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SHORT COMMUNICATION

Parasitological and molecular diagnostic of a clinical *Babesia caballi* outbreak in Southern Romania

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Abstract

Equine piroplasmosis (EP) is a tick-borne disease of equids caused by *Babesia caballi* and/or *Theileria equi*, which is endemic in many tropical and temperate areas of the world. However, clinical outbreaks of EP in Romania during the last decades have not been reported Therefore, the aim of this paper is (i) to describe a clinical *B. caballi* outbreak in horses on several farms in Southern Romania using a diagnostic and therapeutic approach and (ii) the molecular diagnostic of EP in an endemic area of Romania. In the first case, a 10-month-old stallion male was presented with lethargy, anorexia, fever (40.9 °C), pale mucosal/mucous/membranes and a marked anemia. In the subsequent weeks, three horses from other farms located in the same area, displayed similar clinical signs. *B. caballi* was diagnosed in all the horses based on Giemsa-stained blood smears and the diagnosis was further confirmed by polymerase chain reaction (PCR), using a single-round and multiplex PCR and sequencing. All four horses were treated with imidocarb dipropionate, at a dose rate of 2.2 mg/kg body weight (two injections at 48 h apart), and all horses clinically recovered within 24–48 h, post-treatment. This report presents the first molecularly characterized *B. caballi* outbreak in Romania in clinically affected horses, confirmed by DNA sequencing.

Keywords Tick-borne disease · *Babesia caballi* · Horses · Romania

Introduction

Equine piroplasmosis (EP), a tick-borne disease, is a serious threat to the horse industry and international movement of horses by causing significant losses and restrictions on horse movement across the borders (Phipps 1996; Thiemann and Phipps 2009; Wise et al. 2013). EP, widely distributed in tropical and subtropical areas (de Waal 1992; Avarzed et al. 1997), regularly occurs in Southern European countries, including Portugal (Bashiruddin et al. 1999), Spain (Camacho et al. 2005), Italy (Savini et al. 1997), and Southern France (Leblond et al. 2005), and extends east to Hungary (Hornok et al. 2007). Currently, EP appears to be spreading to more temperate climates of Europe (Butler et al. 2012; Jongejan et al. 2015) and may represent one of the major limitations on horse movements, e.g. for horse sports and commerce between countries (Friedhoff et al. 1990; USDA 2011; OIE 2014).

The causative agents of EP are *Babesia caballi* and/or *Theileria equi* (syn. *Babesia equi*) (Mehlhorn and Schein 1998). The infection can occur in varying degrees of severity from subclinical to acute. Although all types of equids are susceptible to both *B. caballi* and *T. equi* infections, clinical cases occur mostly in horses (Uilenberg 2006; Laus et al. 2015). *Theileria equi* is more pathogenic than *B. caballi* and often causes hemoglobinuria and even death of horses (de Waal 1992). Natural transmission and geographical distribution of EP rely on the presence of pathogen-transmitting ticks. The proven tick vectors for these infections belong to the genera *Dermacentor*, *Rhipicephalus*, and *Hyalomma* (reviewed in Wise et al. 2013). Transmission can also occur

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iatrogenically by transfer of blood from infected to naïve horses or through contaminated equipment (Hermann et al. 1987; Gerstenberg et al. 1998; Short et al. 2012).

Diagnosis of EP relies upon the identification of intraerythrocytic parasites by microscopic examination of Giemsa-stained peripheral blood smears, especially from acutely infected animals (OIE 2014). However, this method has certain limitations, particularly when the parasitemia is low. During clinical infections with *B. caballi* the percentage of erythrocytes parasitized is typically less than 1% and may be less than 0.1%, while in *T. equi* infections it is usually between 1 and 5% but in severe cases may exceed 20% (Wise et al. 2013). Serological tests, such as complement fixation test (CFT), indirect fluorescent antibody test (IFAT), and competitive inhibition enzyme-linked immunosorbent assay (cELISA), are available for large-scale studies and also to assess infections during the latent carrier stage characterized by microscopically undetectable parasitemia (Leblond et al. 2005; OIE 2014). Recently, more specific, sensitive, and accurate PCR-based techniques for molecular diagnosis are available, such as real-time PCR, nested PCR, and nested PCR with hybridization and multiplex PCR for simultaneous detection and identification of *Theileria* and *Babesia* species in horses (Alhassan et al. 2005; Wise et al. 2013).

