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Transplacental infection of a foal with *Anaplasma phagocytophilum*

Running title: Transplacental anaplasmosis in a foal

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Summary:

This is the first report to document transplacental transmission of *Anaplasma phagocytophillum* in the horse. A 4-year old late term pregnant mare presented for a recent onset of pyrexia due to equine granulocytic anaplasmosis (EGA). She was hospitalized for treatment with oxytetracycline and monitoring of high-risk foaling due to significant thrombocytopenia. Parturition occurred overnight and the foal was PCR positive for *A. phagocytophilum* at birth. The foal was slow to stand and nurse, with signs of neonatal encephalopathy and anaplasmosis (thrombocytopenia). Therapy with oxytetracycline resulted in complete clinical recovery of the mare and foal within 5 days. Congenital anaplasmosis should be considered in any foal delivered to a mare suffering from EGA during late term pregnancy and guide appropriate antimicrobial therapy.

Key words: horse, anaplasma, pregnancy, critical care-large animal, internal medicine-equine

Abbreviations:

BPM, beats per minute; EGA, Equine granulocytic anaplasmosis; IV, intravenous; PCR, polymerase chain reaction; PCV, packed cell volume; rr, reference range; SAA, serum amyloid A

Case History

A 4-year-old 670 kg Oldenburg maiden mare presented on day 341 of gestation with a two-day history of decreased appetite, and a fever of 40 °C which developed that morning. She was bred using frozen semen via artificial insemination, with normal reproductive ultrasonography on days 15, 30 and 60 of gestation. The mare was routinely vaccinated for equine herpes virus (Pneumabort-K+1b[1]) during gestation. She lived with one other unaffected horse in an area known to be endemic for *A. phagocytophilum* and with a high tick population. The mare was fed free choice Timothy hay, 4.5 kg Timothy/alfalfa cubes, and 2.7 kg grain per day. Flunixin meglumine was administered intravenously (1.1 mg/kg IV) the morning of admission.

Clinical Findings

At presentation, the mare showed a normal rectal temperature (38.1 °C), mild tachycardia (48 beats per min (BPM)), and tachypnea (42 breaths per min). No nasal discharge was present, auscultation of the upper airway and thorax was unremarkable, and the tachypnoea resolved once the mare adjusted to the hospital environment. Abdominal ultrasonography was unremarkable for a late term pregnant mare, with normal foetal fluid depth and echogenicity, normal foetal activity, and an average foetal heart rate of 96 BPM.

Initial bloodwork indicated normovolaemia (packed cell volume (PCV) 40% (reference range (rr): 32-53%), total solids 70 g/L (rr: 52-79 g/L), lactate 0.6 mmol/L (rr: <2 mmol/L)). Complete haematology showed a slight leukopenia at 4.12 10^9/L (rr: 5.9-11.2 10^9/L) and mild neutropenia of 2.0 10^9/L (rr: 2.3-9.1 10^9/L) with morulae of the intracellular bacterium *A. phagocytophilum* being observed within neutrophils (Figure 1). Significant thrombocytopenia was present at 19 10^9/L platelets (rr: 92-253 10^9/L). Biochemistry was unremarkable, aside from a hyperfibrinogenemia of 5.0 g/L (rr: 1.0-4.0 g/L) and an elevated indirect bilirubin of 145 µmol/L (rr: 3-51 µmol/L). An intravenous catheter (14g 13cm IV polyurethane catheter-

over-needle[2]) was placed in the left jugular vein. The mare was sedated with 0.15 mg/kg xylazine (AnaSed LA)[3] IV and, following local anaesthetic administration (2% lidocaine)[4], a Foalert system[5] was sutured in place according to manufacturer's instructions. Therapy with 7.5 mg/kg IV oxytetracycline (Oxytet 100)[6] every 12 hours was initiated and adjusted to 10 mg/kg IV twice daily post-partum, with the mare treated for five days in total. The mare was fed according to her usual diet and monitored closely for signs of foaling.

The mare became restless at 12am the evening after presentation and a filly was naturally delivered at 3.30 am, following 15 minutes of Stage II labour. The foal was in anterior longitudinal and dorsosacral position with one forelimb flexed at the elbow. Spontaneous breathing was observed immediately following birth with the heart rate increasing to 120 BPM. Post-partum physical examination was unremarkable for a 52 kg full-term foal, with a grade 2 systolic murmur identified over the heart base. The umbilicus was dipped in 0.5% chlorohexidine gluconate (1:4 dilution of 2% Chlorohexidine)[3] and the mare and foal were allowed to bond under close observation.

