted on cytology following sedimentation. Transcutaneous abdominal ultrasound was performed using a 1.9 MHz convex probe. Multifocal, hyperechoic foci (≤ 1 cm diameter) were identified within the left aspect of the liver parenchyma (Figure 1). Larger subcapsular hyperechoic areas were associated with reverberation artifact consistent with gas. The right aspect of the liver had fewer, smaller hyperechoic foci deep in the parenchyma (Figure 2). The small intestines were within normal limits for size and wall thickness with minimal motility. There were pockets of hypoechoic free abdominal fluid in the left abdomen.

During this time, the mare was administered Lactated Ringer's Solution (Baxter Corporation, Mississauga, Ontario) at twice maintenance (100 mL/kg BW, IV per day) and antibiotic therapy consisting of sodium penicillin G (Pharmaceutical Partners of Canada, Richmond Hill, Ontario), 22 000 IU/kg BW, IV, q6h and gentamicin (Gentocin; Merck Animal Health, Kirkland, Quebec), 6.6 mg/kg BW, IV, q24h. Flunixin meglumine at 1.1 mg/kg BW, IV, q12h was also administered. The pony was depressed but did not show any other neurological signs and continued to eat small amounts of hay and concentrate. The pony continued to deteriorate over the next 24 h, showing signs of systemic inflammatory response syndrome (SIRS) and abdominal pain. Further diagnostic and treatment options were discussed with the owner; however, euthanasia followed by necropsy was elected due to the mare's poor response to medical therapy and declining systemic condition.

At necropsy, the body was fresh, in good nutritional condition, and there was no evidence of dehydration. There was severe icterus. Within the peritoneal cavity there were approximately 15 L of opaque, reddish fluid. There were numerous petechiae and ecchymoses on the small intestinal serosa. Visible from both the visceral and diaphragmatic surfaces of the left liver lobe, there was an extensive, moderately well-defined, approximately 35 cm in diameter focus where the parenchyma was elevated, firm, and dark red to tan. This area was covered by a thick layer of fibrin on the capsular surface with adhesion to the diaphragm and spleen (Figure 3). On section, there were extensive, malodorous areas of necrosis and hemorrhage that were accompanied by emphysema. Thrombi were observed within blood vessels (Figure 4).

Sections of liver, spleen, lung, kidney, adrenal gland, thyroid gland, pancreas, heart, stomach, small intestine, large intestine and brain were fixed in 10% buffered formalin for 24 h, processed routinely for histological examination, and stained with hematoxylin and eosin (H&E). Gram stain was used on selected sections of liver. Fresh specimens of liver were submitted for aerobic and anaerobic culture and fluorescent antibody testing (FAT) for *C. novyi, C. sordellii, C. chauvoei,* and *C. septicum.* For immunohistochemistry, formalin-fixed, paraffin-embedded 4 μm thick sections of liver were processed by a streptavidin-biotin technique for *C. novyi, C. sordellii, C. septicum,* and

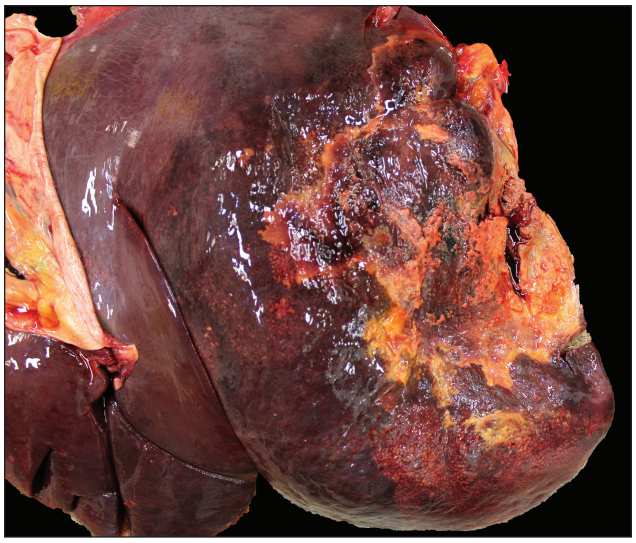

Figure 3. Diaphragmatic surface of the liver of the pony. Involving the left liver lobe, there is an extensive, moderately well-defined, approximately 35 cm focus where the parenchyma is swollen, firm, and dark red to tan. This area was accompanied by a thick layer of fibrin coating the capsular surface.

