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Common and atypical presentations of *Anaplasma phagocytophilum* infection in equids with emphasis on neurologic and muscle disease

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Abstract

Background: Comprehensive descriptions of equids with granulocytic anaplasmosis (EGA) with neurologic or muscle disease and other atypical presentations are scarce in the literature.

Objective: Describe the clinical signs, laboratory findings, treatment, and outcome of equids with EGA with emphasis on neurologic and muscle disease.

Animals: Thirty-eight horses, 1 donkey.

Methods: Retrospective study. Equids with EGA were included. The electronic data base was searched from January 2000 to December 2022 using the words anaplasmosis, ehrlichiosis, granulocytic, and rickettsia. Signalment and clinical data were reviewed. Data were evaluated for normality using Shapiro-Wilk test. Parametric and nonparametric statistics were used for normally and non-normally distributed data.

Results: Common (41%) and other (59%) presentations were seen in horses ≥ 4 years of age (median, 14 years) with an overrepresentation of males (77%). Neurologic disease was common (41%), mainly presenting as diffuse symmetrical proprioceptive ataxia. Brain disease was less common manifesting as obtundation and cranial nerve deficits. Muscle disease was less common, with QH breeds with the variant causing myosin heavy chain myopathy (MYHM) having severe disease. Cavitary effusion, cardiomyopathy and disseminated intravascular coagulation (DIC) were uncommon. Clinical laboratory results varied depending on disease stage. Muscle enzyme activities were significantly higher in horses with muscle disease. Outcome was favorable with prompt tetracycline treatment. Death and long-term sequelae were not reported.

Abbreviations: DIC, disseminated intravascular coagulation; EGA, equine granulocytic anaplasmosis; GBED, glycogen branching enzyme deficiency; HGA, human granulocytic anaplasmosis; HYPP, hyperkalemic periodic paralysis; IMM, immune-mediated myositis; MC, myotonia congenita; MH, malignant hyperthermia; My, MYHM allele; MYHM, myosin heavy chain myopathy; NM, neurologic or muscle disease; No-NM, no neurologic or muscle disease; PSSM1, polysaccharide storage myopathy 1.

Conclusions and Clinical Importance: Common and atypical presentations of EGA have a favorable outcome with prompt tetracycline treatment. Quarter horse breeds with muscle disease should be genotyped for MYHM.

KEYWORDS
anaplasmosis, ehrlichiosis, equine, granulocytic, morulae

1 | INTRODUCTION

Granulocytic anaplasmosis in horses (EGA) is caused by an obligate intracellular gram-negative rickettsial organism, *Anaplasma phagocytophilum*, that infects and replicates in leukocytes.[1] The first report of EGA occurred in northern California in 1969.[2] The organism previously was known as *Ehrlichia equi*, hence the name of equine granulocytic ehrlichiosis, but reclassified based on genetic taxonomical analysis.[3,4] Cases of EGA have been reported in North America, Brazil, Europe, and North Africa.[1,5,6] The organism is transmitted by ixodid ticks such as *Ixodes scapularis* and *I. pacificus* in North America, and *I. ricinus* in Europe.[7-10] Infection with *A. phagocytophilum* has been detected in humans and other species worldwide.[11-15] Ticks involved in granulocytic anaplasmosis in humans (HGA) include *I. scapularis* and *I. pacificus* in North America, *I. ricinus* in Europe, and *I. persulcatus* in Asia.[16]

Clinical signs result from vasculitis and vary depending on severity and organ or tissue affected.[16,17] After an incubation period of a few days of a tick bite, common clinical signs include fever that can range from 38.9 to 41.7°C (102 to 107 °F), lethargy, anorexia, icterus, mild petechiation, limb edema, stiffness and, in some cases, ataxia.[18] Tachycardia and tachypnea are common during the febrile period, which can last a few days.[1,18] Younger animals (<3 years of age) appear to experience milder signs with minimal limb edema.[1] Laminitis and abortion in pregnant mares have not been reported with anaplasmosis.[1]

Common laboratory findings include anemia, leukopenia caused by neutropenia and lymphopenia, thrombocytopenia, and hyperbilirubinemia.[1] Inclusion bodies (morulae) in neutrophils or eosinophils are characteristic hematologic findings observed in Giemsa or Wright-stained buffy coat smears from horses and donkeys with EGA.[1] The use of qPCR for the detection of the organism's DNA in buffy coat samples has high sensitivity and specificity.[19] Positive qPCR results for *A. phagocytophilum* can be detected 2 to 3 days before clinical signs, whereas inclusion bodies in neutrophils can be detected in a buffy coat smear 2 to 3 days after the onset of fever.[20]

