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Short communication

Diversity of tick species and associated pathogens on peri-urban wild boars – First report of the zoonotic *Babesia* cf. *crassa* from Hungary

Sándor Hornok [a,*], Sándor Szekeres [a], Gábor Horváth [b], Nóra Takács [a], Katinka Bekő [c], Jenő Kontschán [d], Miklós Gyuranecz [c], Barnabás Tóth [a], Attila D. Sándor [a,e], Alexandra Juhász [a], Relja Beck [f], Róbert Farkas [a]

[a] Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary
[b] Veterinary Authority, Csurgó, Hungary
[c] Veterinary Medical Research Institute, Budapest, Hungary
[d] Plant Protection Institute, Centre for Agricultural Research, ELKH, Budapest, Hungary
[e] Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[f] Department for Bacteriology and Parasitology, Croatian Veterinary Institute, Zagreb, Croatia

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ABSTRACT

Wild boars show increasing numbers and population densities throughout Europe, including Hungary. While their presence is appreciated as game animals, they are also responsible for significant agricultural damage, habitat degradation and water quality issues. In addition, wild boars may harbor ticks and can act as reservoirs of tick-borne pathogens, thus posing a risk of transmission towards humans and domestic animals. This latter aspect of their veterinary-medical and epidemiological significance has become especially important in recent years, because increasing numbers of wild boars are reported to enter urban areas. Despite of this, reports on tick infestations of wild boars are scarce in Europe.

For this study, 333 ixodid ticks were collected from 51 wild boars at 32 peri-urban locations in 14 counties of Hungary, during 2005-2008 (older samples) and 2019-2020 (new samples). Five species of ticks were identified: *Dermacentor reticulatus* ($n = 165$), *Ixodes ricinus* ($n = 90$) and *Haemaphysalis concinna* ($n = 29$) in both sample groups, while *H. inermis* ($n = 29$) and *D. marginatus* ($n = 20$) were only found among the old samples. The seasonality of collected ticks corresponded to their known activities.

After DNA extraction, ticks were screened for three groups of tick-borne pathogens. All samples were negative for brucellae, recently reported to be carried and transmitted transovarially by *D. marginatus*. Four *D. reticulatus* contained *Babesia canis* DNA, while in one *H. concinna* nymph the recently discovered zoonotic *B.* cf. *crassa* (reported in Slovenia within 80 km of our sampling site) was detected. In addition, *Anaplasma phagocytophilum* was identified in *D. reticulatus* ($n = 1$), *H. concinna* ($n = 3$) and in its known vector, *I. ricinus* ($n = 15$). Phylogenetically, three out of four *A. phagocytophilum* genotypes clustered with zoonotic ones.

In conclusion, despite of the high prevalence of *Brucella suis* in wild boars in Hungary, no evidence was found in support of the epidemiological role of ticks in transmitting brucellae. On the other hand, wild boars might introduce *B. canis*-carrier *D. reticulatus* into urban areas, unlike birds (which are not known to carry this tick species in the country). Most importantly, tick-infested wild boars can contribute to the spread of a novel zoonotic *Babesia* sp. and of the zoonotic variants of *A. phagocytophilum*.

1. Introduction

Wild boars (*Sus scrofa*) show rising population densities throughout Europe, including Hungary (Massei et al., 2015). While their presence is appreciated as game animals, they are also responsible for significant agricultural damage, habitat degradation and water quality issues (Helcel et al., 2016). Apart from these ecologic and economic problems, which increasingly affect Europe (Keuling et al., 2008), wild boars pose long-known health hazards by carrying, maintaining infectious diseases of veterinary-medical importance (Meng et al., 2009). Among these,

* Corresponding author.
E-mail address: hornok.sandor@univet.hu (S. Hornok).

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wild boars may harbor ticks and can act as reservoirs of tick-borne viruses, bacteria and parasites, thus contributing to the risk of transmission of these pathogens by blood-sucking arthropods to domestic animals and humans (Helcel et al., 2016).