In Romania, various tick species known to be vectors for EP have been reported (Ionita et al. 2010, 2013; Mihalca et al. 2012). However, documented reports about equine piroplasmosis in Romanian horses are rare. The first record of this disease was from the Eastern part of Romania (Moldova) and dates back to the first decade of the previous century (Nicolau and Calinescu 1912, cited in Cernaianu 1958). More recently, only one molecular-based survey has been carried out on EP in Romania, showing a prevalence of *B. caballi* and *T. equi* infection of 4.5 and 38.8%, respectively in asymptomatic feral and domestic equines in the Danube Delta (South-eastern Romania) (Gallusová et al. 2014). The aim of this study was to document a clinical *B. caballi* outbreak and to provide molecular epidemiological data on *B. caballi* in an endemic area of Romania.

Material and methods

Case reports

Over a period of 4 weeks (from March 31 to May 2, 2015), four crossbred horses, originating from a lowland area in Southern Romania (Giurgiu county: 44° 15′ 5″ N; 26° 2′ 42″ E) were referred to the local veterinary practices with depression and anorexia. The horses, two males and two females, aged between 10 months to 21 years, originated from three different small farms located within the same village. The first two horses (H1, H2) belonged to the same farm, while the others (H3, H4) belonged each to a different farm. In the originating farms of the clinically affected animals, no other horses were present.

The history of all horses, as recalled by the owners, did not include any travel outside the village border. All horses spent most of the day outdoors and were stabled at night. All the horses had access to pasture and a history of tick infestations over the previous decade of the month.

A routine physical examination of the horses was performed All the horses showed lethargy/depression, pale mucous membranes and fever (39.5 to 40.9 °C) (Table 1). One of them had a per-acute clinical disease, while the others showed moderate to mild forms.

Treatment

The horses were treated with two intramuscular injections of imidocarb-dipropionate (Imizol ®; Schering Plough Animal Health) at a dose rate of 2.2 mg/kg BW at a 48-h interval (de Waal 1992; Friedhoff and Soule 1996).

Ticks and tick spectrum

During the clinical examination, 38 adult ixodid ticks were collected on three of the horses (18 ticks on the horse H1, 7 ticks on the H2, and 13 on the horse H3). They were identified to species level using standard taxonomic keys under a stereomicroscope.

Table 1 Clinical features and results of the *Babesia*-specific PCR and subsequent sequence analysis in symptomatic horses from Romania

Horse	Gender	Age (years)	Date of diagnosis	Clinical signs	Smear exam	PCR results
H1	Male	0.10	31.03. 2015	Anorexia, depression; pale and jaundice conjunctiva; 40.9 °C [+++]	Positive	*Babesia caballi*
H2	Female	7	01.04.2015	Anorexia, depression; pale and jaundice conjunctiva 40.2 °C [++]	Positive	*Babesia caballi*
H3	Female	5	23.04.2015	Lethargy, anorexia; 39.5 °C [+]	Positive	*Babesia caballi*
H4	Male	21	02.05.2015	Lethargy, anorexia; 39.7 °C [+]	Positive	*Babesia caballi*

+ mild /, ++ moderate /, +++ acute clinical presentation

Laboratory investigations

Blood samples were collected from the four horses and tested for the presence of piroplasms using the blood smear method. Briefly, thin blood smear slides were prepared; the smears were air-dried, stained using a Dia-quick Panoptic staining kit (Reagens Ltd., Budapest, Hungary), and then examined by light microscopy at ×1000.

For one horse (H1), a complete blood count (CBC) was performed using an IDEXX LaserCyte and VetTest according to the manufacturer's instructions.