Currently, there are only 2 case studies reporting large numbers of EGA cases (49 and 54 cases) in the literature.[1,21] Atypical presentations of EGA occur, as evidenced by sporadic case reports that include horses with rhabdomyolysis, respiratory distress, cavitary effusion, disseminated intravascular coagulation (DIC), recumbency associated with neurologic disease, and death.[22-27] Furthermore, specific detailed descriptions and frequency of neurologic and muscle disease associated with anaplasmosis in equids are scarce in the literature.[1,21-24,28-30] Therefore, our objective was to review retrospectively cases diagnosed with EGA at a referral hospital, to determine the frequency of neurologic and muscle disease, and to describe their clinical signs, laboratory findings, treatment and outcome compared to cases with no neurologic or muscle disease. Other atypical presentations also were reviewed.

2 | MATERIALS AND METHODS

2.1 | Animals

The electronic medical records of equids seen at the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California Davis (UCD) from January 2000 through December 2022 were searched using the key words *anaplasma*, anaplasmosis, *phagocytophilum*, *ehrlichia*, ehrlichiosis, granulocytic, and rickettsia. Signalment, history, presenting complaint, and current clinical signs were recorded. Cases were included if a definitive diagnosis of anaplasmosis was made. Equids were grouped under 1 of 2 groups: (1) No neurologic or muscle disease (no-NM), and (2) Neurologic or muscle disease (NM) if any of these, or a combination of neurologic and muscle disease was identified. Genotype information was retrieved from the Neuromuscular Disease Laboratory (NDL) at UCD if available to investigate the presence of genetic variants known to cause muscle disease.[23] These included hyperkalemic periodic paralysis (HYPP), malignant hyperthermia (MH), polysaccharide storage myopathy type 1 (PSSM1), glycogen branching enzyme deficiency (GBED), myosin-heavy chain myopathy (MYHM), and myotonia congenita (MC). An institutional animal care and use committee protocol was not required for this retrospective study.

2.2 | Clinical laboratory

Diagnosis of anaplasmosis was achieved by cytological analysis or molecular testing for *Anaplasma phagocytophilum* from buffy coat samples. Clinical laboratory testing included CBC, serum biochemistry panel, and urinalysis. Muscle enzyme activities recorded included creatine kinase (CK) and amino aspartate transferase (AST). Other diagnostic tests such as troponin I, clotting panel, abdominal fluid analysis, muscle biopsy and cerebrospinal fluid (CSF) analysis were recorded when available.

2.3 | Muscle biopsy

Fresh frozen muscle specimens stored at −80°C were processed for the following stains and reactions: Hematoxylin and eosin, modified-Gomori trichrome, periodic acid Schiff, phosphorylase, esterase, Staphylococcal

protein A-horseradish peroxidase, myosin ATPase at preincubation pH of 9.8, 4.6, and 4.3, nicotinamide adenine dinucleotide, succinate dehydrogenase, acid phosphatase, alkaline phosphatase, and oil red O. When available, muscles specimens were processed for DNA extraction and qPCR analysis for the detection of *Anaplasma phagocytophilum*.

2.4 | Statistical analysis

Our hospital population during the study period consisted of 89 253 equids of multiple breeds consisting of 42 913 females and 45 679 males (castrated, 35 170; intact, 10 509). To investigate potential overrepresentation by breed or sex in horses with EGA, a Chi-squared test was conducted. The collected data underwent normality assessment using the Shapiro-Wilk test. For non-normally distributed data, nonparametric tests were utilized, and median values with 95% confidence intervals (CI) were reported. To compare laboratory variables among groups (no-NM, neuro, muscle), a nonparametric one way analysis of variance (ANOVA), Kruskal-Wallis test, was applied. For comparisons among groups, a post hoc test (Dunn's multiple comparison tests) was employed to obtain *P*-values. The threshold for statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Animals

Thirty-nine equids were diagnosed with EGA, 30 males (castrated = 28, intact = 2) and 9 females. Breeds included Quarter horse (QH) and related breeds (n = 14), Warmblood breeds (n = 8), Arabian (n = 3), Friesian (n = 3), mixed breeds (n = 2), and 1 of each: Thoroughbred, Tennessee Walker, Saddlebred, Mustang, Fjord, Gypsy Vanner, and 1 donkey. These equids were presented to our hospital from a few hours to 5 days after onset of fever. There was no apparent overrepresentation of breed with QH being the most popular breed at our hospital. Although, there was no overrepresentation of age, no horses < 4 years old were diagnosed at our hospital with EGA. Ages ranged from 4 to 30 years old (median, 14) with 11 equids between 4 and 9 years, 20 between 10 and 20 years, and 7 > 20 years of age. However, males were overrepresented compared to females ($P < .0001$; Chi-square, 20.98). During the study period, 0 to 3 cases were presented per year except for the years of 2014 and 2022 during which 6 horses were admitted each year. Cases were seen all year round except during July, August, and September. Cases were seen during the spring (n = 12; March = 4, April = 7, May = 1), summer (n = 4 in June), fall (n = 3; October 1, November 2), and winter (n = 20; December 8, January 4, February 8).

