CLINICAL DISEASE ASSOCIATED WITH *ANAPLASMA PHAGOCYTOPHILUM* INFECTION IN CAPTIVE PRZEWALSKI'S HORSES (*EQUUS FERUS PRZEWALSKII*)

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Abstract: *Anaplasma phagocytophilum* is a tick-borne pathogen of domestic horses and the causative agent of Equine Granulocytic Anaplasmosis. This case series describes three confirmed cases of clinical anaplasmosis, and a fourth case of presumptive anaplasmosis in Przewalski's horses (*Equus ferus przewalskii*) housed at the Smithsonian Conservation Biology Institute from 2008 – 2014. Clinical signs varied among individuals with affected horses exhibiting lethargy, weakness, pyrexia, hypophagia, reluctance to move, or ataxia. Anaplasmosis was confirmed with a combination of identification of neutrophilic inclusions (morulae) on peripheral blood smear, positive polymerase chain reaction (PCR) testing of whole blood, or convalescent titers. All animals recovered after antimicrobial therapy with oxytetracycline. Diagnosis should be made by a combination of clinical signs plus identification of morulae or positive *A. phagocytophilum* PCR. Disease is curative with treatment using oxytetracyline intramuscularly or intravenously followed by daily therapy with oxytetracyline or minocycline for 14 – 30 days. The authors recommend that *A. phagocytophilum* infection be included on any differential list for Przewalski's horses presenting with fever or ataxia within or near an enzootic area.

Key words: *Anaplasma phagocytophilum*, anaplasmosis, *Equus ferus przewalskii*, PCR, Przewalski's horse.

INTRODUCTION

The Przewalski's horse (P-horse; *Equus ferus przewalskii*) is a subspecies of the wild horse (*Equus ferus*) that is native to central Asia. P-horses became extinct in the wild in the late 1960s primarily due to habitat loss, hunting, and competition with domestic livestock.[6] With the assistance of ex-situ conservation programs, the species has been successfully reintroduced to Mongolia and China, and is now classified as endangered by the International Union for Conservation of Nature.[6]

The Smithsonian Conservation Biology Institute (SCBI) in Virginia, USA has contributed to P-horse ex-situ conservation for nearly four decades with P-horses housed in modified domestic animal barns with access to grazing pastures surrounded by forest and agricultural fields. Annual vaccination, at the time of this manuscript development, has been consistent for the last decade, and includes a killed rabies virus and *Neorickettsia* (*Ehrlichia*) *risticii* bacterin combination vaccine (POTOMAVAC + IMRAB, Merial Limited, Inc., Duluth, Georgia 30096, USA), and a killed West Nile Virus (WNV), eastern equine encephalomyelitis virus (EEE), western equine encephalomyelitis virus (WEE), and tetanus toxoid combination vaccine (WEST NILE-INNOVATOR +EWT, Zoetis, Florham Park, New Jersey 07932, USA).

Anaplasma phagocytophilum (formerly *Ehrlichia equi*) is an emerging tick-borne, non-contagious pathogen that causes Equine Granulocytic Anaplasmosis (EGA, formerly Equine Granulocytic Erhlichiosis) in domestic horses, and Human Granulocytic Anaplasmosis.[2,8] This pathogen is seasonally transmitted by the *Ixodes* spp. tick vector and has a worldwide distribution throughout North America, Europe, Africa, and Asia.[3,8,11] This gram-negative cocci rickettsial organism has a tropism for granulocytes.[8] Acute infection results in the formation of

morulae, which are granular aggregates (inclusion bodies) within the cytoplasm of neutrophils that stain bluish-gray with Giemsa or Wright-Leishman stains.[3,8,11] Domestic horse EGA was first described in 1969 in California, USA, and recently has been reported in Colorado, Connecticut, Florida, Illinois, Minnesota, Virginia, and Wisconsin.[3,7,8] Ticks of varying species are present throughout the SCBI property including *I. scapularis*, and *A. phagocytophilum* is endemic in this region.[1]