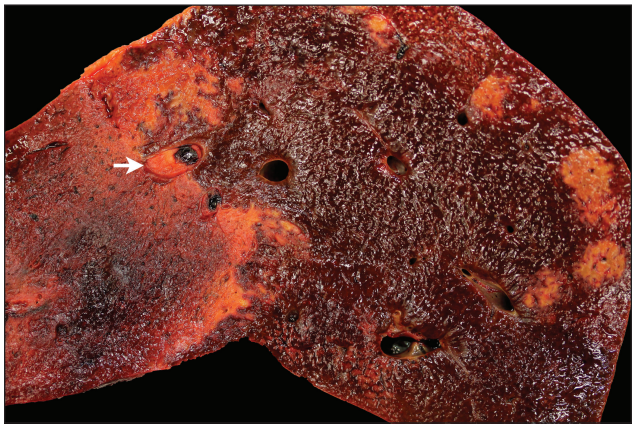

Figure 4. Cut surface of the liver of the pony. There are multifocal to extensive, well-demarcated, tan to dark red, dry, dull foci consistent with coagulation necrosis. Necrosis is accompanied by hemorrhage and emphysema. Blood vessels contain thrombi (arrow).

C. chauvoei using the Dako EnVision kit (Dako, Carpenteria, California, USA) according to the manufacturer's instructions. Rabbit polyclonal anti-*C. novyi,* anti-*C. sordellii,* anti-*C. septicum,* and anti-*C. chauveoi* antibodies (Veterinary Medical Research and Development, Pullman, Washington, USA) were used. Negative controls consisted of sections incubated with normal rabbit serum instead of the primary antibody. Positive controls consisted of sections of liver or muscle known to contain the clostridial species investigated.

Microscopically, there were multifocal to coalescent zones of acute coagulation necrosis bordered by an intense band of viable and degenerate neutrophils. Areas of emphysema frequently accompanied the necrosis. The tunica media of blood vessels was disrupted by fibrinoid change, nuclear debris, neutrophils, and red blood cells consistent with vasculitis. Affected blood vessels often contained thrombi. Within the zone of coagulation necrosis there

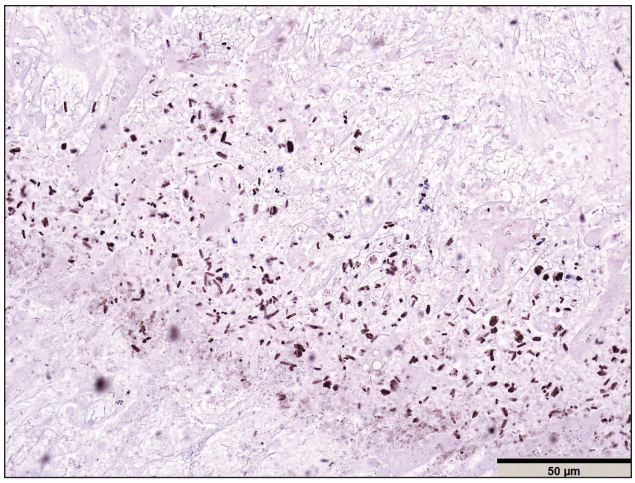

Figure 5. Immunohistochemical staining of a section of liver for *Clostridium novyi*. Large numbers of strongly positive staining rods are present within an area of hepatic necrosis. Bar = 50 µm. Stain: rabbit polyclonal anti-*C. novyi* antibodies (Veterinary Medical Research and Development, Pullman, Washington, USA).

were variable numbers of large (~6 µm × 1 µm), Gram-positive rods compatible with *Clostridium* species. There was no evidence of hemoglobinuric nephrosis in the sections of kidney examined. Diffuse splenic congestion was a feature and the splenic capsule was lined by a layer of fibrin and degenerate neutrophils.

Fluorescent antibody testing on sections of fresh liver was negative for *C. novyi, C. sordellii, C. chauveoi,* and *C. septicum.* No bacterial pathogens were isolated on aerobic or anaerobic culture of liver. Bacteria in formalin-fixed, paraffin-embedded sections of liver demonstrated strongly positive immunohistochemical staining for *C. novyi* (Figure 5) and were negative for *C. sordellii, C. chauveoi,* and *C. septicum.*

Discussion

Gross pathology, histopathology, and immunohistochemistry in this case were consistent with a diagnosis of clostridial hepatitis caused by *C. novyi*-group bacteria. Clostridial hepatitis is a common disease of ruminants, and a rare disease of equids.

