

Anaplasma phagocytophilum infection in a horse from Switzerland with severe neurological symptoms

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Summary

A 22-year old mare from Switzerland was admitted to an equine clinic in May 2011. She presented with fever, lethargy, icteric mucous membranes, reduced alertness, an unsteady gait and ataxia. An *Anaplasma phagocytophilum* infection was confirmed by blood smear and PCR. The mare was treated with oxytetracylin and recovered rapidly, but she still suffered from a slight atactic gait disturbance at 3 weeks post infection.

Keywords: equine granulocytic anaplasmosis, Switzerland, neurological symptoms, oxytetracylin

Anaplasma phagocytophilum Infektion bei einem Pferd aus der Schweiz mit ausgeprägter neurologischer Symptomatik

Eine 22 Jahre alte Stute aus der Schweiz wurde im Mai 2011 in eine Pferdeklīnik aufgenommen. Die klinischen Symptome bestanden in Fieber, Abgeschlagenheit, ikterischen Schleimhäuten, reduzierter Aufmerksamkeit, einem unsicherem Gang und einer Ataxie. Eine Infektion mit *Anaplasma phagocytophilum* wurde durch den Erregernachweis im Blutausstrich und in der PCR bestätigt. Die Stute wurde mit Oxytetracyclin behandelt, worunter eine rasche Besserung eintrat. Trotzdem war drei Wochen nach der Infektion noch eine geringgradige ataktische Gangstörung nachweisbar.

Schlüsselwörter: Equine granulozytäre Anaplasrose, Schweiz, Neurologische Symptome, Oxytetracyclin

Introduction

Anaplasma phagocytophilum is a Gram-negative, obligate intracellular bacterium. It is transmitted via tick-bite and replicates in neutrophils. In Europe, its main vector is *Ixodes ricinus* (Ismail et al., 2010). *A. phagocytophilum* causes febrile disease in animals and humans (Stuen et al., 2013). Due to changes in the taxonomy in 2001, the former species *Ehrlichia phagocytophila*, *E. equi* and the agent of human granulocytic ehrlichiosis (HGE) were united to the new species *A. phagocytophilum* (Dumler et al., 2001). *A. phagocytophilum* is the causative agent of tick-borne fever in sheep and cattle in Europe (Stuen, 2007; Woldehiwet, 2010), whereas canine and feline granulocytic anaplasmosis do occur in North America and Europe (Carrade et al., 2009; Little, 2010). In contrast, human granulocytic anaplasmosis is much more common in the United States (Ismail et al., 2010) than in Europe, where it is still a rare disease (Blanco and Oteo, 2002; Strle, 2004).

The first case of equine granulocytic anaplasmosis (EGA) was described in California in 1969 (Gribble, 1969). EGA occurs in North America (Madigan and Pusterla, 2000), in Europe (Dzięgiel et al., 2013) and North Africa (M'ghirbi et al., 2012). In Switzerland, the first case proven by microscopic detection of the pathogen was published in 1985 (Hermann et al., 1985). In 1998, *A. phagocytophilum* was primarily identified by molecular characterization as infectious agent in a Swiss horse (Pusterla et al., 1998). Since then no further cases from Switzerland have been published. We here report an *A. phagocytophilum* infection in a mare from Switzerland which showed severe neurological symptoms.

Case presentation

A 22-year-old mare (Westphalian breed) was admitted to an Equine Clinic in May 2011 because of a marked re-

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duced general condition, fever (41.5 °C), dry icteric mucous membranes and a mild colic with diarrhea. Before the transport, she was treated symptomatically with flunixin (0.25 mg/kg of body weight), metamizol (50 mg/kg of body weight) and 10 l Ringer's lactate solution.

At the clinic the rectal temperature had decreased to 38.2 °C. The clinical examination most prominently showed reduced alertness, an unsteady gait and ataxia. In the back and in serpentine the hindquarters were found to be unstable. Cranial nerve examination revealed no deficiencies and the anal reflex, the tail tone as well as cutaneous sensation appeared to be normal. Collection of the cerebral spinal fluid and a radiograph of the spinal column were refused by the owner.

The heart rate was raised (68 beats/min) and a holosystolic heart murmur was audible. Additionally, the pulsation of the digital arteries over the fetlocks was increased. The laboratory test results (Tab. 1) revealed thrombopenia, neutrophilia, lymphopenia, a mild anemia and hyperbilirubinemia. An empiric antibiotic treatment with Penicillin (20'000 IU/kg of body weight i.v. q 8 h) and Gentamicin (6.6 mg/kg of body weight i.v. q 24 h) was begun.