Clinical disease in multiple P-horses at SCBI with evidence of *A. phagocytophilum* infection prompted a retrospective review of medical records for cases of anaplasmosis. The medical records of 31 P-horses housed at SCBI from 2008 to 2014 were reviewed to develop a description of clinical anaplasmosis in P-horses, including diagnostic methods and treatments. Data compiled from the records included gender, age, presenting signs, physical examination findings, diagnostic testing results, treatment, and outcome (Tables 1-2). From 2008-2014, three confirmed cases of clinical anaplasmosis in captive P-horses were diagnosed and treated at SCBI; one of these P-horses was diagnosed with *A. phagocytophilum* re-infection or recrudescence five years after the initial diagnosis. A fourth case of presumptive anaplasmosis was tentatively diagnosed based on clinical signs, exclusion of other etiologies, and response to empirical therapy.

CASE REPORTS

Case 1

An estimated 14-yr-old, female P-horse (238 kg) that had been in the collection for five months presented for acute lethargy and severe ataxia in hind limbs in July 2008. The animal had no known clinically relevant history. At presentation, the horse collapsed into lateral recumbency while being transported to the onsite veterinary hospital for evaluation. Under chemical restraint, the animal was examined revealing dehydration (tacky mucous membranes and prolonged capillary refill time) and pyrexia (rectal temperature 103 ˚F). The P-horse was administered crystalloid fluid therapy (5% Dextrose and 0.9% Sodium Chloride Injection; Abbott Laboratories, Abbott Park, Illinois 60064, USA; 11 L i.v.), ceftiofur sodium (Naxcel; Zoetis; 4.2 mg/kg i.v.), flunixin meglumine (Banamine; Merck Animal Health, Kenilworth, New Jersey 07033, USA; 1.3 mg/kg i.v.), vitamin E (Natural E-300; Neogen Corp., Lexington, Kentucky 40511, USA; 12.6 IU/kg s.c.), and dexamethasone sodium phosphate (Dexium-SP; Bimeda Inc., Riverside, Missouri 64150, USA; 0.13 mg/kg s.c.).

Contusions were noted following placement of a jugular intravenous catheter and at other injection and phlebotomy sites during the exam. Following anesthetic recovery, the animal was unable to stand, so was supported with a sling positioned under the ventrum with its hooves contacting the ground. A complete blood count (CBC) and serum chemistry panel revealed leukopenia due to a lymphopenia (WBC count 4.1 $x10^3$ cells/µl; reference range 4.8 - 12.36 $x10^3$ cells/µl; lymphocyte count 0.5 $x10^3$ cells/µl; reference range 0.87 - 5.58 $x10^3$ cells/µl), thrombocytopenia (55 $x10^3$ cells/µl; median 190 cells/µl, range 14 – 474 $x10^3$ cells/µl), and hypophosphatemia (1.7 mg/dL; reference range 2.2 – 7.8 mg/dL).[16]

Morulae were identified within neutrophils by microscopic evaluation of a peripheral blood smear, resulting in a diagnosis of presumptive anaplasmosis. Oxytetracycline HCl (Oxytet-100; Norbrook Inc., Lenexa, Kansas 66219, USA; 8.4 mg/kg i.m. q. 12 hr for 10 days)

was initiated that day. Polymerase chain reaction (PCR) assays on whole blood were positive for *A. phagocytophilum* and negative for *N. risticii* (Cornell Animal Health Diagnostic Center [CAHDC], Ithaca, New York 14853, USA). By serum neutralization (SN), the P-horse was seropositive for WNV (1:768; CAHDC). Enzyme-linked immunosorbent assay (ELISA) tests revealed the P-horse was seronegative for EEE and WEE viruses (Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, Texas 77843, USA).

Improved alertness and responsiveness were noted the day after presentation, but ataxia persisted, and the P-horse remained sling-assisted due to generalized weakness. A recheck CBC revealed a mild neutrophilic leukocytosis (17.6 $x10^3$ cells/µl; neutrophil count 14.6 $x10^3$ cells/µl; reference range 1.74 - 8.05 $x10^3$ cells/µl) with lymphopenia (0.4 $x10^3$ cells/µl) and monocytosis (2.5 $x10^3$ cells/µl; reference range 0.06 – 0.66 $x10^3$ cells/µl).[16] Thrombocytopenia persisted (77 $x10^3$ cells/µl). The P-horse's attitude was considered normal at day 4 as the animal was unapproachable without sedation. Eight days after presentation, standing sedation was implemented for follow-up venipuncture. A CBC revealed persistent thrombocytopenia (81 $x10^3$ cells/µl), and morulae were no longer observed in the neutrophils. The animal was PCR-negative for *A. phagocytophilum* (CAHDC). Ten days after presentation, the P-horse was no longer showing any clinical signs.