This latter aspect of their veterinary-medical and epidemiological significance became especially important during the past years, when increasing numbers of wild boars have been reported to enter urban areas (gardens, city parks and even streets) in a worldwide context, particularly in Europe (Cahill et al., 2012; Stillfried et al., 2017; Ikeda et al., 2019). Background factors responsible for this aggravating situation include natural (environmental) effects, as well as human activity and disturbance. For instance, climate change may assist the spread of wild boars into northern regions by generating milder winters (Snow et al., 2017). On the other hand, wild boars tend to cause minor problems when undisturbed but are assumed to become nocturnal and wide-ranging under hunting pressure (Keuling et al., 2008). In Hungary, the emergence of invasive wild boars in urban habitats, including the capital city, shows an increasing tendency. Underlying causes include permanent availability of food sources and absence of hunting in such environments (Heltai et al., 2016). As a consequence, a significant ratio of wild boars showing up in human settlements live in stable urban populations (Bogdán and Heltai, 2014).

Despite all this, reports focusing on the tick infestation of wild boars are scarce in Europe, and most studies are based in the western and southern parts of the continent (e.g., de la Fuente et al., 2004; Selmi et al., 2009). Although data on tick-borne pathogens in wild boar ticks are also available from central Europe, some of these otherwise excellent surveys include relatively small sample size (e.g., Kazimírová et al., 2018) or analysis of only one tick species (*Ixodes ricinus*) from wild boars (Michalik et al., 2012; Silaghi et al., 2014). Thus, the aim of the present study was to extend the scope of a previously (in 2005-2008) initiated study on wild boar ticks in Hungary with more recently (in 2019-2020) collected samples, as well as to analyze these ticks for three important groups of pathogens with high veterinary-medical significance.

2. Materials and methods

2.1. Sample collection

Hard ticks (Acari: Ixodidae) were collected from wild boars in peri-urban areas (i.e., at the outskirts of 32 cities and villages) in Hungary in two periods. "Old samples" were provided by hunters between May, 2005 and April, 2008 (including March, April, May, July and October as sampling months). These ticks were removed from 19 wild boars at 19 locations in eleven counties of Hungary (i.e., Győr-Moson-Sopron, Veszprém, Fejér, Somogy, Baranya, Nógrád, Heves, Borsod-Abaúj-Zemplén, Pest, Bács-Kiskun, Jász-Nagykun-Szolnok). "New samples" were obtained from hunters between September, 2019 and June, 2020 (including September, February, April, May, June as sampling months). These ticks were removed from 32 wild boars at 13 locations in five counties of Hungary (i.e., Zala, Somogy, Tolna, Pest, Hajdú-Bihar). The locations of samplings are shown in Fig. 1. The ticks were stored in 70% ethanol, and their species were identified according to Estrada-Peña et al. (2017).

2.2. DNA extraction

Adults of *Dermacentor* and *Haemaphysalis* spp., as well as nymphs, larvae of *Haemaphysalis concinna* and *I. ricinus* ($n = 253$) were included in molecular analyses. DNA was extracted from whole ticks individually, with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer and 6.6% Proteinase-K at 56°C, as reported by Hornok et al. (2014). An extraction control (tissue lysis buffer without DNA) was also processed in each set of samples.

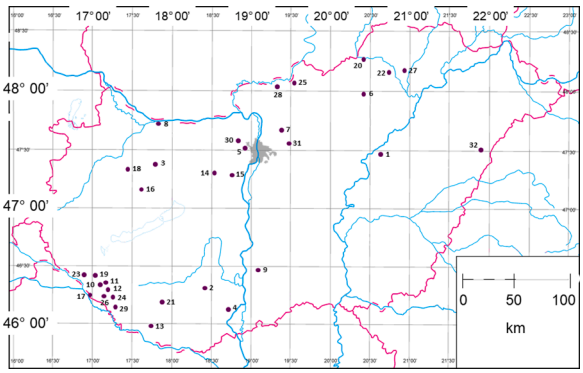

Fig. 1. Map of Hungary showing locations where ticks were collected from wild boars, according to this numbering: 1-Abádszalók, 2-Aparhant, 3-Bakonyszentlászló, 4-Bátaszék, 5-Budakeszi, 6-Felsőtárkány, 7-Galgamácsa, 8-Gönyű, 9-Homokmégy, 10-Iharosberény, 11-Inke, 12-Kaszó, 13-Kétújfalu, 14-Lovasberény, 15-Martonvásár, 16-Németbánya, 17-Őrtilos, 18-Pápa, 19-Pogányszentpéter, 20-Putnok, 21-Ropoly, 22-Sajóbábony, 23-Sand, 24-Somogyszob, 25-Szécsény, 26-Szenta, 27-Szikszó, 28-Szügy, 29-Tarany, 30-Telki, 31-Valkó, 32-Vámospércs.

