Veterinary Parasitology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Piroplasms in feral and domestic equines in rural areas of the Danube Delta, Romania, with survey of dogs as a possible reservoir

Martina Gallusová[a,d,*], Moneeb A. Qablan[a,d], Gianluca D'Amico[b], Miroslav Oborník[c], Klára J. Petrželková[a,c,e,f], Andrei D. Mihalca[b], David Modrý[a,c,d]

[a] Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1, 612 42 Brno, Czech Republic
[b] Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania
[c] Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Branišovská 31, 37005 České Budějovice, Czech Republic
[d] CEITEC-VFU, University of Veterinary and Pharmaceutical Sciences, Palackého1, 612 42 Brno, Czech Republic
[e] Institute of Vertebrate Biology Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic
[f] Liberec Zoo, Masarykova, 1347/31, 460 01 Liberec, Czech Republic

ARTICLE INFO

Article history:
Received 16 June 2014
Received in revised form 7 October 2014
Accepted 8 October 2014

Keywords:
T. equi
Babesia caballi
Equine piroplasmoses
Danube Delta
Genetic diversity

ABSTRACT

Rural areas of Romania, particularly the localities covering Danube Delta, are still not sufficiently explored in terms of epidemiological aspects, despite the large density of domestic animals living in close contact with people and natural environment of the Danube Delta Biosphere Reserve. Between 2010 and 2012, a survey on equine piroplasmids species was performed in this area, using a total of 178 horses, 15 donkeys and 177 dogs from 18 localities inside and outside the Danube Delta. None of the sampled hosts showed any clinical symptoms typical for equine piroplasmoses. A 25.4% overall prevalence for both equine species of piroplasmids was detected by PCR. Detection by "catch-all" primers followed by multiplex PCR revealed 20.3% prevalence of *T. equi*, beside 2.2% of *B. caballi* and 3.0% of mixed infections for all examined animals. Based on sequencing of 67 PCR amplicons of 18S rRNA gene of *T. equi*, four genotypes (A, C, D and E) were detected, whereas four *B. caballi* sequences clustered within a single genotype (A). A single dog infected with *T. equi* was found and confirmed by sequencing, clustering within genotype D. Our study further proved limited host specificity of piroplasmid. Higher prevalence was revealed inside the Danube delta, presumably caused by different environmental conditions, different tick densities and lower availability of veterinary services.

1. Introduction

Danube Delta is one of the largest wetlands in Europe, and the greater part of the delta is situated in the south-eastern part of Romania (Navodaru et al., 2001). The Danube Delta Biosphere Reserve is (DDBR) recognised under UNESCO's Man and Biosphere program since 1992

* Corresponding author at: Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1, 612 42 Brno, Czech Republic. Tel.: +420 541 562 474; fax: +420 541 562 266.
E-mail addresses: gallusovam@vfu.cz, MartaGall@seznam.cz (M. Gallusová).

http://dx.doi.org/10.1016/j.vetpar.2014.10.018
0304-4017/

and represents a unique mosaic of wetland and rural ecosystems (Ioras, 2003). Feral horse population is distributed in areas confounded to Letea forest and the region enclosed by Sulina and Chilia branches of the Danube. Based on their appearance, they are considered a cross between the 'Huţul' and Arabic breeds (Simon et al., 2012), however, currently they are a mixture between original feral horses and those abandoned recently (DDBRA, 2011).

Theileria equi and *Babesia caballi* are the causative agents of equine piroplasmoses, and transmitted by several ticks belongs to genera *Rhipicephalus*, *Dermacentor* and *Hyalomma* (Rothschild, 2013). In Romania, most of the previous epidemiological studies on equine pathogens were connected to equine infectious anaemia (Cappelli et al., 2011) and West Nile virus (Ludu Oslobanu et al., 2014). The first record of equine piroplasmoses comes from Moldova region and dates back to the first decade of the previous century (Nicolau and Calinescu, 1912, cited by Cernaianu, 1958). Recently, *T. equi* was detected in Romanian *Hyalomma marginatum* (Ioniţă et al., 2013); however, molecular studies on the occurrence of piroplasmids in Romanian equines are absent.