Following this episode of clinical anaplasmosis in 2008, the P-horse remained clinically healthy for several years. One year after infection, this animal was PCR-negative (CAHDC) and seronegative (<1:20) for *A. phagocytophilum* by IFA (University Tennessee College of Veterinary Medicine Diagnostic Laboratory Services [UTDLS], Knoxville, Tennessee 37996, USA). The UTDLS laboratory considers titers ≥ 1:80 to be moderate to high in level of antibody and likely more indicative of current or recent exposure. For the purposes of this manuscript,

titers ≥ 1:80 will be referred to as positive. Three years from initial presentation, it had seroconverted (1:1280; UTDLS) with no evidence of infection in the intervening years.

Five years after the original clinical diagnosis of anaplasmosis, this individual (19-yr-old, 277-kg), presented with a 24-hour history of moderate bilateral ataxia in the hind limbs and conscious proprioceptive deficits. The horse was restrained in a hydraulic mechanical restraint device (Fauna Hydraulic TAMER, Fauna Research Inc., Red Hook, New York 12571, USA) for exam with a towel as an eye cover, and found to be quiet, alert, responsive, and euthermic. Blood was collected. CBC and serum chemistry results were unremarkable with platelets scored as 'adequate,' but not quantified. No morulae were seen on microscopic examination of the blood smear. Despite laboratory findings, anaplasmosis was suspected and long-acting oxytetracycline (Noromycin 300 LA; Norbrook Inc.; 10 mg/kg i.v.), flunixin meglumine (1.1 mg/kg i.v. once, then p.o. q. 24 hr for 7 days), and vitamin E (10.2 IU/kg s.c.) were administered. Three days after presentation, therapy continued with oral minocycline HCl (Ranbaxy Pharmaceuticals Inc., Jacksonville, Florida 32257, USA; 100 mg capsules; 4.1 mg/kg p.o. q. 12 hr for 28 days). Ataxia was mild by eight days after presentation. The horse was considered neurologically normal one month after presentation. Paired convalescent titers showed a four-fold increase in *A. phagocytophilum* from presentation to 36 days later (1:320 versus 1:1280; UTDLS), and the animal was PCR-negative at both of these timepoints (CAHDC).

Case 2

A 21-yr-old, male P-horse (345 kg) presented with a 24-hour history of lethargy and ataxia in October 2009. The animal had been in the SCBI collection for 1.5 years prior to presentation with no relevant medical history. On presentation, the animal exhibited depression, ataxia in all four limbs, and decreased blink reflexes on the right side. Under chemical restraint, the P-horse was examined, revealing pyrexia (rectal temperature 103.7 ˚F). Cytology from a mucus sample off the endotracheal tube revealed moderate suppurative and histiocytic inflammation, and very rare, morulae in the neutrophils. Blood was collected; a CBC and serum chemistry revealed marked thrombocytopenia (38 x10^3 cells/µl) and hypophosphatemia (1.3 mg/dL). Morulae were observed, but rarely, in neutrophils in a peripheral blood smear. Lateral cervical vertebral radiographs were unremarkable. The P-horse was administered oxytetracycline HCl (11.6 mg/kg, i.m.), flunixin meglumine (0.58 mg/kg i.v.), vitamin E (8.7 IU/kg s.c.), dexamethasone sodium phosphate (0.012 mg/kg i.v.), and crystalloid fluids (Lactated Ringer's Injection USP; Abbott Laboratories; 6 L i.v.).