Of 39 equids, 23 horses (59%) had ≥1 of the following: neurologic or muscle disease, cardiomyopathy, cavitary effusion, and DIC. The remaining 16 equids (41%, 1 donkey included) were classified as having other presentations (Table 1; for complete individual information see Table S1 [ST1]). Twenty horses had neurologic or muscle disease (NM), and 18 horses and 1 donkey did not (Table 1, ST1). These 19 equids classified in the no-NM group presented with fever, lethargy, anorexia, icterus, and limb edema with no apparent signs of neurologic or muscle disease. The remaining 20 equids classified in the NM group also presented with similar signs and had neurologic (n = 13), muscle (n = 4), or a combination of neurologic and muscle (n = 3) disease. Most horses with rectal temperature ≥ 103 °F appeared lethargic.

3.1.1 | Neurologic disease group

Sixteen horses (13 males: 12 castrated, 1 intact; 3 females) presented with central nervous disease involving the forebrain and brainstem (n = 3), spinal cord (n = 8), or a combination of both localizations (n = 5). Horses with brain disease displayed obtundation (n = 8/8), altered behavior (n = 3/8; compulsive walking, pressing or leaning the entire body against a wall), dysphagia (n = 3/8), tongue paresis (n = 2/8), multiple cranial nerve abnormalities (n = 1/8), and absent menace response (n = 1/8). Cranial nerve abnormalities consisted of asymmetrical mild facial paresis with ipsilateral head tilt, ipsilateral decreased facial sensation, dysphagia, and stertor presumed to be caused by laryngeal dysfunction of neurologic origin (not confirmed). Horses considered obtunded had other concurrent central nervous disease signs. Spinal cord disease manifested as diffuse general proprioceptive (spinal) ataxia in 13 horses and graded as grades 2 (n = 7/13), 3 (n = 5/13), and 4 (n = 1) of 5 according to a modified published scoring system.[31] Neurologic signs were symmetrical in 15 of 16 horses and asymmetrical in 1 horse with obtundation, multiple cranial nerve abnormalities, and spinal cord disease. Three of 16 horses with neurologic dysfunction also had muscle disease as described below.

3.1.2 | Muscle disease group

Seven geldings had clinical manifestations of muscle disease, 5 QH, 1 Paint horse (PT), and 1 Standardbred (SDB; Table 1, ST1). All 6 QH had apparent firm, painful, swollen muscles upon palpation, reluctance to walk, and stiff gait when walked. These horses were genotyped as part of the diagnostic evaluation for muscle disease, and all 6 were heterozygous for the variant known to cause MYHM (My = MYHM allele). Four of these 6 horses (data available from the UCD NDL) also were tested for other variants causing muscle disease (HYPP, GBED, MH, PSSM1, MC) and found to be N/N (normal). Two of these 6 horses with N/My also had neurologic disease (obtunded = 1, ataxia grade 2 = 1). Quarter horse breeds with the My allele had painful swollen muscles on presentation and developed rapid diffuse muscle atrophy within days. The SDB horse had diffuse marked muscle fasciculations, ataxia grade 2, and reluctance to walk, but did not appear painful upon palpation. Muscle fasciculations could have been associated with neuromuscular or nerve disease but because of concurrent mild increases in CK and AST activity with no identified cause (eg, recumbency, trauma), this case was grouped under muscle disease.

TABLE 1 Clinical laboratory.