Molecular diagnostic assays followed by sequencing allow the detection of piroplasmids, even during latent infections, as well as determination of genotypes (Qablan et al., 2013). During the last decade, several molecular studies illustrated lower host-specificity of piroplasmids (Fritz, 2010; Hamel et al., 2012; Qablan et al., 2012) and suggested possible transmissions between equines and dogs. So far, five *T. equi* and three *B. caballi* genotypes have been described, with more diversity reported within *T. equi* (Munkhjargal et al., 2013). The aims of this study were (i) to determine the prevalence and diversity of piroplasmids infecting feral and domestic equines in the Danube Delta area, (ii) to assess the intraspecific genetic diversity of detected piroplasmids, (iii) to assess the extent of cross transmissions between equines and domestic dogs.

2. Materials and methods

2.1. Location and sample collection

A cross-sectional molecular epidemiological survey was carried out during three consecutive years (2010–2012). The study was conducted in several localities inside and adjacent to the Danube delta in Romania (Table 1). Horses examined in this study were classified into three categories based on their husbandry status: domestic, semi-feral and feral. In total, 193 equines and 177 dogs were sampled (Table 1). The data regarding the age, sex, breed, and clinical signs were collected from the owner or after basic examination. Blood samples were obtained via puncture of the jugular vein using plastic containers Hemos H-01 (Gama group, Czech Republic) fitted with 18G needles (for equines) or from the cephalic vein using syringes fitted with 21G needles (for dogs). Two blood smears were prepared from each animal and subsequently fixed in methanol. Blood for later DNA analysis was preserved either in 5 ml EDTA test-tube, or in 2 ml 96% ethanol and kept at −20 °C prior to examination.

2.2. Sample analyses

2.2.1. Microscopy, DNA extraction and PCR

Blood smears were stained with Giemsa solution (Merck, Germany) and examined using light microscopy (Olympus AX 70). Genomic DNA was isolated from blood samples (using 200 μl preserved in EDTA or same amount preserved in ethanol) by modified phenol-chloroform method. For equine piroplasmids detection, two PCR assays ("catch-all" and multiplex) were followed (for further details see Sloboda et al., 2011). Detection of canine piroplasmids was commenced with nested PCR (Jefferies et al., 2007). All PCR reactions were conducted in a total volume of 25 μl consisting of; ~30 ng of DNA template, 12.5 μl of

Table 1
Material collected from equids in sampled localities.

	Locality	Horse			Donkey
		Domestic	Feral	Semi-feral	
Outside delta	Satu Nou	4	–	–	1
	Turda	6	–	–	1
	Cataloi	7	–	–	4
	Visterna	16	–	–	1
	Enisala	–	–	–	1
	Săcele	12	–	–	–
	Nuntaşi	7	–	–	4
	Istria	1	–	–	–
Inside delta	Mineri	3	–	–	–
	Nufăru	2	–	–	–
	Iligani de Jos	1	–	–	1
	Maliuc	11	–	13	2
	Ceatalchioi	2	–	–	–
	Sălcieni	5	–	–	–
	Sfântu Gheorghe	11	9	–	–
	Caraorman	6	–	–	–
	Chilia Veche	20	42	–	–
Total		114	51	13	15

Please cite this article in press as: Gallusová, M., et al., Piroplasms in feral and domestic equines in rural areas of the Danube Delta, Romania, with survey of dogs as a possible reservoir. Vet. Parasitol. (2014), http://dx.doi.org/10.1016/j.vetpar.2014.10.018

Table 2
The numbers of sampled animals and results of examination by "catch-all" and multiplex primers.

	Primers					
	"Catch-all"		Multiplex			
Host	*n*	%	*Theileria* (*n*)	%	*Babesia* (*n*)	%
Horse	87	48.9	69	38.8	8	4.5
Donkey	6	40	5	1.3	0	–

Combi PPP Master Mix (Top-Bio), 1 μl of each primers and 8.5 μl of PCR water.

Positive controls were: DNA isolated from blood of naturally infected horse and donkey from Italy (for equine piroplasmids) and DNA isolated from two dogs infected by *B. canis* (for canine piroplasmids). Negative controls were DNA isolated from piroplasmids-free horse and dog provided by animal clinics at the University of Veterinary and Pharmaceutical Sciences (UVPS), Brno, Czech Republic. The amplified PCR products were subjected to 1.5% agarose gel electrophoresis stained with Gold-View.