Anaplasmosis was diagnosed in this P-horse by observation of morulae, positive PCR testing for *A. phagocytophilum* (CAHDC), and negative IFA (<1:20; UTDLS). The P-horse was found to be negative for equine herpesvirus-1 (EHV; SN, 1:24), EHV-2 (SN, 1:48), and *N. risticii* (IFA, <1:200) (CAHDC). The P-horse was negative for WNV acute infection (IgM capture ELISA, 2.125), and a high serum neutralization titer (1:1536) demonstrated strong humoral response, likely from vaccination (CAHDC). The P-horse was seropositive for *Sarcocystis neurona* (IFA, 1:640, CAHDC).

Within hours of treatment the P-horse was bright, alert, and responsive with mild ataxia in all limbs. Oxytetracycline HCl administration continued for 7 days (5.8 mg/kg i.m., q. 12 hr

for 2 days, then q. 24 hr for 5 days). Clinical resolution of neurological signs occurred four days after presentation/initial treatment with no residual ataxia or other neurological abnormalities.

Case 3

A 24-yr-old, male P-horse (273 kg) presented with a three-day history of hypophagia, lethargy, and hind limb ataxia in November 2012. The horse had been in the collection for 12 years and had no relevant medical history. With a dull attitude, poor appetite, and hind limb ataxia, empirical therapy with minocycline HCl (4.4 mg/kg p.o. q. 12 hr for 14 days) was initiated. After three days with minimal improvement the animal was chemically restrained for evaluation, and there were no relevant findings on examination. Lateral cervical radiographs revealed moderate osteoarthritis at C4/C5 and C5/C6, but no apparent narrowing of the spinal canal. Blood was collected; a CBC and serum chemistry were unremarkable. No morulae were evident on peripheral blood smear. PCR for *A. phagocytophilum* was negative with a moderate positive titer by IFA (1:160; UTDLS). Ultrasound-guided cervical centesis was performed to obtain a cerebrospinal fluid (CSF) sample. The CSF was considered normal compared to the domestic horse: clear in character with very low cellularity (WBC 1.1 cells/µl, RBC 1.1 cells/µl) and a specific gravity of 1.006.[14] *Sarcocystis neurona* Surface Antigen (SAG) 2/3/4 assays revealed a serum:CSF titer ratio of 200, which indicates exposure, but not active infection in the domestic horse (Equine Diagnostic Solutions [EDS], Lexington, Kentucky 40511, USA), and was interpreted the same in this P-horse.

The animal was administered oxytetracycline HCl (10 mg/kg i.v.), vitamin E (11 IU/kg s.c.), and ceftiofur crystalline free acid (Excede; Zoetis; 6.6 mg/kg s.c.). Nearly immediate

improvement in mentation was noted after evaluation and treatment with intravenous oxytetracycline. The following day, no ataxia was noted, but hypophagia persisted. The animal was clinically normal four days after intravenous treatment with oxytetracycline began (one week after onset of clinical signs). A follow up *A. phagocytophilum* titer conducted three years later was negative (<1:20; UTDLS).

Case 4

A 1.5-yr-old, female P-horse (213 kg) presented with sudden onset lethargy and an abnormal gait in April 2014. The P-horse was hanging its head low with bilateral ptosis, and had a wide hind limb stance and gait. Under physical restraint in a hydraulic mechanical restraint device, examination found pyrexia (rectal temperature, 106.5 ˚F), tachypnea, weight loss (10-kg in one week), and dehydration. Following venipuncture to sample blood, the P-horse was treated with oxytetracycline HCl (10 mg/kg i.v.), flunixin meglumine (1.1 mg/kg i.v.) and crystalloid fluids (Lactated Ringer's Injection USP; 1.5 L i.v. and 1 L s.c.). Later that day, the P-horse was sedated with hydraulic restraint for further therapy, and the P-horse's temperature and respiratory rate had normalized. Crystalloid fluid therapy (0.9% Sodium Chloride Injection; Abbott Laboratories; 6 L i.v.) and ceftiofur crystalline free acid (2.3 mg/kg i.m.) were administered. Topical permethrin (Equi-spot; Farnam Companies, Inc., Phoenix, Arizona 85013, USA; 5 mL) was applied to the withers and dorsal hindquarters for tick control. The animal was neurologically appropriate with a normal gait when released from the restraint device.