Blood for subsequent DNA analysis was preserved in EDTA test tubes and kept at −20 °C prior to examination.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from 200 μl of EDTA whole blood using DNeasy Blood and tissue Kit (QIAGEN), according to the manufacturer's instructions. Quality and quantity of the extracted DNA were checked with a spectrophotometer (NanoDrop 1000, Peqlab, Erlangen, Germany). The extracted DNA was stored at −20 °C prior to the molecular analysis.

A single-round and multiplex polymerase chain reaction (PCR) method was carried out on all the samples for molecular detection of *B. caballi* and *T. equi* DNA, as described before by Alhassan et al. 2005. The single-round, multiplex PCR protocol used a set of primer combinations in which a forward universal primer (Bec-UF2) that amplifies a fragment of the 18S rRNA gene common for both species and two reverse primers (Cab-R and Equi-R) that are species-specific for *B. caballi* and *T. equi*, respectively.

All PCR reactions were conducted in a total volume of 25 μl consisting of buffer ×1, $MgCl_2$ 2 mM, 0.2 mM of each primer, 0.2 mM dNTP mixture, 2.5 U of Maxima Hot Start DNA polymerase (Thermo Fisher Scientific, USA), and 3 μl DNA template. PCR-clean water was used as a negative control.

The PCR was carried out on a MJ Mini Thermal Cycler (Bio-Rad®). Cycle conditions were as follows: an initial activation (96 °C 10 min), 40 cycles (96 °C 1 min, 60.5 °C 1 min, 72 °C 1 min), and a final extension (72 °C 10 min).

The PCR products were examined under UV light, after 2% agarose gel electrophoresis and staining with ethidium bromide. Positive amplicons were purified using QIAQuick PCR purification kit (QIAGEN) and were sequenced on both DNA strands (CeMIA (Greek)).

The resulting sequences were analyzed and compared with homologous sequences available in GenBank using the Basic Local Alignment Search Tool (BLAST) as described before (Ionita et al. 2012).

The partial 18S rDNA sequences were deposited in GenBank® (accession numbers: MG760619 - MG760622).

Results

Clinical and laboratory findings

The clinical symptoms of the four affected horses included depression, anorexia, fever (39.5–40.9 °C), weakness, and pale mucous membranes, which were consistent with a diagnosis of equine piroplasmosis. One horse (H1) showed severe clinical signs; the others showed only moderate clinical signs (Table 1).

The hematological examination of the first horse in the outbreak (H1) revealed an anemia characterized by a decreased PCV (19.9%), erytrocytopenia (5010×10^3 cells/μl and a reduced hemoglobin level (9.1 g/dl) (Table 2).

The examination of the blood smears revealed intraerythrocyte parasites in all four clinically affected horses. Large (3–5 μm) microorganisms, single or paired, characteristic for *B. caballi* (Mehlhorn and Schein 1998) were detectable (Fig. 1a, b).

All 38 tick specimens collected from the horses were identified as *Dermacentor reticulatus* (27 females, 11 males).

Molecular analysis—sequencing

The molecular analysis confirmed *B. caballi*; the multiplex PCR amplified DNA fragments (of 540 bp) compatible with *B. caballi* (Alhassan et al,. 2005) in all four samples (Fig. 2). The BLAST analysis of all sequences obtained in this study showed 100% identity to the *B. caballi* genotype A (accession number: EU642512.1) and of the horse isolates from Serbia (accession numbers: KR527220; KR527221) and 99% identity to the horse isolates from the Danube Delta (Southeastern Romania) (accession numbers: KJ908939; KJ908940) and Spain (accession numbers: AY534883; AY309955) (Fig. 3).

All sequences of *B. caballi* obtained in this study were identical; alignment of the 538 bp sequences showed a complete identity.

Treatment outcome

After the treatment with imidocarb-dipropionate, none of the treated horses showed any relevant side effects except a slight hyper-salivation in two horses after the first injection (H1, H2). To prevent clinical side effects, the calculated dosage for the second imidocarb-dipropionate injection was split into two and administered 30 min apart. Additionally, supportive therapy was administered to two horses (H1, H2).

Following the imidocarb-dipropionate treatment, the horses' health improved rapidly and they clinically recovered within 1 week.