2.2.2. Sequencing, phylogenetic analyses and statistics

Selected PCR amplicons from the "catch-all" essay were purified using QIAquick gel extraction kit (Qiagen, Germany) and sequenced in both directions (Macrogen, Netherlands). Obtained sequences were aligned to homologues available from GenBank™ using KALIGN (Lassmann and Sonnhammer, 2005) and edited in Bioedit (Hall, 1999). Phylogenetic trees were constructed using maximum parsimony (MP) as implemented in PAUP* 4b10 (Swofford, 2002), maximum likelihood (ML) with gamma corrected GTR model (PhyML; Guindon and Gascuel, 2003), and Bayesian inference with gamma corrected GTR model (MrBayes 3.2; Ronquist et al., 2012). Robustness of MP and ML trees was inferred from bootstrap analyses computed from 1000 replicates in the frame of used programs.

The effect of several factors on the occurrence of *T. equi* was tested using General Linear Model with binomial distribution. The factors tested include: locality (inside the delta, outside the delta), sex (male, female), domestication status (feral, semiferal, domestic), age (0–4 years, 4–12 years, above 12 years) and species (donkey, horse). Data from *B. caballi* were not included due to a low number of detected cases. The analysis were performed in R v. 2.13.1 (R Development Core Team, 2011).

3. Results

None of the animals included in the study showed clinical symptoms typical for piroplasmoses, except for mild anaemia reported from three horses that later appeared positive for *T. equi* in PCR. Neither *Babesia* nor *Theileria* stages were detected by microscopy.

3.1. Prevalence based on PCR

While the PCR result shows that 93 equines (48.1%) were positive for piroplasmids with "catch-all", only 82 animals were positive with the multiplex PCR (Table 2). The exact prevalence of *T. equi* and *B. caballi* based multiplex PCR are indexed in Table 2. Five horses (three domestic and two feral) were co-infected with both species of piroplasmids (2.8%). The highest prevalence of piroplasmids was among the feral horses (64.7%), followed by semi-feral (46.2%) and domestic (33.3%). The occurrences of *T. equi* and *B. caballi* among different horse groups are shown in Table 3. Only *T. equi* was detected from positive donkeys. The prevalence of both *T. equi* and *B. caballi* was significantly higher among equids sampled from localities inside of the delta (34.8% for *T. equi*, 3.9% for *B. caballi*), compared to localities outside of the delta (1.8% for *T. equi*, 0% for *B. caballi*).

Among the dogs examined, "catch-all" primers revealed a single positive dog which was also positive for *T. equi* by multiplex PCR. The specific nested PCR-assay for canine piroplasmids revealed another dog positive for *B. canis* (data not shown).

Only location and age were statistically significant factors concerning *T. equi* infection (GLM, location: $z = 5.246$, $p < 0.001$; age: $z = 3.349$, $p < 0.001$), while other factors were not significant (GLM: domestic status: $z = -1.260$, $p = 0.20$; species: $z = 0.904$, $p = 0.366$; sex: $z = 0.904$, $p = 0.366$).

3.2. Sequencing and phylogenetic analyses

Overall, we retrieved 71 sequences from 93 amplicons amplified with the "catch-all" PCR assay. Among them, 67 sequences were identified as *T. equi*, the rest as *B. caballi*. Phylogenetic trees of the obtained sequences were constructed to infer the intraspecific diversity of each equine species separately (Fig. 1a and b). All sequences of *B. caballi*

Table 3
Prevalence's of *T. equi* and *B. caballi* among different horses groups based on the "catch-all" primers.