The CBC and serum chemistry from the sample taken during the morning restraint revealed hypophosphatemia (1.6 mg/dL) with morulae in the neutrophils on a peripheral blood

smear and buffy coat smear. PCR for *A. phagocytophilum* was positive (CAHDC), and the animal was seronegative by IFA (<1:20; UTDLS). A western blot for *S. neurona* was negative, and a SAG 2/3/4 ELISA titer on serum was positive at the lowest threshold (1:250), which suggests exposure but not clinical disease (EDS). WNV IgM capture ELISA was positive, which was likely due to vaccination which had occurred one week prior (EDS). The Lyme Equine multiplex titer revealed that the horse was negative for *Borrellia burgdorferi* SAGs OspA, OspC, and OspF (CAHDC).

The following day, the P-horse was clinically normal. Treatment was continued with oxytetracycline HCl (10 mg/kg i.m. q. 24 hr for 4 days) without return of clinical signs. The P-horse was restrained in a hydraulic mechanical restraint device for blood sampling 2, 28, and 63 days after presentation; the horse remained PCR-positive for *A. phagocytophilum* at two days, but negative at 28 days after presentation (CAHDC). The P-horse did not seroconvert and was titer negative at 28 and 63 days post-presentation (UTDLS).

DISCUSSION

This case series documents clinical anaplasmosis in P-horses at SCBI with clinical presentations that are similar to those seen with EGA in the domestic horse. Lethargy, ataxia, and pyrexia were the most common observed clinical signs in the P-horses infected at SCBI. The P-horses ranged from 1.5 to 24 years of age; all older P-horses (cases 1-3, aged 14-24 years) exhibited moderate to severe lethargy, severe ataxia, and mild pyrexia. The younger P-horse (case 4) was noted to have mild lethargy, mild ataxia, and severe pyrexia. This demarcation of clinical signs with respect to age is consistent with EGA in the domestic horse, where younger

242 animals have been found to have less severe general clinical signs, but have a more severe
243 pyrexia.[3,7]

244 Spontaneous petechiation, icterus, and dependent limb edema are common clinical signs
245 of EGA in the domestic horse that were not seen in these P-horses.[3,7,11] Petechiation in the
246 domestic horse is likely due to a coagulopathy secondary to consumptive thrombocytopenia.[3]
247 One P-horse with thrombocytopenia did exhibit multiple, iatrogenic contusions, but the other
248 case with thrombocytopenia did not show overt evidence of coagulopathy.

249 Hematologic and serum chemistry findings associated with anaplamosis in P-horses
250 differed from those seen in the domestic horse with EGA. Blood cell line aberrations were
251 minimally seen, which is markedly different findings than the pancytopenia that is common in
252 the domestic horse.[11] Hypophosphatemia occurred in the P-horses with anaplasmosis; however,
253 a conclusive cause for hypophosphatemia and the clinical relevance is unclear. This finding is
254 not reported in domestic horses with EGA.[3,7,8,11,14] Hypophosphatemia in the domestic horse can
255 be associated with chronic renal failure, hemoglobinuria, *Brassica* toxicity, inadequate dietary
256 intake, and hyperparathyroidism.[14] With the exception of inadequate dietary intake, there was no
257 evidence of any of these disease processes in the P-horses evaluated. Hypophagia was
258 confirmed in one case (case 3, presumptive anaplasmosis) that did not exhibit
259 hypophosphatemia.

260 Anaplasmosis was diagnosed in 3 of 4 clinically abnormal P-horses based on the
261 identification of morulae in the neutrophils, positive *A. phagocytophilum* PCR, or four-fold or
262 greater increase in paired convalescent IFA titers. Importantly, all individuals with morulae in
263 their neutrophils were also PCR-positive for *A. phagocytophilum* and were febrile at exam. The
264 humoral response to *A. phagocytophilum,* as measured by IFA serology, appears less indicative

of clinical disease as two of the three confirmed cases did not seroconvert, so does not have much value based on one time point. Serosurveillence for *A. phagocytophilum* in P-horses at SCBI was conducted, but is outside the scope of this report and has been reported separately.[13]

Case 1 remained seronegative one year following its initial infection, but was found to have a high titer two years later without clinical signs of disease, then developed clinical disease again five years after the initial infection. This individual may have undergone different episodes of reinfection, recrudescence, or perhaps the magnitude of titers does not correspond to the progression of this disease or antibody response in the P-horse. Historically, it has been asserted that domestic horses do not maintain a chronic carrier state for *A. phagocytophilum*,[7,11] but molecular persistence of the pathogen was recently demonstrated in the bloodstream of asymptomatic, domestic horses for up to four months after experimental infection.[4] Notably, immunosuppression in the five domestic horses of this study was induced with dexamethasone treatment or trailer transport before PCR-positive results were achieved.[4] It is unknown whether persistent *A. phagocytophilum* infection occurs in P-horses.