Following parturition the mare became extremely protective and required sedation (0.3 mg/kg xylazine IV once; 0.02 mg/kg acepromazine IV twice (Aceproject)[7] to permit veterinary care of her foal. Flunixin (1 mg/kg IV; Flunixiject)[8] was also administered to supply analgesia. The mare's placenta was retained for 7 hours post foaling, despite repeated administration of oxytocin (7 units intramuscularly; Oxytocin)[8], hand-walking and gradual traction by a water-filled glove attached to the exterior portion of the placenta. Following expulsion of the main body of the placenta, the tip of the non-gravid horn remained retained. Uterine lavage was performed following standard protocols (15L of 0.9% sodium chloride[9]) and the residual placenta was weighted to provide continuous gentle traction. Intravenous crystalloid fluid therapy (30 ml/kg bolus Plasma-lyte A)[10] was also initiated, as the mare remained agitated following foaling with no water intake. Additionally, she was treated with Domperidone (6cc

Equidone Gel by mouth q24h)[11] due to limited milk production. Based on the mare's thrombocytopenia, retained placenta, and risk of haemorrhage, coagulation testing and blood cross matching were performed pre-emptively, demonstrating a normal prothrombin time (12.2 s; rr: 10.9-14.5 s) and a fast partial thromboplastin time (40.9 s; rr: 54.7-69.9s). Repeat haematology confirmed ongoing thrombocytopenia (15 10^9/L), but resolution of neutropenia (4.0 10^9/L) with *A. phagocytophilum* still observed. Administration of oxytocin (7-10 units intramuscularly q4-6h) was continued and treatment with enrofloxacin (7.5 mg/kg IV q24h; Baytril 100)[12] and polymyxin B[13] (2500 units/kg IV q12h for 2 doses) were added to counteract infection and endotoxaemia, respectively. The application of traction (by a water-filled glove connected via umbilical tape) on the retained placental tip resulted in its successful and complete expulsion at 8 hours post-partum. Histopathology of examined sections of placenta was unremarkable, with no evidence on inflammation. Uterine lavage was repeated 3 times over the next 2 days, followed by infusion of 6g ampicillin[14]. Enrofloxacin and oxytocin therapy were discontinued after resolution of all clinical abnormalities on day 3.

The filly failed to stand within 1 hour following delivery, despite a normal gestational length and parturition. Initial bloodwork showed an elevated PCV, marked hyperlactataemia, hypoglycaemia, and hypercreatininemia (Table 1). No morulae were identified on a blood smear. An intravenous catheter (14 Ga 15cm polyurethane over-the-wire catheter)[2] was, therefore, placed in the left cephalic vein, and 40 ml/kg Plasma-lyte A with 2.5% dextrose (Dextrose 50%)[15] was administered IV over the following hour. Blood was obtained for culture and anaplasma polymerase chain reaction (PCR), prior to any intake of colostrum. Anaplasma phagocytophilum (MSP2 gene) qPCR (Real-time PCR Research and Diagnostic Core Facility, University of California, Davis School of Veterinary Medicine, CA 95616, USA) was positive, whilst blood culture yielded no growth.

Four hours after delivery the filly's vital parameters were normal (temperature 38.1 °C, heart rate 100 bpm, respiratory rate 40 breaths per min), but she remained unable to stand unaided. Complete haematology at this time was unremarkable aside from a mild thrombocytopenia of 87 10^9/L, low globulin levels, increased indirect bilirubin and elevated creatine kinase (Table 1). Serum creatinine concentrations had improved to 168 /µmolL and the initial serum amyloid A (SAA) was normal at 0 µg/mL (rr: <50 µg/mL). Pending blood culture and anaplasma PCR results, the foal was started on oxytetracycline (7.5 mg/kg IV q12h) and amikacin (20 mg/kg IV q24h; Amiglyde-V)[16]. Vitamin E and selenium was supplemented (1ml IM once; E-Se)[17].