Group of horses	Number of individuals	Positive individuals		Prevalence (%)		Total prevalence (%)
		T. equi	*B. caballi*	*T. equi*	*B. caballi*	
Domestic	114	36	2	31.6	1.8	33.3
Semi-feral	13	3	3	23.1	23.1	46.2
Feral	51	30	3	58.8	5.9	64.7
Total	178	69	8	38.8	4.5	47.8

Please cite this article in press as: Gallusová, M., et al., Piroplasms in feral and domestic equines in rural areas of the Danube Delta, Romania, with survey of dogs as a possible reservoir. Vet. Parasitol. (2014), http://dx.doi.org/10.1016/j.vetpar.2014.10.018

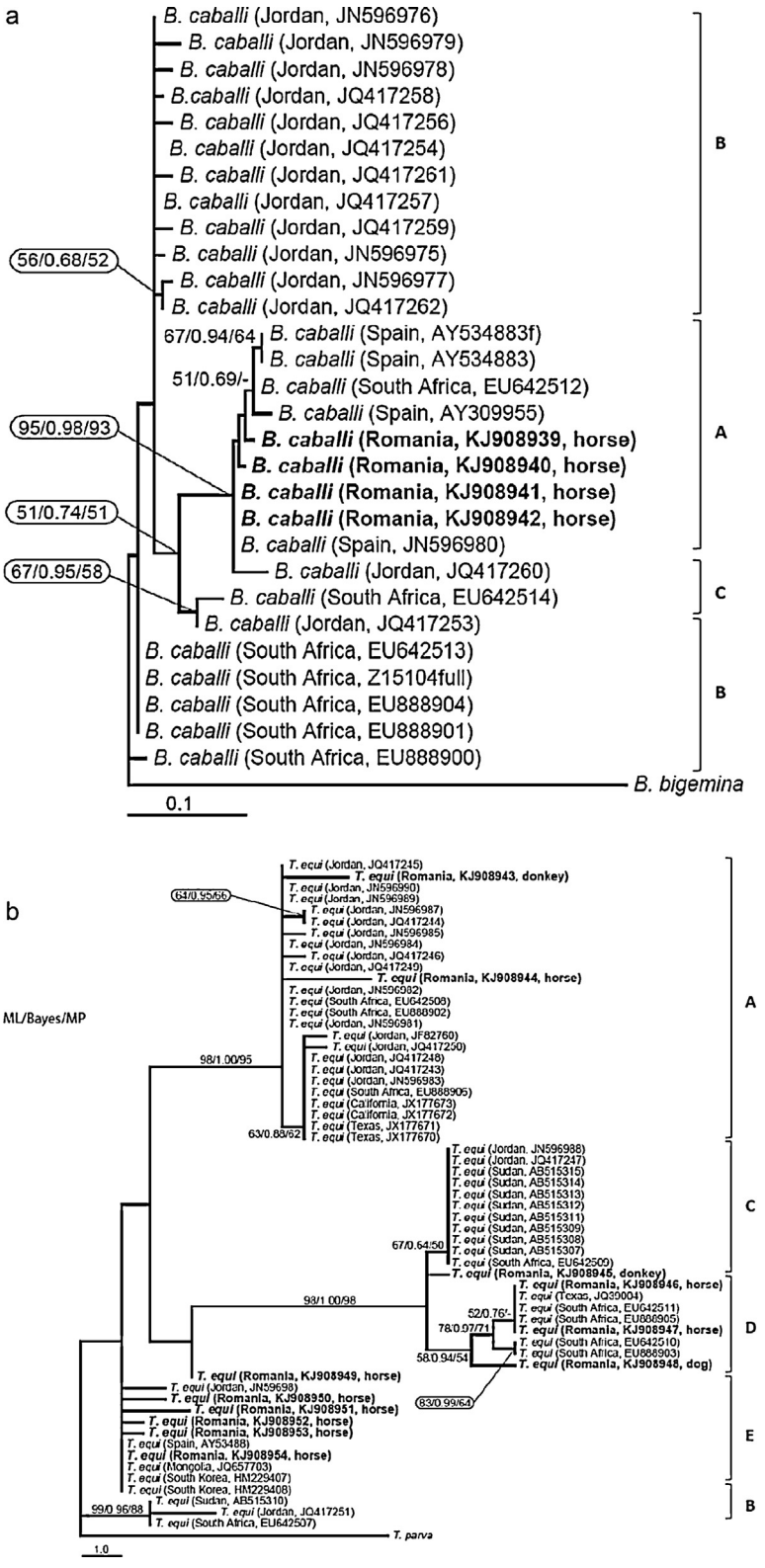

Fig. 1. a. Phylogenetic tree of *B. caballi* as derived from partial sequences of the 18S rRNA gene. The numbers above branches label maximum likelihood/maximum parsimony/Bayesian inference bootstrap supports values. Sequences in bold represent those obtained in this study. b. Phylogenetic tree of *T. equi* as derived from partial sequences of the 18S rRNA gene. The numbers above branches label maximum likelihood/maximum parsimony/Bayesian inference bootstrap supports values. Sequences in bold represent those obtained in this study.

