VETERINARSKI ARHIV 87 (1), 113-120, 2017

A first case of equine granulocytic anaplasmosis in Croatia - a case report

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GOTIĆ, J., N. BRKLJAČA BOTTEGARO, I. KIŠ, M. CRNOGAJ, V. MRLJAK, R. BECK: A first case of equine granulocytic anaplasmosis in Croatia. Vet. arhiv 87, 113-120, 2017.

ABSTRACT

We report on the first confirmed case of equine granulocytic anaplasmosis in Croatia. Physical examination of a diseased mare at admission revealed depression, ataxia, high fever, pale mucous membranes and limb edema. A hematology profile showed mild anemia, thrombocytopenia and leukopenia, with no blood parasites or inclusions visual in the blood smear, while PCR detected specific DNA of *Anaplasma phagocytophilum*. The mare was treated with oxytetracycline for seven consecutive days, which resulted in a rapid recovery. Equine granulocytic anaplasmosis is a "newly" recognized disease in southeastern Europe, and should be considered as a major differential diagnosis in similar cases.

Key words: equine granulocytic anaplasmosis, *Anaplasma phagocytophilum*, horse, PCR, southeastern Europe

Introduction

Equine granulocytic anaplasmosis (EGA) is a vector borne disease caused by the intracellular bacterium *Anaplasma phagocytophilum.* It is transmitted by ticks of the genus *Ixodes*, in Europe mainly *I. ricinus* (STRLE, 2004; RYMASZEWSKA and GRENDA, 2008). Three species of granulocytic bacteria, causing the disease in horses *(Ehrlichia equi)*, ruminants *(Ehrlichia phagocytophilum)* and humans (human granulocytic ehrlichiosis agent), respectively, have been designated as variants of the same species,

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ISSN 0372-5480
Printed in Croatia

Anaplasma phagocytophilum (DUMLER et al., 2001). More than one genetic variant of *A. phagocytophilum* infects and causes EGA in horses, suggesting that the bacterium may exist in different natural cycles, involving different reservoir hosts (SILAGHI et al., 2011). Equine granulocytic anaplasmosis is mostly a self-limiting disease, although any concurrent infection can be exacerbating. In horses it causes fever, partial anorexia, depression, ataxia, icterus, reluctance to move, limb edema, petechiae and jaundice (GRIBBLE, 1969; MADIGAN and PUSTERLA, 2000; FRANZEN et al., 2005; PUSTERLA and MADIGAN, 2013). Subclinical self-limiting infections explain the high seropositivity in certain countries, reaching 67%, or even 73% (PRASKOVA et al., 2011; M'GHIRBI et al., 2012).

After the initial published report on EGA in California (GRIBBLE, 1969), it has been described in other parts of North and South America (LEWIS et al., 2009; UEHLINGER et al., 2011), Asia (CHAHAN et al., 2005) and recently in North African countries (M'GHIRBI et al., 2012). In the last three decades EGA has been described in many European countries: Germany (BÜSCHER et al., 1984), Switzerland (HERMANN et al., 1985), Scandinavia (BJÖERSDORFF et al., 1990), UK (KORBUTIAK and SCHNEIDERS, 1994), Austria (FRÖHLICH and EDELHOFER, 1998), France (BERMANN et al., 2002), Italy (MORETTI et al., 2010), the Netherlands (BUTLER et al., 2008), Portugal (SANTOS et al., 2009), Poland (ADASZEK et al., 2009), and the Czech Republic (JAHN et al., 2010). So far, cases of EGA have not been described in southeastern Europe, thus this report describes the first confirmed case of EGA in the region.

Case presentation

A Croatian Warmblood mare, 14 years old, residing in the northeast part of Croatia (Slavonia) was presented in late spring to the clinic for inappetence, persisting fever (above 40˚C), reluctance to move, slight submandibular edema and weight loss, lasting for more than a week. Vaccinations for equine influenza, equine rhinopneumonitis and tetanus had been performed regularly. The mare was kept in a combined stall and pasture confinement together with nine other horses, that showed no similar symptoms. The owners had not found ticks on the mare in the past weeks, although attached ticks had been seen on their horses before. Prior to admission the mare was treated with flunixin meglumine and imidocarb diproprionate for two consecutive days without major clinical improvement.

On the day of presentation the mare was depressed and slightly ataxic, but responsive. Rectal temperature was 38.9 ˚C, heart rate was 36 beats per minute with a normal rhythm, and the respiratory rate was 18 breaths per minute. The mucous membranes were pale with pronounced jaundice. Edema was present in the distal part of all four limbs. Initially complete blood cell count (CBC) and serum biochemistry profile (Table 1 and 2) were

performed. CBC showed mild anemia, thrombocytopenia and leukopenia, with no visual blood merozoites or morulae in the blood smear.