On the third day after admission morulae of *A. phagocytophilum* were detected in neutrophil granulocytes in a Giemsa-stained blood smear from venous EDTA-blood (Fig. 1) and *A. phagocytophilum* DNA was detected by real-time PCR (Schaarschmidt-Kiener and Müller, 2007). This result was confirmed by amplification and sequencing of the 16S rRNA (Massung et al., 1998; von Loewenich et al., 2003) and the *ankA* gene (Massung et al., 2000). A titer of 1:200 (cut-off 1:50) was found for anti-*A. phagocytophilum* IgG antibodies by indirect immunofluorescence (MegaCor, Hörbranz, Austria). Based on these results EGA was diagnosed and the antibiotic treatment was switched to oxytetracyclin (7.5 mg/kg of body weight i.v. q 12 h).

The mare rapidly recovered, but was still found to suffer from a slight atactic gait disturbance at 3 weeks post infection. At this time morulae were no longer detected in

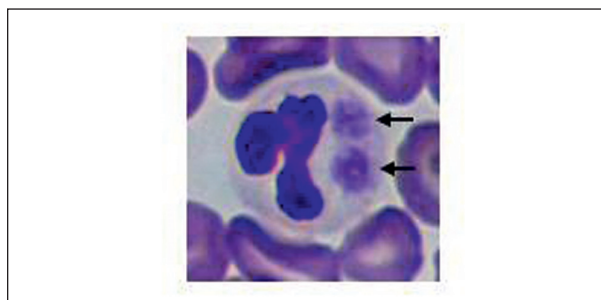


Figure 1: Morulae (arrows) of *Anaplasma phagocytophilum* in a neutrophil in a Giemsa-stained blood smear of the mare. Magnification: 10 × 100.

the blood smear and the real-time PCR for *A. phagocytophilum* was negative. Further, a significant rise in the IgG titer to 1:800 was found.

Discussion

EGA has an incubation period of approximately 10 days after tick-bite (Dzięgiel et al., 2013). Typical symptoms include fever, depression, anorexia, lower limb edema, icterus, petechiation, reluctance to move and ataxia (Madigan and Pusterla, 2000). A transient systolic heart murmur has been reported in experimentally infected horses that has been ascribed to physiologic turbulence (Madigan, 1993; Franzén et al., 2005). Laboratory findings are thrombopenia, leukocytosis or leukopenia, anemia and hyperbilirubinemia (Dzięgiel et al., 2013). The diagnosis is made by detection of typical inclusions (morulae) in neutrophil granulocytes in a Giemsa-stained blood smear and detection of *A. phagocytophilum* DNA from EDTA-blood by PCR (Stuen et al., 2013). The PCR has been shown to be much more sensitive than the microscopic investigation (Franzén et al., 2005). Although often performed, serology is of very limited use, because animals in the acute phase of

Table 1: Laboratory test results of the mare with suspected *A. phagocytophilum* infection.

	On admission	Reconvalescence ¹	Reference range
Thrombocytes (× 10 ⁹ /l)	79	127	100–300
White blood cell count (× 10 ⁹ /l)	9.53	6.59	5.00–10.00
Granulocytes (× 10 ⁹ /l)	8.40	5.10	1.20–6.80
Lymphocytes (× 10 ⁹ /l)	0.90	1.40	1.20–32.00
Monocytes (× 10 ⁹ /l)	0.20	0.10	0.00–1.80
Red blood cell count (× 10 ¹² /l)	5.57	6.25	6.00–10.00
Hematocrit (%)	27.7	31.6	28.0–46.0
Hemoglobin (g/l)	100	109	103–170
Total bilirubin (μmol/l)	99	nd ²	0–60

¹ three weeks after admission. ²nd = not done.

the infection are usually seronegative (Franzén et al., 2005). Further, investigation of a single serum samples does not allow the distinction between current or past infection. Oxytetracylin is usually used as rapidly effective treatment, although self-limiting disease has been observed as well (Madigan and Pusterla, 2000). Usually, a full recovery including the neurological symptoms is achieved within 2 to 3 weeks (Madigan, 1993; Franzén et al., 2005).

In our case, the mare showed most of the clinical symptoms and laboratory findings described above. She rapidly responded to treatment with oxytetracylin, which could have been administered earlier on clinical grounds alone. However, the neurological impairment was most impressive and still slightly present at 3 weeks post infection. Severe neurological symptoms in EGA have been described before (Madigan and Gribble, 1987). If they do occur, differential diagnostic considerations might be broadened. In contrast to horses, neurological involvement is uncommon in sheep and cattle (Stuen, 2007; Woldehiwet, 2010) as well as in dogs (Carrade et al., 2009) and humans (Ismail et al., 2010). Although clinical data on cats are still limited, incoordination has been described in cats (Tarello, 2005).

Many *A. phagocytophilum* infections probably pass unrecognized because subclinical infections are frequent and because many of the symptoms are rather unspecific (Madigan and Pusterla, 2000). Therefore, disease awareness is important to diagnose EGA.

GenBank accession numbers

The sequences obtained during this study are available at GenBank (accession numbers JN247406 and JN247407).

Consent

Informed consent was obtained from the owner for publication of this report.