Table 2 Hematological parameters of a 10-month-old cross-bred horse male with clinical piroplasmosis caused by *Babesia caballi*, Romania

Variable (unit)	Data	Reference interval	Interpretation
Red blood cells (/10^6µl)	5.01	6.80–12.90	↓
Packed cell volume (%)	19.9	32.0–53.0	↓
Hemoglobin (g/dl)	9.1	11.0–19.0	↓
Mean corpuscular volume (fl)	39.6	37.0–60.0	=
Mean corpuscular hemoglobin (pg)	18.1	13.0–20.0	=
Mean corpuscular hemoglobin concentration (g/dl)	37	30–42	=
White blood cells (/10^3µl)	4.81	5.4–14.30	↓
Neutrophils (/10^3µl)	1.3	2.3–8.7	↓
Lymphocytes (/10^3µl)	2.99	1.5–7.7	=
Monocytes (/10^3µl)	0.33	0.10–1.0	=
Eosinophils (/10^3µl)	0.16	0.10–1.00	=
Basophils (/10^3µl)	0.02	0.00–0.3	=
Platelet (/10^3µl)	132	100–500	=

"↓": lower than normal, "↑": higher than normal, "=": between marginal values of reference interval

Discussion

Although *B. caballi* and *Theileria* / *(Babesia) equi* had been reported in Romania in the early 1900s (Cernaianu 1958), clinical outbreaks of equine piroplasmosis in Romania during recent decades have not been reported. The presently described EP—cases occurred in horses on several farms in the Giurgiu County (Southern Romania), which had not ever left the area, confirms the existence of autochtonus clinical *B. caballi* infections in Romanian horses. Since all the affected horses lived in an area known to have an increasing abundance of *Dermacentor reticulatus* (Ionita et al., 2013), such outbreaks are not surprising and should always been considered in clinical cases associated with anemia in tick-infected areas.

The presence of both, *B. caballi* and *T. equi*, respectively, in tick-endemic areas of South-Eastern Romania has recently also been confirmed in a molecular-based survey in asymptomatic Romanian horses (Gallusová et al. 2014), and in *Hyalomma marginatum* ticks collected in various parts of

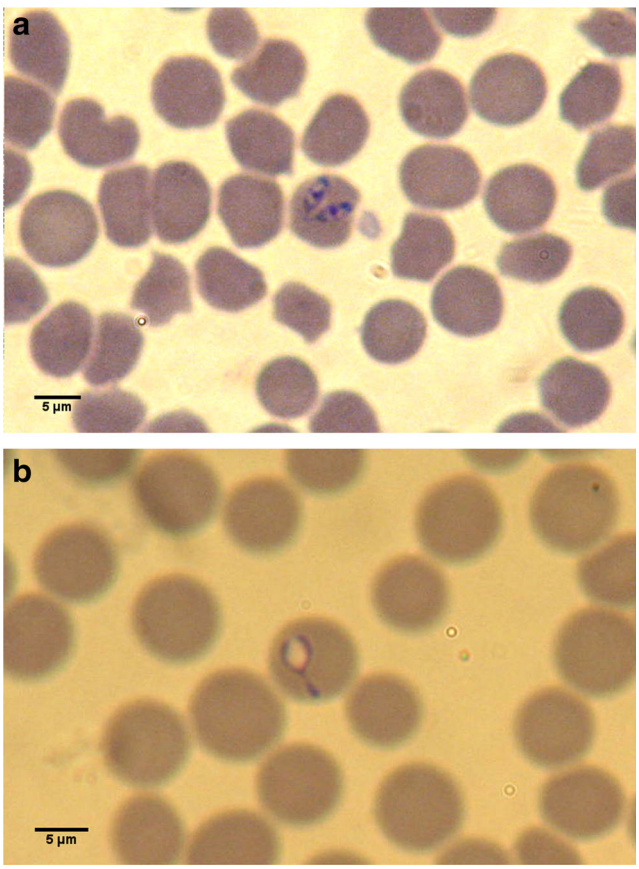

Fig. 1 Peripheral blood smear of a horse from Romania showing intraerythrocytic large piroplasms compatible with *Babesia caballi* [**a** paired forms lied in a sharp angle; **b** single annular and oval shaped forms]

