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Case Report
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Cavitary Effusion Associated with *Anaplasma phagocytophilum* Infection in 2 Equids

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Case 1

A 15-year-old, 558-kg Quarter Horse gelding was examined because of peripheral edema and bicavitary (pleural and abdominal) effusion, identified via field ultrasonography before referral. Two years earlier, the horse was evaluated for a similar clinical presentation, including peripheral edema, bicavitary effusion, and fever. The gelding was diagnosed with *Anaplasma phagocytophilum* infection by polymerase chain reaction (PCR)[a] on peripheral blood. He was treated with oxytetracycline[b] (approximately 10 mg/kg IV q12h for 5 days) and made a full clinical recovery.

Initial evaluation during the most recent episode revealed a normal body temperature, tachycardia (60 BPM; reference range (RR): 26–50[1]), and a normal respiratory rate and effort. Thoracic auscultation identified muffled left-sided heart sounds and bilateral absence of bronchovesicular sounds ventrally. The horse had marked pitting ventral, preputial, and pectoral edema. There were prominent jugular pulses, extending one-half to one-third up the neck.

Arterial oxygen tension[c] was within reference range. A subsequent serum chemistry analysis revealed low total protein levels (3.9 g/dL; RR: 5.6–7.0) characterized by panhypoproteinemia, but was otherwise unremarkable.

Complete blood count (CBC)[d] analysis revealed mild anemia (hematocrit [HCT] 30%; RR: 32–50), leukopenia (4.8×10^3/μL; RR: 5.9–11.2), and lymphopenia (0.96×10^3/μL; RR: 1.6–5.2), consistent with inflammation. The serum fibrinogen (375 mg/dL; RR: 100–400) and platelet count (159×10^3/μL; RR: 100–400) were within normal limits. No granulocytic inclusions were noted.

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Abbreviations:

AST	aspartate aminotransferase
BPM	beats per minute
CK	creatine kinase
CSVM	Cummings School of Veterinary Medicine
EGA	equine granulocytic anaplasmosis
F	fahrenheit
GGT	gamma-glutamyl transpeptidase
PCR	polymerase chain reaction
RR	reference range
SCID	severe combined immunodeficiency disease

Abdominal and thoracic ultrasound examination confirmed the presence of moderate, hypoechoic bicavitary effusion, and identified mild dilatation of the liver sinusoids. Bilateral thoracentesis yielded moderate volumes (4 L from the left hemithorax, 3 L from the right hemithorax) of a neutrophilic transudate. As the horse's clinical signs could be attributable to heart failure, an echocardiogram was subsequently performed. The examination revealed a structurally normal heart with normal cardiac measurements, but mild pulmonic valve insufficiency and mild tricuspid valve regurgitation. Moderate pericardial effusion was present, resulting in irregular motion of the right cardiac chambers, consistent with mild tamponade. Regional epicardial thickening with fibrin accumulation was also noted. An electrocardiogram identified a second-degree atrioventricular block. Subsequent pericardiocentesis yielded 5 L of serosanguinous fluid, which was characterized as a mixed inflammatory exudate with a total protein of 3.8 g/dL (RR: <2), HCT of 2%, red blood cell count of 0.334 M/μL, and a total nucleated cell count of 6.02×10^3/μL (RR: <5). Cytologic evaluation revealed 52% nondegenerate neutrophils, 39% lymphocytes, and 9% monocytes. While peripheral blood contamination could not be entirely ruled out, the lack of visible platelets in the sample, as well as the relative increase in inflammatory cells compared to peripheral blood made a primary serosanguinous effusion probable. Multiple neutrophils contained pale basophilic inclusions consistent with degenerating *A. phagocytophilum* organisms; however, peripheral blood leukocytes exhibited normal morphology with no noted inclusions. Samples of the pericardial fluid and peripheral blood were PCR[a] positive for *A. phagocytophilum*, consistent with a diagnosis of equine granulocytic anaplasmosis (EGA; formerly equine ehrlichiosis).