Please cite this article in press as: Gallusová, M., et al., Piroplasms in feral and domestic equines in rural areas of the Danube Delta, Romania, with survey of dogs as a possible reservoir. Vet. Parasitol. (2014), http://dx.doi.org/10.1016/j.vetpar.2014.10.018

obtained in this study belong to genotype A (Fig. 1a). The cluster of our samples within the genotype A clade was supported with high bootstrap values. Sequences of *B. caballi* previously classified as genotype B appeared in our analyses further split into two sub-clades, however, the bootstrap values were low.

Sixty-seven sequences were identified as *T. equi*, and among them 55 sequences were 100% identical. A total of 12 unique sequences were indexed in the GenBank™ (KC908939–KJ908954). Four previously known *T. equi* genotypes (A, C, D and E) were identified and they were represented by 2, 1, 3 and 6 unique sequences, respectively (Fig. 1b). All clades were supported with significant bootstrap values. The distribution of *T. equi* genotypes differ between three groups of horses; while all four determined genotypes were found among domestic horses, only genotypes D and E were detected among feral horses and, finally, genotype E was the only one found in semi-feral horses. Single sequence retrieved from a dog was identified as *T. equi* genotype D. Genotype E of *T. equi* was found only inside the Danube Delta, however, in two locations (Caraorman, Sfântu Gheorghe) it occurred together with genotype D.

4. Discussion

The mutual contact of a variety of domestic, feral and wild animals sharing not only the environment, but also a wide spectrum of vectors, undoubtedly makes the Danube Delta one of the unique places to study the vector-borne infections. Previously, the horse population in Danube delta was only screened for equine strongyles (Răileanu et al., 2008; Cernea et al., 2009). Herein, we addressed the genetic diversity of *T. equi* and *B. caballi* in horses and donkeys for the first time, not only in Romanian feral horses, but also in Romania as such.

Both *T. equi* and *B. caballi* were more frequently encountered among the feral horses. Although the populations of feral horses are distributed in several areas around the world, studies addressing piroplasmid infections in those populations are limited to Mongolia, where Robert et al. (2005) and Rüegg et al. (2006) studied the impact of equine piroplasmoses on reintroduced Przewalski's horses. In contrast to our finding, *B. caballi* was more prevalent than *T. equi*, using serological diagnostics.

The multiplex PCR assay and sequencing confirmed that *T. equi* was, in our study, more prevalent than *B. caballi*, which can be explained by the life-long persistence of *T. equi* infection compared to effective elimination of *B. caballi* (Chauvin et al., 2009). Such finding is in agreement with previous studies from South Africa (Bhoora et al., 2009), Greece (Kouam et al., 2010), Mongolia (Sloboda et al., 2011) and Jordan (Qablan et al., 2013).

Previously, using the PCR targeting various genes (18S rRNA, BC48 gene and EMA-1 gene), Munkhjargal et al. (2013) found *B. caballi* more prevalent than *T. equi* among horses from Tov province in Mongolia, which corresponds with serological data from Przewalski's horses. This contradiction can be explained by different affinity of *B. caballi* to different tick species and by differences in tick's abundance (Munkhjargal et al., 2013). In our study, the ticks were absent on the animals examined, probably due to seasonality. Drawing any conclusions on the possible influence of particular tick species on the prevalence of equine piroplasmids within surveyed localities would require further sampling effort during spring months.

Among horses, the piroplasmids were only found in animals from inside the Delta localities. Such a geographical delimitation may result from higher humidity (Gray et al., 2013) as well as the ecological diversity of the habitats (Mihalca et al., 2012; Sándor et al., 2014). These qualities, collectively, represent more suitable conditions for the ticks.