The foal received 2L frozen hyperimmune plasma (HiGamm-Equi)[18] within the first 24 hours of life, as the mare lacked colostrum production and frozen colostrum was unavailable. To facilitate nutritional support, the filly was initially fed via an indwelling nasogastric tube (12Fr 108cm feeding tube with stylet)[2]. She was frequently assisted to rise and encouraged to nurse, successfully latching on and nursing at 10 hours of age. By 18 hours post-partum the foal was urinating normally, with a urine specific gravity of 1.005. However, her blood lactate remained elevated (3.2-4.2 mmol/L) despite normohydration (PCV 38-40 % and total solids 48-52 g/L) and fluid administration (170 ml/kg Plasma-lyte A over 24 hours), suggesting type B hyperlactataemia (Table 1). Adequate immunoglobulin levels were confirmed (IgG > 8.0 g/L) at 24 hours of age, but the SAA had increased to 145 µg/mL, suggesting an ongoing inflammatory or infectious process. Intravenous fluid therapy was discontinued by 36 hours of age, as the foal could rise unassisted and was nursing independently. Subsequent, repeat haematology at 60 hours post-partum showed a mild neutrophilia (11.17 10^9/L) and progressive rise in SAA (196 µg/mL) which decreased to 140 µg/mL by day 5. Mild hyperlactataemia persisted until 72 hours of age.

Outcome

The filly remained clinically normal with an average daily weight gain of 0.75 kg/day and was discharged on day 5 with one week of minocycline treatment (4 mg/kg orally q12h; Minocycline hydrochloride capsules)[19] until lab work had normalized. The filly was PCR negative for *A. phagocytophilum* at the time of discharge. The mare required no further treatment at home, and both patients recovered uneventfully.

Discussion

This report documents the trans-placental transmission of *A. phagocytophilum* in a late-term pregnant mare, based on pre-colostral (immediate peri-partum) confirmation of infection in the foal. Variants of *A. phagocytophilum* are the causative agents of tick-borne fever in cattle, sheep, camelids and goats; canine, human and equine granulocytic anaplasmosis (EGA) (Woldehiwet, 2010). *A. phagocytophilum* is a tick-borne, gram negative, pleomorphic, intracellular bacteria, which forms macrocolonies known as morulae within infected granulocytes. Typical clinical signs observed in adult horses with EGA include pyrexia, anorexia, lower limb oedema, icterus, petechiation, reluctance to move, and ataxia, while laboratory findings may indicate thrombocytopenia, leukocytosis or leucopenia, anaemia, and hyperbilirubinaemia. Of these, historical pyrexia, mild leukopenia, hyperbilirubinaemia and significant thrombocytopenia were observed in the mare of the current report. Rhabdomyolysis (Hilton et al., 2008), cavitary effusion (Restifo et al., 2015), and premature parturition (Tinkler et al., 2012) are less commonly identified manifestations of EGA in various species. Neonatal anaplasmosis is infrequently observed and the route of infection remains uncertain (Dhand et al., 2007), but may include *in utero* infection, transmission at the time of delivery, or through milk (Horowitz et al., 1998).

Transplacental transmission was the most likely route of infection in this foal, due to a lack of colostrum intake prior to the time of diagnostic (PCR) testing, immediately post-partum. The latter timing further precluded exposure to ticks and made disease transmission at the time of

158 delivery unlikely. Transplacental, intrauterine, or congenital infection with *A.*
159 *phagocytophilum* (although a different strain to that which affects equines) has been previously
160 reported in calves (Pusterla et al., 1997; Henniger et al., 2013), lambs (Reppert et al., 2013;
161 Stuen et al., 2018), and humans (Horowitz et al., 1998; Dhand et al., 2007). For example,
162 experimental intrauterine infection was induced in a cow, where the calf did not receive
163 colostrum or milk from its dam and had no exposure to ticks (Pusterla et al., 1997).
164 Additionally, naturally occurring *in utero* infection was identified in a calf based on the
165 presence of morulae in the calf's precolostral blood sample (Henniger et al., 2013), where the
166 dam was thought to have been infected 2-3 weeks prior to delivery. In ewes, *in utero* infection
167 has been documented after both acute infection during gestation (Reppert et al., 2013), and in
168 chronically infected ewes that were exposed prior to the beginning of gestation (Stuen et al.,
169 2018). Of the chronically infected ewes, only those (n=3) that delivered positive lambs were
170 identified as PCR positive at the time of parturition. Similarly, the mare in the current report
171 showed anaplasma morulae on blood smear evaluation the day prior to parturition of a PCR
172 positive foal.