To date, there are 7 reported cases of clostridial hepatitis in equids occurring in Australia, New Zealand, the United Kingdom, and the United States (1–3,5,6). Similar to the current case, clinical disease in horses described before was characterized by acute onset of depression, fever, abdominal pain, icterus, tachycardia, and tachypnea with rapid deterioration and death in 12 to 48 h (2,7). To date, successful therapy has not been described; this is not surprising given the peracute nature of the disease, rapid development of toxemia, and difficulty in establishing an antemortem diagnosis. Reported necropsy findings include serosanguinous pericardial, pleural and peritoneal effusions, serosal hemorrhages, icterus, fibrinous peritonitis, and hepatic necrosis (1,2,6,7). Interestingly, the hepatic necrosis is frequently described as being restricted to the left side of the liver (1,2,6,7) as was observed in this pony. The reason for this seemingly unique distribution within the liver remains unknown.

Key to the pathogenesis of clostridial hepatitis is the development of anaerobic conditions in the liver favoring spore germination. In ruminants, spore germination is most frequently associated with liver fluke migration. The inciting cause of the suitable anaerobic conditions for spore germination within the equine liver has not been definitively determined. *Strongyle* spp. migration though the liver has been proposed as an initiating cause, but has been inconsistently reported (3,5). In the current case, there was no evidence of larval migration on gross or microscopic examination of the liver. Interestingly, many of the reported cases have a recent history of anthelminthic therapy prior to the onset of clinical signs (1,3,7) suggesting the possibility of liver damage following destruction of migrating parasites. Recent use of anthelminthics was not reported in the current case.

Arriving at an etiologic diagnosis was problematic in this case. Liver was submitted to 2 laboratories for anaerobic culture and FAT for *Clostridium* spp. *Clostridium novyi*-group organisms were not detected by FAT or by culture, highlighting the challenge at arriving at an etiologic diagnosis in cases of clostridial hepatitis. Both *C. novyi* and *C. haemolyticum* are extremely oxygen sensitive and fastidious in their nutritional requirements making culture challenging and an unreliable diagnostic tool (8). The reason for the negative FAT result is unknown, but may reflect sampling as the organisms were not uniformly distributed throughout the liver. In the current case, immunohistochemistry was positive for *C. novyi* and was instrumental in confirming the diagnosis of clostridial hepatitis. It is important to note that *C. novyi* type B and *C. haemolyticum* are very closely related and polyclonal antiserum made for either is typically cross-reactive and unable to differentiate between the 2 species (8). Similarly, the generic *C. novyi* antibody used for immunohistochemistry in the current case cross-reacts with *C. haemolyticum*. As a result, we were able to establish a diagnosis of clostridial hepatitis caused by *C. novyi*-group bacteria, but were unable to further discriminate to the species level. Although red urine was reported in the clinical history, there was no evidence of hemolysis on CBC nor was there evidence of hemoglobinuric nephrosis on histopathology and the bladder was devoid of urine at necropsy. The lack of clear evidence for intravascular hemolysis in this case may make it more comparable to infectious necrotic hepatitis caused by *C. novyi* rather than bacillary hemoglobinuria caused by *C. haemolyticum*. The ongoing advancement of molecular diagnostic techniques will hopefully allow for discrimination between these organisms in the future.

In conclusion, this appears to be the first reported case of clostridial hepatitis in an equid in Canada and only the third report of this disease in North America. While clostridial hepatitis appears to be a rare disease of horses, it should still be considered as a differential diagnosis for acute hepatic disease in this species. The ability to rapidly recognize the clinical features of this acute, fulminating disease in horses will be crucial to future successful therapy. CVJ

References

1. Gay CC, Lording PM, McNeil P, Richards WPC. Infectious necrotic hepatitis (black disease) in a horse. Equine Vet J 1980;12:26–27.
2. Sweeny HJ. Infectious necrotic hepatitis in a horse. Equine Vet J 1986; 18:150–151.
3. Oaks JL, Kanaly ST, Fisher TJ, Besser TE. Apparent *Clostridium haemolyticum/Clostridium novyi* infection and exotoxemia in two horses. J Vet Diagn Invest 1997;9:324–325.
4. Stalker MJ, Hayes MA. Liver and biliary system. In: Maxie MG, ed. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. 5th ed. Vol 2. London, UK: Saunders Elsevier, 2007:354–356.
5. Dumaresq JA. A case of black disease in a horse. Aust Vet J 1939;15: 53–57.
6. Hollingsworth TC, Green VJD. Focal necrotizing hepatitis caused by *Clostridium novyi* in a horse. Aust Vet J 1978;54:48.
7. Whitfield LK, Cypher E, Gordon SJG, et al. Necrotic hepatitis associated with *Clostridium novyi* infection (black disease) in a horse in New Zealand. N Z Vet J 2015;63:177–179.
8. Hatheway CL. Toxigenic clostridia. Clin Microbiol Rev 1990;3: 66–98.