Few studies, based on molecular detection, found the age a significant factor influencing the prevalence of equine piroplasmids (Kouam et al., 2010; Sloboda et al., 2011; Qablan et al., 2013). The same effect of age is apparent also among our dataset, as *T. equi* infection was more prevalent among equids aged 4 years and older. The increasing prevalence of *T. equi* with age is mainly attributed to the life persistence nature of *T. equi* infection (Kouam et al., 2010), while donkeys are more refractory to the infection (Pearson et al., 2000).

Molecular taxonomy revealed the host specificity of piroplasmids to be lower than previously supposed (Moretti et al., 2010; Qablan et al., 2012). Although those were mainly the zoonotic species (*Babesia* EU1, *Babesia microti* and *Babesia divergens*) attracting the attention (Häselbarth et al., 2007; Gray et al., 2010), the role of dogs in life cycle of "non-canine" piroplasmids species can have epidemiological consequences. Detection of a single *T. equi* case among 177 dogs examined corresponds to findings from France (Fritz, 2010), Croatia (Beck et al., 2009) and from Jordan (Qablan et al., 2012), however, does not suggest high epidemiological importance of dogs as reservoirs. Interestingly, *B. canis* was almost absent in studied dog population, which can be related to the absence of *D. reticulatus* and corresponds well to the absence of clinical babesioses in dogs in the area.

The intraspecific genetic diversity of piroplasmids is gradually increasing as new sequences are introduced into the analyses (Bhoora et al., 2009; Qablan et al., 2013). However, in both species we identified only previously known genotypes (*B. caballi* genotype A and *T. equi* genotypes A, C, D and E), with a notably high degree of diversity among *T. equi* isolates. In study describing the diversity of equine piroplasmoses in the Near East, Qablan et al. (2013) suggested the existence of one or more species within *T. equi* complex. Recently, alongside the SSU rRNA gene, Munkhjargal et al. (2013) included also the *T. equi* merozoite antigen1 (EMA1) gene in their phylogenetic analysis and reported four different groups of *T. equi* isolates, however, clustering arrangement were different from SSU rRNA tree topology. As long as we do not have clear species concept and data on association of piroplasmid genotypes with particular vectors, the relevance of intraspecific diversity of equine piroplasmids remains in mist, mainly due to their common co-occurrence and broad distribution.

Acknowledgements

This work was, in part, funded by European Social Fund and state budget of the Czech Republic by projects OP VK

Please cite this article in press as: Gallusová, M., et al., Piroplasms in feral and domestic equines in rural areas of the Danube Delta, Romania, with survey of dogs as a possible reservoir. Vet. Parasitol. (2014), http://dx.doi.org/10.1016/j.vetpar.2014.10.018

CZ.1.07/2.2.00/28.0288 and OP VK CZ.1.07/2.3.00/30.0014. The laboratory part of the study was supported by grant COST CZ LD14048. The survey was organized in the framework of the EurNegVec COST Action TD1303 and partly financed by the IDEI PCE 236/2011 grant. We thank all veterinarians (especially Gheţu Gabriela, DVM from Chillia-Veche), horsemen and students participating in the survey.

References

Beck, R., Vojta, L., Mrljak, V., Marinculić, A., Beck, A., Zivicnjak, T., Cacciò, S.M., 2009. Diversity of Babesia and Theileria species in symptomatic and asymptomatic dogs in Croatia. Int. J. Parasitol. 39, 843–848.

Bhoora, R., Franssen, L., Oosthuizen, M.C., Guthrie, A.J., Zweygarth, E., Penzhorn, B.L., Jongejan, F., Collins, N.E., 2009. Sequence heterogeneity in the 18S rRNA gene within *Theileria equi* and *Babesia caballi* from horses in South Africa. Vet. Parasitol. 159, 112–120.

Cappelli, K., Capomaccio, S., Cook, F.R., Felicetti, M., Marenzoni, M.L., Coppola, G., Verini-Supplizi, A., Coletti, M., Passamonti, F., 2011. Molecular detection, epidemiology, and genetic characterization of novel European field isolates of equine infectious anemia virus. J. Clin. Microbiol. 49, 27–33.