173 The pathophysiology of intra-uterine transmission of *A. phagocytopilum* may be associated
174 with a vasculitis, resulting in placentitis, or be facilitated by the type of placentation, which
175 varies between species. The mare has a nondeciduate, epitheliochorial, diffuse,
176 microcotyledonary placenta. In general, the chorioallantoic placenta of domestic animals is
177 classified by the number of distinct tissue layers that separate maternal and fetal blood
178 (Stabenfeldt and Edqyist, 1993). Ruminants and camelids, like horses, have an epitheliochorial
179 placentation, while dogs and cats show endotheliochorial, and primates haemochorial
180 placentation. This would suggest that ruminants and horses are less likely to experience intra-
181 uterine transmission of pathogens compared to other species with less circulatory separation.
182 Anaplasma-associated vasculitis is likely to occur due to one of two main mechanisms. *A.*

183 *phagocytophilum* may colonize endothelial cells with infection of microvascular endothelium,
184 as demonstrated *in vitro* and in severe combined immune-deficiency mice (Munderloh et al.,
185 2004; Herron et al., 2005). Alternatively, production of myelosuppressive and proinflammatory
186 cytokines by neutrophils can contribute to the development of vasculitis and oedema (Carlyon
187 and Fikrig, 2003; Davies et al., 2011). Of these, INF-γ may be of particular importance given
188 its potential role in pre-term birth due to placentitis (Lyle, 2014). However, in the current
189 report, histopathology of the placenta did not identify evidence of placentitis. Nonetheless, the
190 foal displayed abnormalities associated with placental insufficiency and tissue hypoxia, based
191 on spurious hypercreatinemia, persistent hyperlactataemia, and slow behavioral development
192 suggestive of neonatal encephalopathy; a manifestation of perinatal asphyxia syndrome.

193 The foal's post-partum inflammatory response and mild thrombocytopenia are comparable to
194 clinical abnormalities identified in previous reports of neonatal anaplasmosis in other species
195 (Horowitz et al., 1998; Henniger et al., 2013). For example, following late-term infection of a
196 mother, her infant developed signs of fever, thrombocytopenia, and leukopenia at 9 days post-
197 partum without other clinical abnormalities, and completely recovered following 5 days of
198 doxycycline therapy (Horowitz et al., 1998). Similarly, a calf developed fever, marked
199 thrombocytopenia and intracellular morulae at 13 days of age, after experimental intrauterine
200 infection (Pusterla et al., 1997). Following natural *in utero* infection, a calf of a second report
201 showed an elevated rectal temperature from birth, with thrombocytopenia, leukopenia and
202 anemia developing rapidly, and was euthanized at 4 days of age (Henniger et al., 2013).
203 Similarly, an experimental study confirmed *in utero* infection in six lambs (delivered to 3 of 9
204 ewes), of which 4 died within 2 days of birth (Stuen et al., 2018). The current literature suggests
205 that the clinical manifestation of congenital anaplasma infection may range from mild to severe
206 disease, supporting the decision to promptly treat the affected foal in the current report, while
207 awaiting test results. The latter strategy may have prevented the development of more severe

thrombocytopenia and clinical illness. The observed elevation in SAA and mild neutrophilia are likely a direct response to inflammation caused by *A. phagocytophilum*.

Alternative differential diagnosis for thrombocytopenia in a neonate include allo-imune disorders, which were ruled out in this case due to the lack of colostrum intake and the dam being a maiden mare. Decreased platelet production is also unlikely, as the platelet count increased during treatment, ruling out a hereditary defect in platelet production. There was also no evidence of increased platelet consumption such as disseminated intravascular coagulation, haemorrhage or thrombosis. Whilst thrombocytopenia secondary to sepsis cannot be fully excluded, anaplasmosis is considered to be the more likely cause in this case.

Anaplasmosis is most often successfully treated with tetracyclines is all species, and more specifically oxytetracycline in horses (Dziegiel et al., 2013). However, use of this antimicrobial class may be undesirable in pregnant animals due to its teratogenic effects. Tetracyclines cross the placenta and form stable calcium complexes with bone-forming tissue, thus abnormalities will occur if administered during bone formation (Tötterman and Saxén, 1969). Evidence of bone abnormalities has not been directly demonstrated in horses, possibly due to the significantly longer gestation length compared to laboratory animals, resulting in fetal exposure to tetracycline for a smaller percentage of gestation in the horse. Late in gestation when tooth development is taking place, permanent discoloration and enamel hypoplasia may occur (Kline et al., 1964). Oxytetracycline administration in humans has the highest tetratogenic risk in the first trimester of pregnancy, with no increased risk (excluding dental discoloration) when exposed in the 9th month of gestation (Czeizel and Rockenbauer, 2000). However, alternative choices should still be considered, with rifampin and fluoroquinolones having some *in vitro* activity against *A. phagocytophilum* (Klein et al., 1997; Horowitz et al., 2001). Fluoroquinolones are known to be inhibitory to *A. phagocytophilum*, but are considered bacteriostatic and not bacteriocidal due to the high minimum inhibitory concentration (MIC)

required, with variable efficacy among different fluoroquinolones (Klein et al., 1997; Horowitz et al., 2001; Wormser et al., 2006). This variability, combined with known risks of fluoroquinolone-associated cartilage injury in neonates, makes them an unsatisfactory choice. The use of IV oxytetracycline in the foal was therefore justifiable, and is supported by the reported use of intravenous doxycycline in a human neonate (Horowitz et al., 1998).