The horse was treated with oxytetracycline[a] (approximately 7.5 mg/kg IV q12h) for 14 days, followed by

14 days of doxycycline[e] (10 mg/kg PO q12h). There was no recurrence of effusion after the initial pericardio- and thoracocentesis, based on repeated ultrasound examination of the thorax. The horse's hypoproteinemia began to improve within 24 hours of initial therapeutic intervention, and his tachycardia and jugular pulses resolved after pericardiocentesis. Peripheral edema improved by day 3 of hospitalization, and had largely resolved at the time of discharge. The horse remained afebrile with a gradual daily improvement in affect and appetite throughout hospitalization. A recheck CBC on day 8 revealed a normal leukocyte count ($8.2 \times 10^3/\mu L$; RR: 5.9–11.2), with a neutrophil count of $5.2 \times 10^3/\mu L$ (RR: 2.3–9.1) and a lymphocyte count of $2.64 \times 10^3/\mu L$ (RR: 1.6–5.2). The horse's platelet count was estimated to be 200,000–500,000/μL and the hematocrit was 31% (RR: 30–51), Serum chemistry indicated resolving hypoproteinemia (4.7 mg/dL; RR: 5.6–7.0). No recurrence of disease has been reported.

Case 2

A 4-month-old, 49-kg miniature donkey filly was examined because of a several-day history of anorexia, weakness, and lethargy, which at the time of admission had progressed to recumbency. Initial evaluation revealed the filly to be weak and centrally lethargic with normal cranial nerve function, deep pain, withdrawal and reflex responses. The foal's body temperature was within normal limits[2]. Auscultation identified sinus tachycardia (90 BPM; RR: 30–50) and a normal respiratory rate with mildly increased bronchovesicular sounds.

Arterial blood gas analysis[b] verified adequate oxygenation and ventilation. However, several abnormalities were present, including mild, uncompensated metabolic acidosis, anemia (PCV 16%; RR: 33–43), hyperlactatemia [94.5 mg/dL (10.5 mmoL/L); RR: <18 (<2)], hyponatremia (121 mEq/L; RR: 137–145), mild hyperkalemia (5.4 mEq/L; RR: 3.6–4.8), hyperglycemia (168 mg/dL; RR: 60–110), and hypercreatininemia (3.2 mg/dL; RR: 0.8–1.9). Serum chemistry further identified hypoalbuminemia (2.0 g/dL; RR: 3.0–4.0), hypochloremia (85 mEq/L; RR: 99–105), and elevations in AST (2,324 U/L; RR: 368–600) and CK (627 U/L; RR: 21–136). A CBC revealed leukocytosis ($16.7 \times 10^3/\mu L$; RR: 5.9–11.2) and hyperfibrinogenemia (500 mg/dL; RR: 100–400)[3], consistent with inflammation, and an estimated platelet count of 200,000–500,000/μL. Neutrophil morphology was normal and no cellular inclusions were noted. Fecal floatation showed a heavy parasite burden of strongyles (6,150 ova/g), Parascaris equorum (300 ova/g), and *Strongyloides* spp. (97,950 ova/g). Serum iron concentrations[a] were subsequently determined to be low (38 μg/dL; RR: 50–198). Serum vitamin E and selenium levels[f] were within published reference ranges. Goal-directed fluid resuscitation with lactated Ringer's solution (LRS)[g] led to a clinical improvement in alertness and gradual resolution of the hypercreatininemia, hyperlactatemia and electrolyte derangements over 24 hours.

After initial stabilization, abdominal ultrasound revealed mildly increased small intestinal wall thickness, a prominent liver, and subjectively small spleen. Thoracic ultrasound demonstrated marked pleural effusion bilaterally with evidence of fibrin strands, but a normal pleural reflection. No other abnormalities of the lungs or heart were appreciated. Bilateral thoracocentesis yielded a moderate volume of pleural effusion (660 mL from the left hemithorax, 215 mL from the right hemithorax). Fluid analysis from the left hemithorax revealed a pure transudate (protein <2.0 g/dL; RR<2.0) with a low nucleated cell count ($1.25 \times 10^3/\mu L$; RR: <8.0) and a mixed population of cells (36% nondegenerate neutrophils, 37% lymphocytes, and 27% monocytes). Analysis of the right hemithorax sample showed a protein rich fluid (4.4 g/dL; RR: <2.0) with a normal leukocyte count ($5.16 \times 10^3/\mu L$; RR: <8.0). Leukocyte examination demonstrated a mixed population of cells (51% nondegenerate neutrophils, 23% lymphocytes, and 26% monocytes) and the presence of morulae within a small percentage of neutrophils. This fluid sample tested PCR[h] positive for *A. phagocytophilum*.