Follow up serologic monitoring was not standardized for this case series, but case 1, during its 2013 infection event, shows that a four-fold increase between paired serum samples (acute versus convalescent) is possible. Case 4 was similarly monitored, but showed no seroconversion at all. In naturally infected domestic horses, a peak antibody titer occurs 19 to 81 days, so a rising convalescent titer is a significant finding and is a definitive means of diagnosing EGA.[7,11,15] Further study monitoring of convalescent titers for *A. phagocytophilum* in P-horses is needed.

The ancillary diagnostics for each P-horse reported in this case series were selected based on clinical judgment at the time of presentation. Investigated pathogens included: *N. risticii*,

WNV, EEE, WEE, EHV-1, EHV-2, *S. neurona*, and *B. burgdorferi*. Ancillary testing results were negative for these pathogens except for *S. neurona* (case 2) and WNV (cases 1, 2, and 4). Case 2 had strong humoral response to *S. neurona* (high enough to expect natural exposure and disease in domestic horses), but clinical signs improved without directed treatment for this pathogen. Serologic findings for WNV were complicated by humoral immunity related to routine vaccination.

Treatment of anaplasmosis in P-horses consisted of oral minocycline, parenteral oxytetracycline, anti-inflammatory medications, and supportive therapies. In domestic horses, treatment with tetracycline drugs is considered critical as this class of drugs can penetrate cells and act on intracellular pathogens.[3,5,11] Initiation of therapy resulted in the majority of P-horses showing improvement of attitude and ataxia within 12-24 hours, and normalization of pyrexia in this time period as well. In the domestic horse, it is suggested that a failure to return to normal rectal temperature within 24-hours of starting tetracycline therapy is evidence that anaplasmosis is not responsible for the horse's illness.[7,11] Of note, the immunomodulatory effects of tetracyclines can benefit the treatment of inflammatory pathologies.[5] In all the cases, oxytetracycline was administered parenterally at least once. Cases 1 and 2 initially received injections of oxytetracycline twice a day, which is a higher treatment frequency than is recommended for the domestic horse.[3,9,11] Once daily therapy of oxytetracycline in case 4 appeared to be effective. No adverse effects associated with oxytetracycline were noted in the P-horses.

Oral minocycline was administered to two of the four cases and was well tolerated at a dosage used in domestic horses.[9] Doxycycline is commonly administered as enteral therapy for domestic horse EGA, but minocycline was chosen in these cases based on manufacturer

311 availability. With high oral bioavailability, minocycline penetrates the central nervous system
312 (CNS) better than doxycycline, but, as the mechanism for ataxia in affected P-horses is
313 vasculitis-associated cerebral edema, CNS penetration is not an advantage of using
314 minocycline.[7,9] Although minocycline successfully treated P-horses after initial intravenous or
315 intramuscular injection of oxytetracycline, the reverse was not true. In case 3, minocycline was
316 an initial empirical therapy, but did not result in rapid resolution of clinical signs as was seen
317 with oxytetracycline therapy. For this reason, case 3 was anesthetized three days into the disease
318 course, oxytetracycline was administered, and the individual improved rapidly afterwards. The
319 authors suggest all suspected cases of anaplasmosis in the P-horse should be initially treated with
320 at least one dose of parenteral oxytetracycline before the use of an oral tetracycline drug.

321 All clinical signs of initial infection resolved within 10 days with re-infection taking
322 longer to resolve (30 days). As such, treatment for initial anaplasmosis is recommended for 14
323 days, but in the case of re-infection treatment may need to be extended.