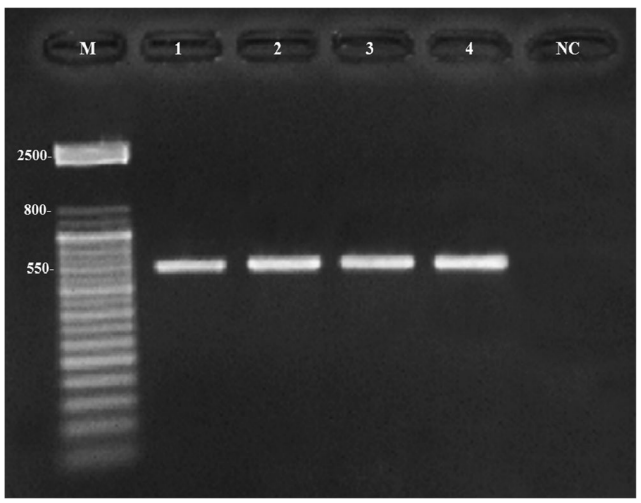

Fig. 2 PCR detection of *Babaesia caballi* DNA in Romanian horses: lanes 1 to 4: PCR products from the four tested horses; NC: negative control M: DNA size marker (50 bp DNA Ladder)

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BcRO            TAGTCCTAACCATAAACTATGCCGACTAGGGATTGGAGGTCGTCATTTTTCCGACTCCTT 60
EU642512.1      TAGTCCTAACCATAAACTATGCCGACTAGGGATTGGAGGTCGTCATTTTTCCGACTCCTT 60
KR527220        TAGTCCTAACCATAAACTATGCCGACTAGGGATTGGAGGTCGTCATTTTTCCGACTCCTT 60
KR527221        TAGTCCTAACCATAAACTATGCCGACTAGGGATTGGAGGTCGTCATTTTTCCGACTCCTT 60
AY534883.1      TAGTCCTAACCATAAACTATGCCGACTAGGGATTGGAGGTCGTCATTTTTCCGACTCCTT 60
AY309955.1      TAGTCCTAACCATAAACTATGCCGACTAGGGATTGGAGGTCGTCATTTTTCCGACTCCTT 60
                ************************************************** *******

BcRO            CAGCACCTTGAGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGTCTGA 120
EU642512.1      CAGCACCTTGAGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGTCTGA 120
KR527220        CAGCACCTTGAGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGTCTGA 120
KR527221        CAGCACCTTGAGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGTCTGA 120
AY534883.1      CAGCACCTTGAGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGTCTGA 120
AY309955.1      CAGCACCTTGAGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGTCTGA 120
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BcRO            AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACT 180
EU642512.1      AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACT 180
KR527220        AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACT 180
KR527221        AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACT 180
AY534883.1      AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACT 180
AY309955.1      AACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACT 180
                ************************************************************

BcRO            CAACACGGGGAAACTCACCAGGTCCAGACAGAGGTAGGATTGACAGATTGATAGCTCTTT 240
EU642512.1      CAACACGGGGAAACTCACCAGGTCCAGACAGAGGTAGGATTGACAGATTGATAGCTCTTT 240
KR527220        CAACACGGGGAAACTCACCAGGTCCAGACAGAGGTAGGATTGACAGATTGATAGCTCTTT 240
KR527221        CAACACGGGGAAACTCACCAGGTCCAGACAGAGGTAGGATTGACAGATTGATAGCTCTTT 240
AY534883.1      CAACACGGGGAAACTCACCAGGTCCAGACAGAGGTAGGATTGACAGATTGATAGCTCTTT 240
AY309955.1      CAACACGGGGAAACTCACCAGGTCCAGACAGAGGTAGGATTGACAGATTGATAGCTCTTT 240
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BcRO            CTTGATTCTTTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTT 300
EU642512.1      CTTGATTCTTTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTT 300
KR527220        CTTGATTCTTTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTT 300
KR527221        CTTGATTCTTTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTT 300
AY534883.1      CTTGATTCTTTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTT 300
AY309955.1      CTTGATTCTTTGGGTGGGGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTT 300
                 *************** ************ ******************************