Breed	Sex	Age (years) Median	Presentation RR	Temp (°F) 99-100.8	PCV (%) 30-46	WBC (cells/μL) 5000-11 600	NEUT (cells/μL) 2600-6800	LYMPH. (cells/μL) 1600-5800	PLAT (cells/μL) 100 000-225 000	FIB (mg/dL) 100-400	CK (IU/L) 119-287	AST (IU/L) 168-494	TB (mg/dL) 0.5-2.3	Hospital (days)
No neurologic or muscle disease (n = 19)														
QHs = 5, WB = 4, ARAB = 3, DONKEY =1, DRAFT = 1, FRIESIAN = 1, GYPSY = 1, MUST = 1, TB = 1	F = 6 (PREG = 1/6), X = 12, M = 1	15	NO NM = 19: DIC = 2/19, PERIT EFFUSION = 1/19	104.1	32.2	4450	3473	663	54 000	400	160.5	285	3.2	3
	Range	6-30		101.7-106.1	20.8-38.9	2590-16 190	2120-7286	231-3800	8000-138 000	300-1000	79-403	157-687	2-8.9	1-7
Neurologic disease (n = 13 [+3*])														
WB = 4, QHs = 3, TW = 2, ARAB = 1, FRIESIAN = 1, NORW = 1, TB = 1	F = 3, X = 9, M = 1	12	NEURO = 13: PERIT EFFUSION = 3/13, CARDIO = 1/13, DIC = 1/13	104.3	35.7	5510	3509	1446	53 500	600	186	258	3.9	5
	Range	6-30		102-106.7	25.3-39.2	2340-16 190	1685-7286	515-2886	30 000-146 000	200-1000	103-823	190-567	1.4-12.2	1-8
Muscle disease (n = 7 [3*]; QH breeds (6 = N/My)														
QHs = 6, SDB = 1	X = 7	17	MUSCLE = 7: NM = 3/7, CARDIO = 3/7	104	33.4	9950	7741	1512	73 000	600	101 077	10 269	3.1	5
	Range	5-26		101.8-105.8	31.6-37.2	2490-11 850	2117-8994	224-3339	27 000-270 000	400-1000	2763-959 453	685-20 705	1-4.2	4-9

Note: Median and range values or concentrations are presented, RR = reference range, Temp = temperature, PCV = pack cell volume, WBC = white blood cells, Neut = neutrophils, Lymph = lymphocytes, Plat = platelets, Fib = fibrinogen, CK = creatine kinase, AST = amino aspartate transferase, TB = total bilirubin, (3*) = 3 horses with neuro/muscle disease concurrently, NM = neurologic, muscle or neurologic and muscle disease, Neuro = neurologic disease, DIC = disseminated intravascular coagulation, and Cardio = cardiomyopathy. For breed, Arab = Arabian, Draft = Draft breed, Gypsy = Gypsy Vanner, Must = Mustang, Norw = Norwegian Fjord, QHs = Quarter Horse breeds (QH, Paint), SDB = Standardbred, TB = Thoroughbred, TW = Tennessee Walker, WB = Warmblood. F = female, Preg = pregnant, X = gelding, M = intact male, N/My = heterozygous for MYHM allele.

3.1.3 | Other presentations

Four horses had suspected cardiomyopathy based on the presence of ventricular tachycardia. These horses included 1 Warmblood with neurologic disease and 3 horses with muscle disease (QH = 2, SDB = 1; Table 1, ST1). Four horses had moderate to marked peritoneal effusion: 2 Warmbloods and 1 Tennessee Walker with neurologic disease, and 1 QH with no-NM disease (Table 1, ST1). Three horses were suspected of having DIC based on severity of disease, marked limb edema, and petechiation. These cases included 2 Friesians (no-NM = 1, neurologic = 1) and 1 Mustang (no-NM; Table 1, ST1).

3.2 | Clinical laboratory

Anaplasma phagocytophilum was identified in blood using qPCR (n = 9), cytological analysis (n = 8), or both (n = 22). Clinical laboratory testing consisted of CBC and serum biochemistry panel (Table 1, ST1). Degenerative left shifts with bands were observed in 13 horses with toxic changes in 7 horses, and metamyelocytes were identified in 2 horses. Seven horses with muscle disease had increased of CK and AST activity. Six horses of QH breeds with the N/My allele had median CK activity of 130 229 IU/L (range, 4043-959 453 IU/L; reference range, 119-287 IU/L) and median AST activity of 10 269 IU/L (range, 4222-20 705 IU/L; reference range, 168-494 IU/L). The SDB horse with muscle disease had CK activity of 2763 IU/L and AST activity of 685 IU/L. Kruskal-Wallis one way ANOVA analysis showed significantly higher CK activity in the muscle group (median, 101 077; 95% CI, −112 952, 520 099) when compared to the neuro group (median, 186; 95% CI, 133.1, 364) and no-NM group (median, 160.5; 95% CI, 126.0, 231.9; $P = .0002$; Figure 1A). Similarly, Kruskal-Wallis one way ANOVA analysis showed significantly higher AST activity in the muscle group (median, 10 269; 95% CI, 3777, 16 081) when compared to the neuro group (median, 258; 95% CI, 231.1, 350.2) and no-NM group (median, 285; 95% CI, 246.7, 424.8; $P = .0003$; Figure 1B). Other laboratory variables such as packed cell volume ($P = .11$), white blood cell counts ($P = .35$), neutrophil count ($P = .13$), and platelet count ($P = .39$) were compared among groups and not found to be significantly different. Azotemia was observed in 6 horses (serum creatinine concentration, 2.1-6.1 mg/dL; reference range, ≤2 mg/dL) and considered prerenal and renal in 4 and 2 horses, respectively. Clinical laboratory results are shown in Table 1 (ST1 shows individual animals).