The foal was treated with oxytetracycline[b] (10 mg/kg IV q12h). Treatment with iron[i] (6.5 mg/kg PO q24h) was initiated based on the filly's low serum iron concentration, and a 5-day course of fenbendazole[j] (10 mg/kg PO q24h) was administered to address the extensive parasitism. Routine supportive therapies, including fluids administered IV (LRS)[g], were also continued.

The foal's clinical signs and HCT steadily improved after the initiation of tetracycline and iron treatment, and by day 5 of hospitalization, her packed cell volume (PCV) had risen to 25%. A repeat serum chemistry performed at that time revealed mild hypertriglyceridemia (169 mg/dL; RR: 32–100) and elevations of GGT (355 U/L; RR: 40–98) and AST (1311 U/L; RR: 368–600), indicating hepatic injury[3]. Hyperglobulinemia (5.8 g/dL; RR: 3.2–4.6) was noted, and was considered consistent with antigenic stimulation. Concurrently, the filly's SDH level[a] was increased (18.4 U/L; RR: 2.0–6.0), consistent with ongoing hepatic insult. The aberrations in liver values potentially occurred in association with EGA infection, although other possible causes of liver damage, including cholestasis, hypoxic injury, hepatic lipidosis, and drug-induced liver damage, were considered. A definitive cause could not be determined with certainty, in the absence of a liver biopsy. On day 8 of hospitalization, the GGT had decreased slightly (260 U/L; RR: 40–98), and a serum iron level was found to be within normal limits.

Thoracic ultrasound evaluation repeated on day 8 of hospitalization demonstrated minimal residual pleural effusion. CBC and serum chemistry performed the next day revealed mild leukocytosis ($11.8 \times 10^3/\mu L$; RR: 5.9–11.2), a HCT of 25%, further improvement in GGT (210 U/L; RR: 40–98), and normalization of AST (391 U/L; RR: 368–600), suggesting cessation of active hepatocyte injury. Thoracic radiographs obtained on day 13 were unremarkable, and the foal was discharged with a 2-week course of doxycycline[k] (10 mg/kg PO q12h) and

continued iron supplementation under the direction of the referring veterinarian. The foal's HCT remained stable throughout the remainder of hospitalization, and was 25% at the time of discharge. CBC and serum chemistry analysis[d] performed 6 weeks after discharge revealed a HCT of 34%, and resolution of all other blood work abnormalities. Serum iron at that time was normal. No recurrence of disease has been noted.

This report describes *Anaplasma*-associated cavitary effusion in equids leading to a primary complaint of cardio-respiratory compromise. In contrast, classic EGA most commonly presents with a fever, partial or total anorexia, lethargy, peripheral edema, ataxia, icterus, and petechiation, secondary to vasculitis and thrombocytopenia. In addition, mild anemia, leukopenia, and thrombocytopenia are frequently reported hematologic abnormalities in affected horses[4,5]. Some of these characteristics were also shared by the equids in this report. A variety of uncommon clinical manifestations of *A. phagocytophilum* include acute recumbency[6], rhabdomyolysis[7], and acute death in an experimentally infected horse, attributed to disseminated intravascular coagulation and circulatory shock[8]. Novel disease presentations in other species have historically included edema, asymptomatic ascites, and premature parturition in a camelid[9], congenital infection in a calf[10], and acute blindness associated with uveitis, intraocular hemorrhage, and retinal detachment in a dog[11]. However, clinically symptomatic multi-cavitary effusion secondary to *A. phagocytophilum* infection is considered to be an atypical clinical presentation of this tick-borne disease.

The ability to document morulae and to obtain a positive PCR for *A. phagocytophilum* in various body fluids was strongly supportive of EGA as the inciting cause of cavitary effusion in both equids of the current report. Visualization of basophilic morulae within the neutrophilic cytoplasm is commonly observed, and most notable on days 3–5 postinfection in horses[12]. Identification of these morulae in whole blood or fluid samples can be a critical element in obtaining a rapid diagnosis in affected equines, although PCR analysis for *A. phagocytophilum* is considered a more sensitive diagnostic test[13].