Table 1. Complete blood cell count parameters; (Reference values: Central Clinical Laboratory, Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb)

	RBC 10^{12}/L	HCT %	HGB g/L	MCV fL	MCH pg	MCHC g/L	MPV fL	WBC 10^9/L	Seg. N.%	Band N%	Ly %	M %	PLT 10^9/L
Ref. values	6-12	32-48	100-180	32-48	13-19	310-370	5-6	6-12	51-65	0-2	29-41	2-6	150-600
Day 1	5.8	30	163	52	17	319	5	4.6	42		58		128
Day 2	6.2	32	102	52	18	337	4	6.1	56	1	43	1	223
Day 3	6.9	36	116	52	17	325	4	7.0	55		42	3	218
Day 4	6.1	32	103	53	17	324	5	8.8	62		38		251
Day 5	7.1	37	125	52	17	337	5	6.5	53		47		204
Day 6	7.3	37	125	53	16	341	6	6.9	60		39		221

RBC = Red Blood Cell Count; HCT = Hematocrit; HGB = Hemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; MPV = Mean Platelet Volume; WBC = White Blood Cell Count; Seg. N = Segmented neutrophiles; Band N = Band Neutrophiles; Ly = Lymphocites; M = Monocites; PLT = Platelet Count

Table 2. Serum biochemistry parameters. (Reference values: Central Clinical Laboratory, Clinic for Internal diseases, Faculty of Veterinary Medicine, University of Zagreb)

	BUN mmol/L	CREA µmol/L	TP g/L	ALB g/L	TBIL µmol/L	GLU mmol/L	AST U/L	GGT U/L	AP U/L	CK U/L	Ca mmol/L	P mmol/L	LDH U/L
Ref. values	3.3-6.6	-115	55-75	26-37	24-50	3.1-5	-490	-28	-302	-130	2.5-3.4	0.8-1.5	162-412
Day 1	4.2	115	58	28	91.1	4.8	480	23	155	381	2.9	1.0	709
Day 2	4.1	113	62	26	22.6	6.2	560	34	190	171	3.0	1.1	
Day 3	4.7	114	63	26	28.4	5.2	553	36	196	151	3.0	1.1	
Day 4	4.9	114	62	27	29.0	5.5	511	38	188	147	3.1	1.0	
Day 5	4.8	110	58	25	28.1	5.9	451	34	204	136	3.0	1.3	472
Day 6	4.2	111	59	26	21.2	5.5	437	34	206	122	3.0	1.5	

BUN = Blood Urea Nitrogen; CREA = Creatinine; TP = Total Protein; ALB = Albumin; TBIL = Total Bilirubin; GLU = Glucose; AST = Aspartate Aminotransferase; GGT = Gamma Glutamyltransferase; AP = Alkaline Phosphatase; CPK = Creatinine Kinase; Ca = Calcium; P = Phosphorous; LDH = Lactate Dehydrogenase

Further diagnostic workup of the mare included polymerase chain reaction (PCR) testing for *Babesia* spp. (BECK et al., 2009) and *Anaplasma* spp. (PAROLA et al., 2000), as well as serological testing for Equine herpes virus (EHV) and Equine Arteritis virus (EAV). EHV and EAV tests showed low antibody titers for both diseases. PCR for *Babesia* spp. gave negative results but the presence of *A. phagocytophilum* was confirmed with

PCR and subsequent sequencing. *A. phagocytophilum* amplification was done on 345 bp 16S rRNA, followed by BLAST analysis for comparison of the sequences in the NCBI database.

Treatment of the horse was initiated on the day of admission with oxytetracycline (Engemycin®, Intervet, USA) 6,6 mg/kg given intravenously once daily diluted in 1 l saline, for seven days. Forty-eight hours after the first dose, the mare was alert and her appetite significantly improved. Ataxia, fever and limb edema disappeared completely. Clinical signs of icterus and inappetence resolved within a week after the treatment was initiated and the mare was discharged from the clinic. During the next 20 months the owners did not complain of any medical condition in the mare.

Discussion

As we experienced with our patient, PCR was an efficient test, which enabled a rapid and sensitive diagnosis, especially when there were no observable pathogens in the blood smear. Neither indirect fluorescent antibody test nor Western blot give reliable information as to whether the elevated antibodies are indicative of a current disease or a previous clinical or subclinical infection (JAHN et al., 2010). A fourfold rise in antibody titer indicates the diagnosis of EGA, as well as the response to treatment with oxytetracycline, but PCR appears to be the best tool for a definite diagnosis of acute EGA (MADIGAN and PUSTERLA, 2000; FRANZEN et al., 2005; BUTLER et al., 2008; RYMASZEWSKA, 2011). Although in this case equine piroplasmosis was initially suspected, we excluded co-infection with *B. caballi* and *T. equi*, since five days after antiprotozoal treatment the mare did not show any significant clinical improvement. Furthermore, our conclusion was confirmed by negative PCR results for both *B. caballi* and *T. equi*.