Serum troponin I concentration was measured in 4 horses with suspected cardiomyopathy and found increased (median, 0.27 ng/mL; range, 0.17 to 0.36; reference range, 0 to 0.06 ng/mL). Two of these 4 horses had concurrent neurologic and muscle disease (1 was N/My), and 1 each had neurologic and muscle (N/My) disease. A clotting panel was performed in 3 horses with suspected DIC and found to be abnormal with increased fibrinogen concentration and fibrinogen degradation products (FDP), and decrease of antithrombin III < 50%. Serum amyloid A concentration was measured in 2 horses and found to be increased at 682 and 1774 μg/dL (reference range, 0-7.5 μg/mL),

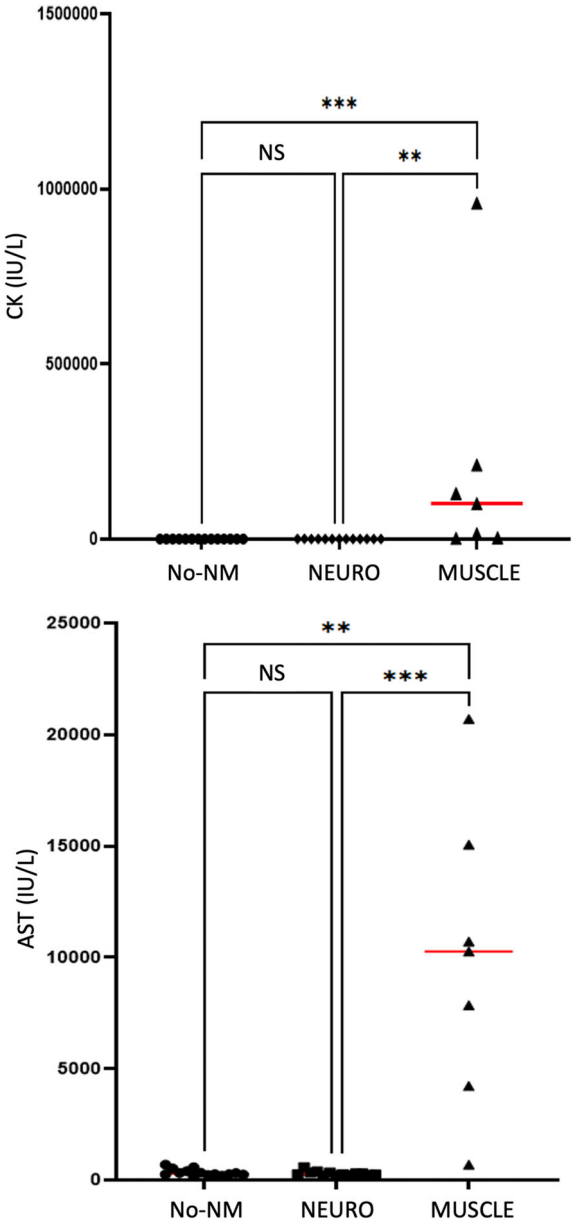

FIGURE 1 Muscle enzyme activities. (A) Creatine kinase (IU/L). (B) AST (IU/L). No-NM = no neuromuscular group, Neuro = neurologic disease, Muscle = muscle disease, NS = not significant, *P .0002 (A), *P .0003 (B) between disease groups. Horizontal line within groups represents the median value.

respectively. Urinalysis was performed in 8 horses and found to be abnormal in 2 horses with muscle disease with proteinuria (n = 2, 100 mg/dL) and isosthenuria of 36 hours' duration (n = 1 hydrated horse, USG 1.015).

Abdominocentesis was performed in 4 horses with peritoneal effusion and the fluid was found to be a modified transudate (total protein concentration, 2.5-4.1 g/dL; total nucleated cell count, 5300-13 580/μL). In 1 horse, morulae were observed in neutrophils in the abdominocentesis fluid. Cerebrospinal fluid collected from the lumbosacral space in 2 horses with neurologic disease appeared xantochromic, otherwise cytology was unremarkable (CSF protein

concentration and total nucleated cell count within reference range) despite mild numbers of RBC present in the fluid (50 and 190 RBC/μL).