2.3. Molecular analysis of ticks for brucellae

Brucella spp. were screened with a real-time PCR which amplifies a 151-bp-long part of the 31-kDa salt-extractable immunogenic protein encoding (*bcsp31*) gene by using the following oligonucleotides: forward primer: 5'-GCT CGG TTG CCA ATA TCA ATG C-3'; reverse primer: 5'-GGG TAA AGC GTC GCC AGA AG-3'; probe: 5'-AAA TCT TCC ACC TTG CCC TTG CCA TCA-3' with 6-FAM fluorophore and BHQ quencher (Probert et al., 2004). PCR was carried out in 12.5 µl total volume, containing 1.25 µl of target DNA solution, 0.5 U of AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA), 400 µM of PCR nucleotide mix, 0.4 µM of each primer, 0.2 µM of TaqMan probe, and 2.5 µl of GeneAmp 10× Gold Buffer (15 mM $MgCl_2$ included) (Thermo Fisher Scientific Inc.). The real-time PCRs were performed using Applied Biosystems Step-One Plus real-time PCR system with StepOne Software version 2.3 (Thermo Fisher Scientific Inc., Waltham, MA, USA). The PCR consisted of initial denaturation for 10 minutes at 95°C followed by 45 amplification cycles of 15 seconds at 95°C and 1 minute at 64°C. *Brucella suis* biovar 2 reference strain Thomsen (ATCC 23445) was used as positive control.

2.4. Molecular analyses of ticks for Anaplasma phagocytophilum

A TaqMan real-time PCR was used for the detection of *A. phagocytophilum*, amplifying part of the gene encoding a major surface protein (*msp*2). The probe was modified as 5′-6-FAM-TGG TGC CAG GGT TGA GCT TGA GAT TG-TAMRA-3′. The assay consisted of 40 cycles, and the results were regarded as positive if the threshold cycle (Ct) value was below 39. The detection limit of this PCR is 0.125 ratio (one-eights) of an *A. phagocytophilum* infected cell (Courtney et al., 2004). Sequence-verified *A. phagocytophilum* DNA from *I. ricinus* (code M33) served as positive control.

To investigate the genetic diversity of *A. phagocytophilum*, amplification of an approx. 600-bp-long fragment of the heat shock chaperonin (*GroEL*) gene was also attempted from all real-time PCR positive samples (Alberti et al., 2005). The primers EphplGroEL(569)F (5′-ATG GTA TGC AGT TTG ATC GC-3′) and EphGroEL(1142)R (5′-TTG AGT ACA GCA ACA CCA CCG GAA-3′) were used in a reaction volume of 25 µl, which included 5 µl of extracted DNA, and 20 µl of reaction mixture containing 1 U of HotStarTaq Plus DNA polymerase (Qiagen), 200 µM of PCR nucleotide mix, 1 µM of each primer and 2.5 µl of 10× Coral Load PCR buffer (15 mM $MgCl_2$ included) (Qiagen). For amplification, an initial denaturation step at 95°C for 5 min was followed by 40 cycles of

denaturation at 95°C for 30 s, annealing at 56°C for 40 s and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 7 min. Sequence-verified *A. phagocytophilum* DNA from a dog (code VE39) was used as positive control.

2.5. Molecular analysis of ticks for piroplasms

Samples were screened for the presence of piroplasms by a conventional PCR modified from Casati et al. (2006). The primers BJ1 (forward: 5′-GTC TTG TAA TTG GAA TGA TGG-3′) and BN2 (reverse: 5′-TAG TTT ATG GTT AGG ACT ACG-3′) were used to amplify an approximately 500-bp-portion of the 18S rRNA gene of *Babesia/Theileria* spp. The reaction volume was 25 µl, i.e., 5 µl of extracted DNA was added to 20 µl reaction mixture containing 1 U of HotStarTaq DNA Plus polymerase (Qiagen), 200 µM of PCR nucleotide mix, 1 µM of each primer and 2.5 µl of 10× CoralLoad PCR buffer (15 mM $MgCl_2$ included) (Qiagen). Cycling conditions included an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 40 s. The final extension was performed at 72 °C for 5 min. Sequence-verified *Babesia canis* DNA from a dog (code EB/SzL.Titok) served as positive control.