The mare in this report developed the clinical signs already described in both naturally and experimentally infected EGA horses (GRIBBLE, 1969; FRANZEN et al., 2005), but since none of them is pathognomonic for the disease, the clinical picture was not considered diagnostic.

Leucopenia, characterized by neutropenia, anemia and thrombocytopenia, typically found in EGA, were present during the febrile stage of infection. Leucopenia was likely to be due to sequestration of infected granulocytes. The ability of *A. phagocytopilum* to evade the bactericidal effects of granulocytes makes it an interesting tool to investigate the mechanisms of neutrophil bacteria obviation (WOLDEHIWET, 2010). Thrombocytopenia appears to be more pronounced in horses than in other animals, with hemorrhages, petechiae, ecchymosis and edema as the main pathological features (GRIBBLE, 1969). *Anaplasma phagocytophilum* morulae are primarily noted in the blood smear during the early phase of the disease, when bacteremia peaks, and as observed in our case, are typically absent in later stages of infection when they may be seen in less than 1% and up

to 20-30% of neutrophils on blood smears (GRIBBLE, 1969; MADIGAN and PUSTERLA, 2000; WOLDEHIWET, 2010). Serum biochemistry revealed an increase in total bilirubin values, lactate dehydrogenase (LDH) and an increase in aspartate aminotransferase (AST) and creatine kinase (CK) enzyme activity. Hyperbilirubinemia was observed together with other hemolytic changes, although prolonged fasting could have resulted in total bilirubin increase. Limited data on biochemistry panel changes in other EGA cases show elevated levels of C-reactive protein and, as in this case, an increase in liver enzymes and bilirubin levels (LEWIS et al., 2009; BERMANN et al., 2002).

Suitable tick habitats and horses' lifestyle, promoting tick exposure in a particular locality, represent the main risk factors for disease acquisition (PRASKOVA et al., 2011). Ticks of the genus *Ixodes* require a relative humidity of at least 80% to survive during their off-host periods, and are therefore restricted to areas of moderate to high rainfall, with vegetation that retains high humidity, which exist in the northern Croatian region during the spring and autumn months. The reservoir species for this infection are wild rodents, and wild and domestic ruminants, but human and companion animal infections are emerging as a significant problem in Europe and North America (WOLDEHIWET, 2010). Although veterinarians in the southeastern European region are commonly confronted with tick-borne diseases, mostly focused on Equine piroplasmosis, they treat horses without etiological diagnosis.

In conclusion, considering that clinical sings of Equine piroplasmosis and Equine anaplasmosis can be similar (ZOBBA et al., 2008), early diagnosis is essential for adequate treatment. The importance of routine blood smear evaluation is well known. However, in acute cases with negative blood smear evaluation, especially together with longer clinical presentation, the relevance of molecular diagnostic methods is irreplaceable. A survey of *A. phagocytophilum*-infected ticks and further studies on prevalence in horses, by serological and molecular methods, are essential to estimate the prevalence and risk of infection in different regions. This report emphasizes EGA as a "newly" recognized disease in southeastern Europe, with the presumption that numerous EGA cases have been misdiagnosed, meaning it should be considered a major differential diagnosis in similar cases.

Acknowledgements

This study was funded by the Croatian Science Foundation Grant 1957 "GENOTICKTRECK". The work of RB was done within the framework of EurNegVec COST Action TD1303.

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Received: 20 October 2015
Accepted: 21 June 2016

GOTIĆ, J., N. BRKLJAČA BOTTEGARO, I. KIŠ, M. CRNOGAJ, V. MRLJAK, R. BECK: Prvi opis slučaja anaplazmoze konja u Hrvatskoj. Vet. arhiv 87, 113-120, 2017.

SAŽETAK

U ovom radu donosimo opis prvoga dokazanog slučaja anaplazmoze konja u Hrvatskoj. Kobila je pri dolasku na kliniku pokazivala ove simptome: depresivno ponašanje, ataksiju, bljedilo sluznica te otečenje svih udova. Hematološki profil pokazao je blagu anemiju, trombocitopeniju i leukopeniju, bez vidljivih parazita u krvnom razmazu. Lančanom reakcijom polimerazom dokazana je prisutnost uzročnika *Anaplasma phagocytophilum*. Kobila je sljedećih sedam dana liječena oksitetraciklinskim antibiotikom. Anaplazmoza konja je „novo" prepoznata bolest na području jugoistočne Europe, te bi se kao takva trebala uvrstiti u diferencijalne dijagnoze u slučajevima anemija konja.

Ključne riječi: anaplazmoza konja, *Anaplasma phagocytophilum*, konj, PCR, jugoistočna Europa