The relationship between EGA infection and the development of cavitary effusions remains speculative. Localized vasculitis is a well described clinical manifestation of EGA infection in equids[4], and has been cited as the most probable pathogenesis of distal limb edema. While it is plausible that localized vasculitis (myocardial, pleural, peritoneal) was the source of cavitary effusion in these cases, it remains unclear why clinically relevant effusion is not more frequently detected in equids infected with *A. phagocytophilum*. Proliferative, necrotizing inflammation of the small arteries and veins, with associated endothelial and smooth muscle cell swelling, cellular thromboses, and perivascular leukocyte infiltration, primarily affecting the distal limbs and reproductive structures, was described in one of the first reported cases of EGA[4]. This study further described a proportion (approximately 11/33) of animals with postmortem effusions of the peritoneum and pericardium, although no mechanism was proposed. In contrast, a more recent report[14], comparing the pathologic findings of *A. phagocytophilum* infection in sheep, humans, and equids, documented localized distal limb vasculitis in horses, but no component of necrosis or proliferation. Evidence of systemic vasculitis as a major underlying pathology was not described in this more recent report.

The currently proposed pathogenesis of vasculitis in EGA infection is 2-fold. First, *A. phagocytophilum* may localize and perpetuate in granulocytes (neutrophils) with subsequent colonization of endothelial cells. Reports focusing on *A. phagocytophilum* have not only determined the organism's ability to colonize the microvascular endothelium and reproduce in vitro[15], but have established this pathogenesis in vivo, using chronically affected SCID (severe combined immunodeficiency) mice[16]. The latter study concluded that microvascular endothelial cells may not only play a key role in the initial infectivity of the bacteria, but also serve as a nidus for continued infection. Vascular endothelium provides an ideal site for colonization, as these cells have a greater longevity than granulocytes, and would allow for easy transfer of bacterial organisms to marginating granulocytes, while altering their behavior to prevent transmigration, and permitting for their return to the general circulation. Furthermore, endothelial colonization allows for avoidance of certain host immune effectors such as immunoglobulin and complement[15], which permits propagation of infection and associated vascular injury.

Alternatively, the production of myelosuppressive and proinflammatory cytokines by neutrophils can contribute to the development of vasculitis and edema[15,17]. Multiple studies have determined that the severity of clinical and histopathologic disease is exacerbated by host immune responses, most notably gamma interferon, IL-12, and IL-10[17]. *A. phagocytophilum* induces increased expression of chemokines, including IL-8, which attracts naïve neutrophils to sites of infection and allows for propagation of the organism[18]. In addition, the variability in host defenses between individuals likely plays a role in the manifestation of disease. This notion is supported by the fact that the horse in case 1 presented twice for EGA infection, with a similar, yet atypical constellation of clinical signs. On both occasions the horse responded well to treatment with oxytetracycline.

Both equids in the current report demonstrated evidence of potential liver damage based on hematology, ultrasonography, or both. While *Anaplasma* spp. infections are associated with liver pathology in humans, including hepatocyte apoptosis and periportal lymphohistiocytic infiltrates[19], this disease manifestation is not routinely recognized in equids. However, one report[14] described a variety of histologic abnormalities of the liver on postmortem examination, including periportal lymphocytic infiltrates, macrophage aggregates, lobular hepatitis, and visible apoptotic cells in a group of horses infected with *A. phagocytophilum*. Subclinical hepatitis may be underdiagnosed in the clinical setting, as most

equines do not undergo routine blood screening in uncomplicated cases of EGA. In addition, horses with more classic clinical signs of EGA may be diagnosed and treated quickly in endemic areas, thus limiting more wide-spread systemic effects of infection.

In summary, this report describes a novel manifestation of clinically relevant cavitary effusion, secondary to *A. phagocytophilum* infection in equids presenting for cardio-respiratory compromise. While classic clinical signs of EGA commonly include fever, anorexia, lethargy, peripheral edema, ataxia, and petechiation, a differential diagnosis of *A. phagocytophilum* infection should be considered in equids presenting for inflammatory pleural, pericardial, or abdominal effusion, especially in endemic areas and in equids with a history of tick exposure.

Footnotes

[a] Idexx Laboratories, N. Grafton, MA
[b] Butler Animal Health, Dublin, OH
[c] Stat Profile pHOx Ultra, NOVA Biomedical, Waltham, MA
[d] Antech Diagnostics, Lake Success, NY
[e] Wedgewood Pharmacy, Swedesboro, NJ
[f] Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI
[g] Abbott Laboratories, N. Chicago, IL
[h] North Carolina State University Vector Borne Disease Laboratory, Raleigh, NC
[i] Ferrous Sulfate, Major Pharmaceuticals, Livonia, MI
[j] Panacur, Intervet Inc., Merck Animal Health, Summit, NJ
[k] Westward Pharmaceutical Corporation, Eatontown, NJ

Acknowledgment

Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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