BcRO            AATTCCGTTAACGAACGAGACCTTAACCTGCTAACTAGCTTCCCttttttttGTTTGGGTT 360
EU642512.1      AATTCCGTTAACGAACGAGACCTTAACCTGCTAACTAGCTTCCCTTTTTTTTGTTTGGGTT 360
KR527220        AATTCCGTTAACGAACGAGACCTTAACCTGCTAACTAGCTTCCCTTTTTTTTGTTTGGGTT 360
KR527221        AATTCCGTTAACGAACGAGACCTTAACCTGCTAACTAGCTTCCCTTTTTTTTGTTTGGGTT 360
AY534883.1      AATTCCGTTAACGAACGAGACCTTAACCTGCTAACTAGCTTCCCTTTTTTTTGTTTGGGTT 360
AY309955.1      AATTCCGTTAACGAACGAGACCTTAACCTGCTAACTAGCTTCCCTTTTTTTTGTTTGGGTT 360
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BcRO            TGCTTCTTAGAGGGACTTTACAACGATAAGTTGTAGGGAAGTTTAAGGCAATAACAGGTC 420
EU642512.1      TGCTTCTTAGAGGGACTTTACAACGATAAGTTGTAGGGAAGTTTAAGGCAATAACAGGTC 420
KR527220        TGCTTCTTAGAGGGACTTTACAACGATAAGTTGTAGGGAAGTTTAAGGCAATAACAGGTC 420
KR527221        TGCTTCTTAGAGGGACTTTACAACGATAAGTTGTAGGGAAGTTTAAGGCAATAACAGGTC 420
AY534883.1      TGCTTCTTAGAAGGACTTTACAACGATAAGTTGTAGGGAAGTTTAAGGCAATAACAGGTC 420
AY309955.1      TGCTTCTTAGAGGGACTTTACAACGATAAGTTGTAGGGAAGTTTAAGGCAATAACAGGTC 420
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BcRO            TGTGATGCCCTTAGATGTCCTGGGCTGCACGCGCGCTACACTGATGCATTCAGTGCGTTT 480
EU642512.1      TGTGATGCCCTTAGATGTCCTGGGCTGCACGCGCGCTACACTGATGCATTCAGTGCGTTT 480
KR527220        TGTGATGCCCTTAGATGTCCTGGGCTGCACGCGCGCTACACTGATGCATTCAGTGCGTTT 480
KR527221        TGTGATGCCCTTAGATGTCCTGGGCTGCACGCGCGCTACACTGATGCATTCAGTGCGTTT 480
AY534883.1      TGTGATGCCCTTAGATGTCCTGGGCTGCACGCGCGCTACACTGATGCATTCAGTGCGTTT 480
AY309955.1      TGTGATGCCCTTAGATGTCCTGGGCTGCACGCGCGCTACACTGATGCATTCAGTGCGTTT 480
                ************************************************************

BcRO            TTCCTGGTCCGAAAGGTCTGGGTAATCTCTAGTATGCATCGTGTCGGGGATTGATTTT 538
EU642512.1      TTCCTGGTCCGAAAGGTCTGGGTAATCTCTAGTATGCATCGTGTCGGGGATTGATTTT 538
KR527220        TTCCTGGTCCGAAAGGTCTGGGTAATCTCTAGTATGCATCGTGTCGGGGATTGATTTT 538
KR527221        TTCCTGGTCCGAAAGGTCTGGGTAATCTCTAGTATGCATCGTGTCGGGGATTGATTTT 538
AY534883.1      TTCCTGGTCCGAAAGGTCTGGGTAATCTCTAGTATGCATCGTGTCGGGGATTGATTTT 538
AY309955.1      TTCCTGGTCCAAAAGGTCTGGGTAATCTCTAGTATGCATCGTGTCGGGGATTGATTTT 538
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Fig. 3 Sequence alignment of the 18S rRNA gene (partial sequence) of the *Babesia caballi* isolates analyzed in this study (BcRO) and homologous (EU642512.1: *B. caballi* genotype A_CABEQ30: USDA reference strain; KR527220: *B. caballi* isolate from Bosnia and Hertegovina; KR527221: *B. caballi* isolate from Montenegro; AY534883.1 and AY309955.1: *B. caballi* strain Spain (in gray are highlighted the differences between the isolates)

Romania (Ionita et al. 2013). But so far nothing is known about the prevalence of clinical *B. caballi* cases in Romania.