3.3 | Muscle biopsy

A muscle biopsy was performed in 6 of 7 horses with muscle disease for the investigation of the underlying cause. Immunohistochemistry indicated marked myofiber size variation, myonecrosis, and mild histiocytic infiltration in the perimysial and endomysial areas in all 6 horses, consistent with the observed rhabdomyolysis. Two horses had occasional predominantly lymphocytic (CD3+, CD4+, CD8+) cellular infiltration in support of immune-mediated myositis (IMM), and *Anaplasma phagocytophilum* was detected by qPCR in skeletal muscle in these 2 horses. In 1 case, lymphocytic infiltration also was observed in intramuscular nerve branches and connective tissue.

3.4 | Treatment and outcome

All horses were treated with tetracyclines as follows: initially with oxytetracycline IV for 1 to 3 days (range, 6-7.5 mg/kg q12-24h) followed by either PO doxycycline (range, 7-10 mg/kg q12h) or minocycline (4.4 mg/kg q12h) for 5 to 14 days. Other treatments consisted of IV non-steroidal anti-inflammatory medications, IV fluids, PO anti-ulcer medication, and PO vitamin E. Marked clinical improvement including resolution of fever occurred within 24 to 96 hours of initiation of treatment. Horses with IMM received corticosteroids (IV dexamethasone, 0.06-0.1 mg/kg q24h) followed by PO prednisolone (1 mg/kg q24h) and muscle relaxants at the clinicians' discretion. Upon initiation of treatment, QH breeds with muscle disease had marked improvement with most signs resolved at the time of hospital discharge except for marked diffuse muscle atrophy. These horses had developed rapid muscle atrophy within days of hospitalization. All 39 horses recovered and were discharged to finish a course of PO tetracyclines at home. In addition, all QH breeds with muscle disease were discharged on gradually tapering doses of PO prednisolone. Horses with no-NM disease were hospitalized for 1 to 7 days (median, 3 days), neurologic disease from 1 to 8 days (median, 5 days), and those with muscle disease from 5 to 9 days (median, 5 days). No recurrence of signs was reported in these 39 horses upon short term follow-up (2 months). Muscle mass improved in QH breeds with the My allele within weeks to months after discharge.

4 | DISCUSSION

We described a cohort of 39 equids with anaplasmosis with both common and atypical presentations. Most cases included horses of multiple breeds and 1 donkey. An overrepresentation of males was found in this sample population. Most cases (82%) occurred during the spring and winter months. Clinical signs such as fever, lethargy, anorexia, icterus, and limb edema were observed in equids in our study with 41% having no other manifestations of disease. Neurologic disease was seen commonly (41%) and most often evidenced by symmetrical diffuse proprioceptive ataxia. Other less common presentations included muscle disease (n = 7/39), peritoneal effusion (n = 4), cardiomyopathy (n = 4), and DIC (n = 3) which in some cases occurred concurrently with neurologic or muscle disease. As expected, horses with muscle disease had increases in muscle enzyme activities. Quarter horse breeds with the My allele had severe muscle disease, evidenced by marked increases in muscle enzymes activities. Despite severe signs of disease, *Anaplasma phagocytophilum* was highly responsive to tetracyclines with signs improving or resolving within hours to days of treatment. Outcome was favorable for all horses, with no recurrence of infection or long-term sequelae.

Similar to humans with HGA, a male overrepresentation was observed in this equine population (humans, 57%-61%; horses, 77%).[16,32] Although this finding is of interest, its significance is unclear. In contrast, a sex predisposition was not found in another report of 54 equids.[21] Experimental infection in pregnant mares results in signs of variable severity with no disruption of fetal development and normal birth of full term foals.[17] Similarly, the 1 pregnant mare in our study recovered fully and the fetus was viable at the time of discharge. A single case report describes natural transplacental infection from a 4-year old Oldenburg mare to a newborn filly that was treated successfully using oxytetracyclines.[33] The median age in our study was similar to that of the other study (14 and 12 years, respectively).[21] No equids <4 years of age were diagnosed with EGA at our hospital. Young animals (<3 years) have been reported to develop mild disease.[1,21] Although naturally-occurring EGA is reported rarely in foals (3 cases in the English literature),[1,33,34] transfer of specific passive immunity to *A. phagocytophilum* was found in 80% of 22 foals born to seropositive mares with a decrease in antibodies by 3 months of age.[35] Despite being rare, the clinician should be prompted to investigate EGA in foals with consistent signs. In people, the median age of HGA is 50 years old.[32] Similar to another report from northern California, most cases were seen during the winter and spring months.[1] Also similar to previous studies, low numbers (1 or 2) or no cases were seen during July, August, September, and October.[1] Prevalence of disease might vary depending upon geographical location, because of seasonal weather conditions, environmental temperature, and tick burden. Therefore, cases might present at different seasons of the year in other parts of the United States. Reports of prevalence of disease in a large population of horses in the midwest and eastern United States are lacking.