Cernaianu, C.C. (Ed.), 1958. Piroplasme si piroplasmoze, vol. 2. Editura Academiei Republici Populare, Romine, p. 779.

Cernea, M., Cernea, C.L., Răileanu, S., Carvalho, L.M., 2009. Determination of resistance reference parameters of Equine Strongyls to Anthelmintic. Vet. Med. Us. 66, 1.

Chauvin, A., Moreau, E., Bonnet, S., Plantard, O., Malandrin, L., 2009. Babesia and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. Vet. Res. 40, 37.

DDBRA, 2011. Danube Delta Biospherical Reserve Administration. The Situation of Abandoned Horses in Letea Forest Area of the Danube Delta Biosphere Reserve, Retrieved from: http://www.self-willedland.org.uk/articles/Horses_from_Letea.pdf,. Accessed: 2 feb 2014.

Fritz, D., 2010. A PCR study of piroplasmids in 166 dogs and 111 horses in France (March 2006 to March 2008). Parasitol. Res. 106, 1339–1342.

Guidon, S., Gascuel, O., 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood, Syst. Biol. 52, 696–704.

Gray, J., Dantas-Torres, F., Estrada-Peña, A., Levin, M., 2013. Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Ticks. Tick. Borne. Dis. 4, 171–180.

Gray, J., Zintl, A., Hildebrandt, A., Hunfeld, K.P., Weiss, L., 2010. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. Ticks. Tick. Borne. Dis. 1, 3–10.

Hall, T.A., 1999. BioEdit. A user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic.Acid.Symp. Ser. 41, 95–98.

Hamel, D., Silaghi, C., Lescai, D., Pfister, K., 2012. Epidemiological aspects on vector-borne infections in stray and pet dogs from Romania and Hungary with focus on *Babesia* spp. Parasitol. Res. 110, 1537–1545.

Häselbarth, K., Tenter, A.M., Brade, V., Krieger, G., Hunfeld, K.P., 2007. First case of human babesiosis in Germany – clinical presentation and molecular characterisation of the pathogen. Int. J. Med. Microbiol. 297, 197–204.

Ioniţă, M., Mitrea, I.L., Pfister, K., Hamel, D., Silaghi, C., 2013. Molecular evidence for bacterial and protozoan pathogens in hard ticks from Romania. Vet. Parasitol. 196, 71–76.

Ioras, F., 2003. Trends in Romanian biodiversity conservation policy. Biodivers. Conserv. 12, 9–23.

Jefferies, R., Ryan, U.M., Irwin, P.J., 2007. PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies. Vet. Parasitol. 144, 20–27.

Kouam, M.K., Kantzoura, V., Masuoka, P.M., Gajadhar, A.A., Theodoropoulos, G., 2010. Genetic diversity of equine piroplasmids in Greece with a note on speciation within *Theileria* genotypes (*T. equi* and *T. equi*-like). Infect. Genet. Evol. 10, 963–968.

Lassmann, T., Sonnhammer, E.L., 2005. Kalign – an accurate and fast multiple sequence alignment algorithm. BMC Bioinf. 6, 298.

Ludu Oslobanu, E.L., Mihu-Pintilie, A., Anită, D., Anita, A., Lecollinet, S., Savuta, G., 2014. West Nile virus reemergence in Romania: a serologic survey in host species. Vector Borne Zoonotic Dis. 14, 330–337.

Mihalca, A.D., Dumitrache, M.O., Magdaş, C., Gherman, C.M., Domşa, C., Mircean, V., Ghira, I.V., Pocora, V., Ionescu, D.T., Sikó Barabási, S., Cozma, V., Sándor, A.D., 2012. Synopsis of the hard ticks (Acari: *Ixodidae*) of Romania with update on host associations and geographical distribution. Exp. Appl. Acarol. 58, 183–206.

Moretti, A., Mangili, V., Salvatori, R., Maresca, C., Scoccia, E., Torina, A., Moretta, I., Gabrielli, S., Tampieri, M.P., Pietrobelli, M., 2010. Prevalence and diagnosis of Babesia and Theileria infections in horses in Italy: a preliminary study. Vet. J. 184, 346–350.