Enrofloxacin was administered to the mare to provide additional gram-negative bacterial coverage and tissue penetration following prolonged retention of fetal membranes. Considering existing concerns of renal injury associated with oxytetracycline to target anaplasma, polymyxin to counteract endotoxemia and flunixin for analgesic purposes, the use of an additional nephrotoxic medication (such as gentamicin) was avoided. Although milk concentrations of enrofloxacin or ciprofloxacin are not reported in mares, data from other species suggests that very low levels of enrofloxacin are found in milk (0.2% of administered dose) (Kaartinen et al., 1995), with higher concentrations of its active metabolite ciprofloxacin (Kaartinen et al., 1995; Aramayona et al., 1996; Haritova et al., 2003). However, studies in humans being treated with ciprofloxacin have identified no harm to the nursing child due to the low milk levels present and consumed (Kaplan and Koren, 2015). Therefore, despite the theoretical risk, enrofloxacin was considered safe to use short-term in lactating mares.

In conclusion, this is the first documented case of transplacental anaplasmosis in a foal. The affected filly showed clinical signs of perinatal asphyxia syndrome despite normal parturition, which can indicate placental dysfunction. Congenital anaplasmosis should be considered in a foal delivered to a mare with EGA and antimicrobial therapy be guided accordingly.

Manufacturers' addresses

[1] Zoetis, Kalamazoo, Michigan, USA

257 [2] Mila International Inc, Florence, Kentucky, USA

258 [3] VetOne, Boise, Idaho, USA

259 [4] Hospira Inc, Lake Forest, Illinois, USA

260 [5] Foalert Inc, Acworth, Georgia, USA

261 [6] Oxytet 100, Norbrook Inc, Lenexa, Kansas, USA

262 [7] Henry Schein, Dublin, Ohio, USA

263 [8] Bimeda-MTC Animal Health Inc, Cambridge, Ontario, Canada

264 [9] Baxter Healthcare Corporation, Deerfield, Illinois, USA

265 [10] Abbott Laboratories, North Chicago, Illinois, USA

266 [11] Dechra, Overland Park, Kansas, USA

267 [12] Bayer HealthCare LLC, Shawnee Mission Kansas, USA

268 [13] Fresenius Kabi USA, Lake Zurich, Illinois, USA

269 [14]AuroMedics Pharma LLC, Dayton, New Jersey, USA

270 [15] Nova-Tech Inc, Grand Island, Nebraska, USA

271 [16] Zoetis Inc, Kalamazoo, Michigan, USA

272 [17] Merck, Germany

273 [18] Lake Immunogenics Inc, Ontario, New York 14519, USA

274 [19] Ohm Laboratories Inc, North Brunswick, New Jersey, USA

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346

347 Figure 1: Photograph demonstrating the presence of morulae in the mare's neutrophils.

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349

350 **Table 1:** Selected hematology and biochemistry values for the foal during hospitalization.

Parameter	Reference range†	Time of sampling from birth (hours)						
		0 h	12 h	24 h	48 h	60 h	72 h	100 h
PCV‡ (%)	30-44	48	39	38	38	36	38	39
Total solids (g/L)	41-66	52	53	48	49	50	52	50
Lactate (mmol/L)	0.6-1.9	11.5	4.2	3.3	2.5	2.8	1.9	2
Glucose (mmol/L)	5.6-12.9	2.6	10.3	8.4	8.8	9.4	7.8	11.1
SAA‡ (µg/mL)	0-50	0		145		196		140
WBC‡ (10^9/L)	6.2-12.4	9.7				11.2		
Neutrophils (10^9/L)	4.1-9.5	8.3				10.1		
Platelets (10^9/L)	129-409	87				102		
Fibrinogen (g/L)	1.0-4.0	1.0				2.0		
Creatinine (µmol/L)	106-380	292		71			71	
Globulin (g/L)	15-46	12						
Creatine Kinase (U/L)	165-761	2368						
Indirect bilirubin (µmol/L)	17-51	89						
Anaplasma PCR‡		Positive						Negative

351 †References ranges for foals at 1 day or 24-48 hours post-partum.(Orsini and Divers, 2014)

352 ‡PCV, packed cell volume; WBC, white blood cell count; SAA, serum amyloid A; PCR,

353 polymerase chain reaction