Clinical anaplasmosis appears to be rare in donkeys based on our study and rare reports of naturally-occurring disease.[2,21] However, serum antibodies against *A. phagocytophilum* have been documented in non-diseased donkeys.[36,37] Disease in donkeys has been experimentally induced resulting in signs similar to those described in horses.[17] The sporadic cases of EGA in donkeys and lack of reports in mules suggests resistance or tolerance to natural infection in these species. However, donkeys and mules are underrepresented species at our hospital.

Reported common clinical signs in horses such as fever, lethargy, anorexia, icterus, and limb edema also were observed in our sample population. Previous studies indicated that clinical signs in horses depend on severity and organ or tissue affected with some organs and body regions more susceptible to vasculitis such as testis, ovaries, lung, kidney, brain, heart and limbs.[2,17] Diarrhea and laminitis have not been reported in horses with EGA. In our study, neurologic disease was common (41%) and involved the brain (forebrain and brainstem) and spinal cord or both based on neurologic examination by a board-certified veterinary neurologist, with signs being diffuse and symmetrical in most cases. General proprioceptive ataxia was the most common neurologic deficit and ranged from mild to severe with some cases affecting the pelvic limbs more than the thoracic limbs. Severe cases of ataxia can result in recumbency.[26] Noting these acute neurologic signs along with fever should prompt the clinician to rule out other acute infectious diseases (eg, equine herpes virus 1 myeloencephalopathy, West Nile, rabies), especially those highly contagious or with zoonotic potential (equine herpes virus 1 myeloencephalopathy, rabies). Multiple cranial nerve abnormalities, tongue paresis, dysphagia, laryngeal dysfunction, and lack of menace were less common in our sample population. These neurologic deficits are rarely reported.[24]

Abnormalities of clinical laboratory variables vary depending on disease stage and often include anemia, leukopenia with neutropenia and lymphopenia, thrombocytopenia, and hyperbilirubinemia caused by indirect hyperbilirubinemia.[1,2] Although these findings also were encountered in our horses, median PCV, WBC, and neutrophils counts were within the reference range in all groups, except for the WBC being below the reference range for the no-NM group (Table 1). Lymphopenia was seen in most horses from all groups. Thrombocytopenia was more commonly seen in horses with no-NM and neurologic disease than in horses with muscle disease. However, the median platelet count was below reference range for all groups. Although hyperfibrinogemia was more severe in horses with neurologic and muscle disease compared to the no-NM group, this difference was not significant. Hyperbilirubinemia caused by indirect hyperbilirubinemia was seen in all groups. Differences in these variables among groups were not significantly different. Horses at our hospital were presented at various stages of disease, which might have accounted for the variability of clinical laboratory test results, especially for those presented with muscle disease. Early diagnosis is essential to initiate appropriate treatment. A previous study reported lymphopenia, hyperbilirubinemia, and hyponatremia as linear predictors, and low creatine kinase activity was a nonlinear predictor of a positive qPCR for *A. phagocytophilum*.[21] Hyponatremia and low CK activity were not features in our horses (Table 1) and would have erroneously predicted the results of qPCR. On the other hand, lymphopenia and hyperbilirubinemia were consistent findings in our equine population, except for inconsistent lymphopenia in horses with muscle disease. Therefore, these potential predictors for positive qPCR results do not seem to apply for horses with muscle disease.

Horses with immune-mediated myositis (IMM: N/My, My/My) can have mild leukocytosis (60%) with neutrophilia, and hyperfibrinogemia.[30] This observation might explain why horses with EGA in the muscle group had higher WBC, although this difference not significant. Creatine kinase and AST activities were significantly higher in horses with muscle disease compared to other groups. Six of these 7 horses were QH breeds with the My allele. Our results suggest that QH with the My allele might be at risk of developing more severe muscle disease or trigger IMM if infected with *Anaplasma*. Reports of severe rhabdomyolysis and cardiomyopathy in horses associated with EGA are scarce.[22] In our study, 2 horses with IMM also had cardiomyopathy which resolved with corticosteroids and supportive treatment. Two horses with IMM had acute kidney injury, presumed to be multifactorial (dehydration, concurrent administration of non-steroidal anti-inflammatory drugs before referral, myoglobinuria, and possible vasculitis from EGA). These latter horses made a full recovery.