2.6. Sequencing and phylogenetic analyses

Purification and sequencing of the PCR products were done by Biomi Ltd. (Gödöllő, Hungary). Obtained sequences were manually edited, then aligned with GenBank sequences by nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). Representative sequences were submitted to GenBank (*A. phagocytophilum GroEL*: MW366833-MW366836; *Babesia* 18S rRNA gene: MW362500-MW362502). Sequences from other studies (retrieved from GenBank) were included in the phylogenetic analyses only if they had nearly 100% coverage with sequences from this study. This dataset was resampled 1,000 times to generate bootstrap values. Unrooted trees were used, because these are beneficial in showing clusters of closely related sequences (Kinene et al., 2016). Phylogenetic analyses were conducted with the Maximum Likelihood method and model selection by the program using MEGA version 7.0.

2.7. Statistical analysis

Prevalence data were analyzed by Fisher's exact test (number of PCR positive ticks belonging to one species *vs* number of PCR positive ticks belonging to another species).

2.8. Ethical permission

No ethical permission was needed, because all samples were provided by licensed hunters who took into account the national regulations of game animal hunting (laws 1996.LV. and 79/2004.V.4. with all their appendices).

3. Results

3.1. Tick infestation of wild boars

Altogether 333 ixodid ticks were collected from 51 wild boars at 32 peri-urban locations in 14 counties of Hungary (Fig. 1). Five species of ticks were identified: *Dermacentor reticulatus* (n = 165, 49.5% of all ticks), *I. ricinus* (n = 90, 27% of all ticks) and *H. concinna* (n = 29, 8.7% of all ticks) in both (old and new) sample groups, while *H. inermis* (n = 29, 8.7% of all ticks) and *D. marginatus* (n = 20, 6% of all ticks) only among the old samples. *Dermacentor* species and *H. inermis* were represented exclusively by adults, whereas *H. concinna* and *I. ricinus* also by larvae and nymphs (Table 1). Regarding the number of infested hosts according to tick species, from the majority of wild boars *D. reticulatus* was collected (n = 29, 56.9% of all boars), followed in decreasing order by *I. ricinus* (n = 23, 45.1% of all boars), *H. concinna* (n = 9, 17.6% of all boars), *D. marginatus* (n = 7, 13.7% of all boars) and *H. inermis* (n = 2, 3.9% of all boars) (Table 1).

Considering the seasonality of ticks from wild boars, *Dermacentor* species infested wild boars both in the autumn (September, October) and in the spring (March to May) (Table 1). However, *D. reticulatus* was also collected three times in the winter (a single male on two occasions and another female) (in February, 2020 at sites No. 24 and 29: Fig. 1), and in one case during the summer (one male and two females in July, 2007 at site No. 15: Fig. 1). *Haemaphysalis inermis* was present on wild boars in the spring (April-May), while *H. concinna* around early summer (May to July). *Ixodes ricinus* was found mostly around late spring (March to June), but also in October (Table 1).

3.2. Molecular investigation of brucellae

None of the molecularly investigated 253 ticks were positive for brucellae.

3.3. Molecular investigation of Anaplasma phagocytophilum

Anaplasma phagocytophilum DNA was identified in a *D. reticulatus* female, in three *H. concinna* nymphs (collected from two wild boars), and in 15 *I. ricinus* (one larva and 14 nymphs, from five wild boars) (Table 1). In one case, all five nymphs collected from the same host were PCR positive for *A. phagocytophilum*. PCR positivity was significantly more frequent among *H. concinna* (10.3%: 3 of 29 ticks) than among *D. reticulatus* (0.6%: 1 of 165 ticks) (P = 0.01). In comparison with the latter, the prevalence was significantly higher in *I. ricinus* (16.7%: 15 of 90 ticks) ($P < 0.0001$).