References

- Blanco J. R., Oteo J. A.: Human granulocytic ehrlichiosis in Europe. *Clin. Microbiol. Infect.* 2002, 8: 763–772.
- Carrade D. D., Foley J. E., Borjesson D. L., Sykes J. E.: Canine granulocytic anaplasmosis: a review. *J. Vet. Intern. Med.* 2009, 23: 1129–1141.
- Dumler J. S., Barbet A. F., Bekker C. P. J., Dasch G. A., Palmer G. H., Ray S. C., Rikihisa Y., Rurangirwa F. R.: Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and “HGE agent” as subjective synonyms of *Ehrlichia phagocytophila*. *Int. J. Syst. Evol. Microbiol.* 2001, 51: 2145–2165.
- Dzięgiel B., Adaszek L., Kalinowski M., Winiarczyk S.: Equine granulocytic anaplasmosis. *Res. Vet. Sci.* 2013, 95: 316–320.
- Franzén P., Aspan A., Egenvall A., Gunnarsson A., Åberg L., Pringle J.: Acute clinical, hematologic, serologic, and polymerase chain reaction findings in horses experimentally infected with a European strain of *Anaplasma phagocytophilum*. *J. Vet. Intern. Med.* 2005, 19: 232–239.
- Gribble D. H.: Equine ehrlichiosis. *J. Am. Vet. Med. Assoc.* 1969, 155: 462–469.
- Hermann M., Baumann D., Lutz H., Wild P.: Erster diagnostizierter Fall von equiner Ehrlichiose in der Schweiz. *Pferdeheilkunde* 1985, 1: 247–250.
- Ismail N., Bloch K. C., McBride J. W.: Human ehrlichiosis and anaplasmosis. *Clin. Lab. Med.* 2010, 30: 261–292.
- Little S. E.: Ehrlichiosis and anaplasmosis in dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* 2010, 40: 1121–1140.
- M'ghirbi Y., Yaïch H., Ghorbel A., Bouattour A.: *Anaplasma phagocytophilum* in horses and ticks in Tunisia. *Parasit. Vectors* 2012, 5: 180.
- Madigan J. E., Gribble D.: Equine ehrlichiosis in northern California: 49 cases (1968–1981). *J. Am. Vet. Med. Assoc.* 1987, 190: 445–448.
- Madigan J. E.: Equine ehrlichiosis. *Vet. Clin. N. Am. Equine Pract.* 1993, 9: 423–428.
- Madigan J. E., Pusterla N.: Ehrlichial diseases. *Vet. Clin. N. Am. Equine Pract.* 2000, 16: 487–499.
- Massung R. F., Slater K., Owens J. H., Nicholson W. L., Mather T. N., Solberg V. B., Olson J. G.: Nested PCR assay for detection of granulocytic ehrlichiae. *J. Clin. Microbiol.* 1998, 36: 1090–1095.
- Massung R. F., Owens J. H., Ross D., Reed K. D., Petrovec M., Björnsdorff A., Coughlin R. T., Beltz G. A., Murphy C. I.: Sequence analysis of the *ank* gene of granulocytic Ehrlichiae. *J. Clin. Microbiol.* 2000, 38: 2917–2922.
- Pusterla N., Huder J. B., Feige K., Lutz H.: Identification of a granulocytic *Ehrlichia* strain isolated from a horse in Switzerland and comparison with other *Rickettsiae* of the *Ehrlichia phagocytophila* genogroup. *J. Clin. Microbiol.* 1998, 36: 2035–2037.
- Schaarschmidt-Kiener D., Müller W.: Diagnostic and clinical aspects of canine anaplasmosis and ehrlichiosis (Article in German). *Tierärztl. Prax.* 2007, 35: 129–136.
- Strle F.: Human granulocytic ehrlichiosis in Europe. *Int. J. Med. Microbiol.* 2004, 293 S37: 27–35.
- Stuen S.: *Anaplasma phagocytophilum* – the most widespread tick-borne infection in animals in Europe. *Vet. Res. Commun.* 2007, 31, Suppl. 1: 79–84.
- Stuen S., Granquist E. G., Silaghi C.: *Anaplasma phagocytophilum* – a widespread multi-host pathogen with highly adaptive strategies. *Front. Cell. Infect. Microbiol.* 2013, 3: 31–33.
- Tarello W.: Microscopic and clinical evidence for *Anaplasma (Ehrlichia) phagocytophilum* infection in Italian cats. *Vet. Rec.* 2005, 11: 772–774.

von Loewenich F. D., Baumgarten B. U., Schröppel K., Geißdörfer W., Röllinghoff M., Bogdan C.: High diversity of *ankA* sequences of *Anaplasma phagocytophilum* among *Ixodes ricinus* ticks in Germany. J. Clin. Microbiol. 2003, 41: 5033–5040.

Woldehiwet Z.: The natural history of *Anaplasma phagocytophilum*. Vet. Parasitol. 2010, 167: 108–122.

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