Muscle biopsy results supported a diagnosis of IMM in 2 of 6 QH with the My allele which prompted clinicians to use corticosteroids early in the disease course. Dexamethasone administration at 0.08 mg/kg suppresses the proinflammatory response caused by experimental infection of horses with *A. phagocytophilum*, resulting in cytokine changes that decrease severity of disease.[38] Dexamethasone decreased interferon gamma transcription, interleukin-8, and interleukin-18, and increased interleukin-4.[38] Clinically, those horses had delayed development of limb edema, decreased anorexia and icterus, and lower fevers compared to horses not treated with dexamethasone.[38] Therefore, the use of corticosteroids in these horses with IMM associated with EGA likely contributed to the rapid favorable outcome. *Anaplasma* was detected by qPCR in 2 horses with muscle disease. The pathogen infects neutrophils and eosinophils and not muscle, and therefore it is likely that blood contamination and DNA from the pathogen resulted in a positive qPCR. However, no obvious blood contamination or neutrophils were observed histologically in these muscles.

Although uncommon, cavitary effusion involving the pleural and peritoneal spaces associated with EGA can occur and is likely the result of leakage associated with vasculitis, and often is necrotizing.[17,25] The observation of morulae within neutrophils from peritoneal fluid has not been reported previously. Disseminated intravascular coagulation is uncommonly reported.[27] Based on clinical signs and severity, DIC was investigated and confirmed in 3 horses in our study. Other cases might have been missed because of lack of signs suggesting DIC. Because of the complete resolution of disease, DIC appears to be a secondary transient event.

Similar to humans with HGA, atypical presentations can occur in horses with EGA.[11] Here, we described rhabdomyolysis, cavitary effusion, cardiomyopathy, and DIC. Hospitalization often is required in people to manage infection and possible complications such as hemodynamic disturbances, myocarditis, heart failure, renal failure, acute respiratory distress syndrome, central (eg, meningoencephalitis) and peripheral (eg, palsies, demyelinating polyneuropathy, plexonopathy) nervous system disease, acute abdominal syndrome, rhabdomyolysis, and opportunistic infections.[11] Although death can occur in people with life-threatening complications, the risk is low (<1%).[11] Death has been reported rarely in horses with naturally-occurring and experimentally-induced EGA and associated with comorbidities or complications.[1,21,27] In our study, all equids survived to discharge and recovered fully.

Limitations of our study included its retrospective nature with potential missing or incomplete data. Some horses were treated with non-steroidal anti-inflammatory drugs before referral, affecting rectal temperatures obtained. Horses were admitted at various stages of disease resulting in variability of clinicopathologic data and detection of morulae within neutrophils. Additionally, different tetracycline protocols were used (type of tetracycline, dosage, dosing interval, and duration). However, all equids had favorable outcomes independent of tetracycline protocol with a minimum treatment duration of 6 days. Overrepresentation of atypical presentations is possible because of the nature of the cases seen at a referral hospital. Furthermore, cases presented throughout the year in our study reflect location of northern California; and might be different in other geographical areas.

In conclusion, EGA can manifest with common and atypical presentations affecting males more commonly. Foals and very young animals do not appear or are less likely to be clinically affected. Although rare, donkeys can suffer from naturally occurring disease. Peak months for infection occur during winter and spring in northern California. Neurologic disease was common in horses with EGA presented at our referral institution and mainly manifested as diffuse symmetrical proprioceptive ataxia. Other neurologic manifestations are less common but can occur involving the brain resulting in obtundation and cranial nerve abnormalities such as dysphagia and dyspnea. Although not common, rhabdomyolysis can occur and should prompt clinicians to investigate for MYHM in QH breeds, especially in severe cases. Cardiomyopathy is rare and can occur in horses with and without concurrent muscle disease. Cavitary effusion and DIC are also uncommon but might be missed unless specifically investigated. Depending on stage of disease, leukopenia with neutropenia and lymphopenia are common in horses with EGA, but less common in horses with muscle disease. Thrombocytopenia and hyperbilirubinemia caused by indirect hyperbilirubinemia are consistent findings. Independent of clinical presentation, outcome appears to be favorable regardless of the tetracycline protocol used, and death or long-term sequelae appear to be rare.

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CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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