In neighboring countries, high serological and/or molecular prevalence of *T. equi* and *B. caballi* have been reported in horses from the central Balkans (Serbia, Muntenegru, Bosnia, and Hertegovine) and Hungary, ranging from 22.5 to 49.0% for *T. equi* (Farkas et al. 2013; Davitkov et al. 2016) and 2.1 to 7.8% in for *B. caballi*, respectively (Hornok et al. 2007; Davitkov et al. 2016). Therefore, further studies on the epidemiology of EP in Romanian horses will elucidate the present status of this infection in Romania.

The molecular analysis not only confirm the presence of *B. caballi* genotype A in all the four infected horses as initially diagnosed by blood smear testing but also allowed to demonstrate a 100% identity with isolates from horses in Serbia (Davitkov et al. 2016) and a 99% identity with isolates from Southern Romania (Danube Delta) (Gallusová et al. 2014) and from Spain (Criado-Fornelio et al. 2004; Nagore et al. 2004). Consequently, these molecular results suggest that the present infections are a part of a geographically extended *B. caballi* genotype A focus in the Balkan region.

Ongoing molecular epidemiological studies will allow a better characterization of this endemic situation of equine piroplasmosis in Romania and surrounding countries.

The observed mild clinical signs including fever, depression, anorexia, and slight jaundice in three out of four horses parasitologically diagnosed (by Giemsa-stained blood smear), together with the more acute course of clinical *B. caballi* infection in one horse (H1), are consistent with the descriptions of clinical *B. caballi* infections in the literature (Camacho et al. 2005). Thus, *B. caballi* infections generally cause rather moderate clinical signs when compared to *T. equi* infections (Zobba et al. 2008; Machado et al. 2012). Acute cases of *B. caballi* infections are characterized by fever that usually exceeds 40 °C, dyspnea, congestion of mucous membranes, edemas icterus, anemia, and hemoglobinuria (Friedhoff and Soule 1996).

It is well established that horses can remain carriers for *B. caballi* for up to 4 years and probably life-long for *T. equi* infection (Wise et al. 2013). Therefore, in endemic regions, premunition (protective immunity) by persistent subclinical infection plays a role in the protection of horses against subsequent infection and clinical disease (de Waal 1992). Accordingly, in the endemic regions, the primary objective for treatment is to reduce clinical disease rather than to clear infection, unless horses have to be moved from an endemic region to an EP-free region (Butler et al. 2005). Two treatments with Imidocarb-dipropionate (Imizol ®; Schering Plow Animal Health), at a dose rate of 2.2 mg/kg BW, administered intramuscularly twice at an interval of 48 h, were highly efficacious in suppressing the clinical signs and, therefore, appropriate for an endemic area. None of the treated horses showed any adverse side effects to the treatment.

Since at present, there are no commercially available effective vaccines for *T. equi* or *B. caballi*; control of EP largely relies on drug therapy, restrictions in the movement of infected horses, premunition strategies, and control of tick vectors, depending on the infection status (endemic versus non-endemic) (Wise et al. 2013). In areas with suspected or even confirmed risk of EP, an acaricidal prophylaxis can be considered in order to protect horses at risk from serious outbreaks. In these endemic areas, strategic tick control should agree with the seasonality of tick infestation. However, development of acaricide resistance is a serious problem in many heavily tick-infested areas in the world and is a consideration for rational use (Rothschild and Knowles 2007).

Conclusion

The findings of the present study emphasize on the potential risks of equine piroplasmosis in Romania. This report presents the first molecularly characterized *B. caballi* outbreak in Romania in clinically affected horses, confirmed by DNA sequencing.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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