324 Case 3 is defined as a suspect case in this series based on a negative PCR result, but the
325 authors believe that this was a false PCR-negative result. Other differential diagnoses for
326 pyrexia and ataxia were adequately ruled out as there was no evidence of cerebral disease,
327 cervical vertebral stenotic myelopathy, or thiamine deficiency, and without xanthochromia,
328 pleocytosis, or an elevated protein level within the CSF sample, bacterial meningitis, verminous
329 meningoencephalomyelitis, and viral meningitis are of low likelihood.[12,14] A normal CSF
330 sample is consistent with anaplasmosis as the pathophysiology of anaplasmosis-associated ataxia
331 is thought to be related to vasculitis and cerebral edema.[7] Samples for PCR for case 3 were
332 obtained after three days of minocycline therapy and resulted in a negative PCR result. Case 4
333 was found to be PCR-positive after receiving parenteral oxytetracycline therapy for two days;

whereas, case 1 was PCR-negative at eight days. Based on case 3, the authors theorize that empirical therapy before samples for PCR confounded the results, and recommend that samples be obtained before tetracyclines are administered if a definitive diagnosis is desired.

In summary, *A. phagocytophilum* can result in clinical anaplasmosis in P-horses with similar disease description to EGA of domestic horses. In the P-horse, lethargy, ataxia, and fever are the most common clinical signs. Thrombocytopenia and leukopenia occurred in the P-horse with anaplasmosis but were less common findings as compared to the domestic horse, and anemia was not documented. A potentially unique characteristic of anaplasmosis in the P-horse is hypophosphatemia, but the clinical significance is unclear. A definitive diagnosis of *A. phagocytophilum* infection in the P-horse can be made based on the combination of clinical signs (i.e. febrile), presence of morulae in neutrophils, and PCR-positive testing. A four-fold or greater rise in convalescent IFA titers is associated with clinical disease of P-horse anaplasmosis, but further studies are needed to understand the immunology of anaplasmosis in the P-horse. Serology should be considered to have limited utility if measured at only one timepoint. Similar to the domestic horse, initiation of tetracycline therapy and supportive care in P-horses results in rapid improvement of clinical signs. Based on this case series, oxytetracyline (8.4 – 11.6 mg/kg i.v. or i.m.) once followed by once daily injectable oxytetracycline or twice daily oral minocycline (4.1 – 4.4 mg/kg) is recommended for the treatment of anaplasmosis in P horses. *A. phagocytophilum* infection should be included as a differential for ataxia and pyrexia in P-horses housed within or near an enzootic area.

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Table 1. Characterization of *Anaplasma phagocytophilum* infection in captive Przewalski's horses (*Equus ferus przewalskii*) at the Smithsonian Conservation Biology Institute in Virginia, USA between 2008-2014 with ancillary diagnostics.[a]

Case	Sex	Age (yr)	Onset	Clinical signs	CBC & morphology	Serum chem.	Testing for *A. phagocytophilum*				Ancillary diagnostics
							PCR[b]		IFA[c]		
							Initial	Follow-up	Initial	Follow-up	
1[*]	F	14	Jul 2008	Lethargy, severe ataxia, mild pyrexia, dehydration	↓WBC, ↓platelets, morulae in neutrophils	↓P	Pos	Neg (8 days)	NT	NT	*Neorickettsia risticii* PCR negative[b] EEE IgM ELISA negative[d] WEE IgM ELISA negative[d] WNV SN, 1:768[b]
		19	Jun 2013	Moderate ataxia	NSF	NSF	Neg	Neg (36 days)	1:320	1:1280 (36 days)	NT
2	M	21	Oct 2009	Lethargy, moderate ataxia, decreased blink reflexes, mild pyrexia	↓platelets, morulae in neutrophils	↓P	Pos	NT	<1:20	NT	EHV-1 SN, 1:24; EHV-2 SN, 1:48[b] *N. risticii* IFA, <1:200[b] WNV: SN, 1:1536; IgM Capture ELISA, 2.125[b] *Sarcocystis neurona* IFA, 1:640[b]
3	M	24	Nov 2012	Lethargy, hypophagia, ataxia	NSF	NSF	Neg	NT	1:160	NT	CSF, clear, WBC 1.1 cells/µl, RBC 1.1 cells/µl & specific gravity 1.006 *S. neurona* SAG 2/3/4 titers: Serum, 1:1000; CSF, 1:5; Serum:CSF titer ratio, 200[e]
4	F	1.5	Apr 2014	Lethargy, ptosis, wide-based stance, marked pyrexia	Morulae in neutrophils	↓P	Pos	Pos at 2 days, Neg at 28 days	<1:20	<1:20 (28 & 63 days)	*S. neurona* Western blot negative; SAG 2/3/4 ELISA titer on serum 1:250[e] WNV IgM capture ELISA positive[e] Lyme Equine multiplex titer, negative for *Borrellia burgdorferi* SAGs OspA, OspC, and OspF[b]