Munkhjargal, T., Sivakumar, T., Battsetseg, B., Nyamjargal, T., Aboulaila, M., Purevtseren, B., Bayarsaikhan, D., Byambaa, B., Terkawi, M.A., Yokoyama, N., Igarashi, I., 2013. Prevalence and genetic diversity of equine piroplasmids in Tov province, Mongolia. Infect. Genet. Evol. 16, 178–185.

Navodaru, I., Staras, M., Cernisencu, I., 2001. The challenge of sustainable use of the Danube Delta Fisheries, Romania. Fisheries. Manag. Ecol. 8, 323–332.

Pearson, R.A., Nengomasha, E., Krecek, R., 2000. The challenge in using donkeys for work in Africa. In: Starkey, P., Fielding, D. (Eds.), Donkeys, People and Development. a Resource Book of the Animal Traction Network for Eastern and Southern Africa (ATNESA), The Netherlands, pp. 194–195.

Qablan, M.A., Oborník, M., Petrželková, K.J., Sloboda, M., Shudiefat, M.F., Hořín, P., Lukeš, J., Modrý, D., 2013. Infections by *Babesia caballi* and *Theileria equi* in Jordanian equids: epidemiology and genetic diversity. Parasitology 140, 1096–1103.

Qablan, M.A., Sloboda, M., Jirků, M., Oborník, M., Dwairi, S., Amr, Z.S., Hořín, P., Lukeš, J., Modrý, D., 2012. Quest for the piroplasmids in camels: identification of *Theileria equi* and *Babesia caballi* in Jordanian dromedaries by PCR. Vet. Parasitol. 186, 456–460.

R Development Core Team, 2011. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, Retrieved from: http://www.R-project.org/

Răileanu, S., Cernea, M., Cernea, L.C., Gut, A., Silberg, R., 2008. The identification of feral-wild horse's strongyles from Danube Delta. In: Danube Delta National Institute for Research and Development, Tulcea, Romania. The XVII Intrnational Symposium, Deltas and Wetlands 2008 18-20 September 2008.

Robert, N., Walzer, C., Rüegg, S.R., Kaczensky, P., Ganbaatar, O., Stauffer, C., 2005. Pathologic findings in reintroduced Przewalski's horses (*Equus caballus przewalskii*) in southwestern Mongolia. J. Zoo. Wildl. Med. 36, 273–285.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Ecient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Rothschild, Ch.M., 2013. Equine piroplasmoses. J. Hered. 33, 497–508.

Rüegg, S.R., Torgerson, P.R., Doherr, M.G., Deplazes, P., Böse, R., Robert, N., Walzer, C., 2006. Equine piroplasmosis at the reintroduction site of the Przewalski's horse (*Equus ferus przewalskii*) in Mongolia. J. Wildl. Dis. 42, 518–526.

Sándor, A.D., Mărcuţan, D.I., D'Amico, G., Gherman, C.M., Dumitrache, M.O., Mihalca, A.D., 2014. Do the ticks of birds at an important migratory hotspot reflect the seasonal dynamics of *Ixodes ricinus* at the migration initiation site? A case study in the Danube Delta. PLoS One 9, e89378.

Simon, T., Carlogea, A., Timotin, V., 2012. Opportunities for the development of tourism in Letea River levee of the Danube Delta biosphere reserve. Forum Geographic. 11, 16–23.

Sloboda, M., Jirků, M., Lukešová, D., Qablan, M., Batsukh, Z., Fiala, I., Hořín, P., Modrý, D., Lukeš, J., 2011. A survey for piroplasmids in horses and Bactrian camels in North-Eastern Mongolia. Vet. Parasitol. 179, 246–249.

D.L. Swofford, PAUP*. Phylogenetic Analyses Using Parsimony (*and Other Methods), Version 4.0b10 (Alvitec). Sinauer Asociates, Sunderland, MA,USA. 2002.

Please cite this article in press as: Gallusová, M., et al., Piroplasms in feral and domestic equines in rural areas of the Danube Delta, Romania, with survey of dogs as a possible reservoir. Vet. Parasitol. (2014), http://dx.doi.org/10.1016/j.vetpar.2014.10.018