[*] Individual had a recurrence of anaplasmosis five years after initial diagnosis.
[a] CBC, Complete blood count; chem., chemistry; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; EEE, eastern equine encephalomyelitis; EHV, Equine herpesvirus; F, female; IgM, immunoglobulin M; IFA, indirect fluorescent antibody assay; M, male; Neg, negative; NSF, no significant findings; NT, not tested; ↓P, hypophosphatemia; PCR, polymerase chain reaction; ↓platelets, thrombocytopenia; Pos, positive; SAG, surface antigen; SN, serum neutralization assay; ↓WBC, leucopenia; WEE, western equine encephalomyelitis; WNV, West Nile Virus.
[b] Cornell Animal Health Diagnostic Center, Ithaca, New York 14853, USA.
[c] University Tennessee College of Veterinary Medicine Diagnostic Laboratory Services, Knoxville, Tennessee 37996, USA.
[d] Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, Texas 77843, USA.
[e] Equine Diagnostic Solutions, Lexington, Kentucky 40511, USA.

Table 2. Treatment of *Anaplasma phagocytophilum* infection in captive Przewalski's horses (*Equus ferus przewalskii*) at the Smithsonian Conservation Biology Institute in Virginia, USA between 2008-2014.[a]

Case #	Onset	Antimicrobial therapy	Supportive therapies	Disease duration (days)
1[*]	Jul 2008	Oxytet HCl 8.4 mg/kg i.m. q. 12 hr for 10 d Ceftiofur sodium 4.2 mg/kg i.v.	BW sling support for 3 d 5% dextrose 11 L i.v. Flunixin meglumine 1.3 mg/kg i.v. Vitamin E 12.6 IU/kg s.c. Dex-SP 0.13 mg/kg s.c.	10
	Jun 2013	Oxytet-LA 10 mg/kg i.v. once Minocycline HCl 4.1 mg/kg p.o. q. 12 hr for 28d, started on day 3	Flunixin meglumine 1.1 mg/kg i.v. once, then p.o. for 7 d Vitamin E 10.2 IU/kg s.c.	30
2	Oct 2009	Oxytet HCl 11.6 mg/kg i.m. once, then 5.8 mg/kg i.m. q. 12 hr for 2 d, then 5.8 mg/kg q. 24 hr for 5 d	LRS 6 L i.v. Flunixin meglumine 0.58 mg/kg i.v. Vitamin E 8.7 IU/kg s.c. Dex-SP 0.012 mg/kg i.v.	7
3	Nov 2012	Minocycline 4.4 mg/kg p.o. q. 12 hr for 14 d Oxytet HCl 10 mg/kg i.v. at day 3 CCFA 6.6 mg/kg s.c. at day 3	Vitamin E 11 IU/kg s.c.	4
4	Apr 2014	Oxytet HCl 10 mg/kg i.v. once, then 10 mg/kg i.m. q. 24 hr for 4 d CCFA 2.3 mg/kg i.m.	Flunixin meglumine 1.1 mg/kg i.v. LRS 1.5 L i.v. and 1 L s.c. 0.9% NaCl 6 L i.v. Permethrin 2.25 g topically	2

[*] Individual had a recurrence of anaplasmosis five years after initial diagnosis.

[a] Oxytet, oxytetracycline; HCl, hydrochloride; BW, body weight; Dex-SP, dexamethasone sodium phosphate; Oxytet-LA; long-acting oxytetracycline; LRS, Lactated Ringer's solution; CCFA, ceftiofur crystalline free acid.