Among the 19 real-time PCR positive samples, 16 samples with the lowest Ct values yielded sequencable products in the *GroEL* PCR. These belonged to four *GroEL* genotypes. One genotype (MW366833) was present in *H. concinna* (in 1 of 29 ticks: prevalence 3.4%), and two others (MW366834, MW366835) in two *I. ricinus*, respectively (in 1 of 90 ticks:

Table 1
Collection data (months and number of hosts, counties and locations) and detected pathogens according to species and sex/stage of ticks removed from wild boars. Superscript numbers 1 or 2 indicate old or new samples, collected in 2005-2008 or 2019-2020, respectively.

Species of ticks	Number of ticks					Number of involved			Months	Pathogens (number of ticks)
	Larva	Nymph	Male	Female	All	boars	counties	locations		
Dermacentor marginatus	-	-	8^1	12^1	**20**	7	7	7	April[1], May[1], October[1]	-
Dermacentor reticulatus	-	-	42^1+53^2	26^1+44^2	**165**	29	9	22	February[2], March[1], April[1, 2], May[1, 2], July[1], September[2]	*Babesia canis* (4) *A. phagocytophilum* (1)
Haemaphysalis inermis	-	-	6^1	23^1	**29**	2	1	2	April[1], May[1]	-
Haemaphysalis concinna	2^2	10^2	4^1+9^2	2^1+2^2	**29**	9	5	8	May[1, 2], June[2], July[1]	*Babesia* cf. *crassa* (1) *A. phagocytophilum* (3)
Ixodes ricinus	3^2	2^1+50^2	10^1+2^2	7^1+16^2	**90**	23	5	11	March[1], April[1], May[1, 2], June[2], October[2]	*A. phagocytophilum* (15)

Abbreviation: *A.* - *Anaplasma*

prevalence 1.1% for both). The fourth (MW366836) was detected in *H. concinna* (in 1 of 29 ticks: prevalence 3.4%) and in *I. ricinus* (in 12 of 90 ticks: prevalence 13.3%). Phylogenetically, three of these (from both *I. ricinus* and *H. concinna*) clustered with zoonotic *A. phagocytophilum* genotypes (Supplementary Fig. 1), including the five PCR-positive nymphs from the same wild boar (accession number: MW366836). PCR positivity for *A. phagocytophilum* was not detected in *D. marginatus* and in *H. inermis* (Table 1).

3.4. Molecular investigation of piroplasms

Four *D. reticulatus* contained *B. canis* DNA (2.4%: 4 of 165 ticks). In addition, one *H. concinna* nymph (3.4%: 1 of 29 ticks; collected in May, 2020 at site No. 19: Fig. 1) contained piroplasm DNA with 100% (431/431 bp) sequence identity to the zoonotic *B.* cf. *crassa* (GenBank: MK240324), recently reported from Murska Sobota in Slovenia close to the Hungarian border, i.e. within 80 km from our sampling site. Phylogenetically, this *Babesia* sp. clustered in the group of Far-Eastern genotypes, most of which are associated with *H. concinna* (Supplementary Fig. 2). Piroplasm DNA was not detected in *I. ricinus, D. marginatus* and *H. inermis* extracts (Table 1).

4. Discussion

The most common tick species collected from wild boars in the present study (i.e., 49.5%: 165 of 333 ticks) was *D. reticulatus,* similarly to what was reported from Croatia, the country neighboring Hungary to the south (Krčmar, 2019). Two further tick species, *D. marginatus* and *H. inermis* infested wild boars in both Hungary (as shown here) and in Croatia (Krčmar, 2019). The significance of these findings lies in the fact that neither *D. reticulatus*, nor *D. marginatus* or *H. inermis* are carried by birds in the study region (Hornok et al., 2014), but according to the present results wild boars may play a significant role in their transportation in peri-urban areas.

Considering that *D. marginatus* and *H. inermis* were found in the same county (Pest) and during the same sampling months (April-May) among the "older samples", where and when these two species could not be collected from wild boars despite of repeated samplings (nine occasions) in 2020, our data might reflect changes in the activity period of ticks infesting wild boars depending on climatic conditions. In particular, the winter of 2019/2020 preceding the collection of "new samples" in this study, was by far the warmest ever recorded in Europe (ECMWF: European Centre for Medium-Range Weather Forecasts, 2020), and Hungary experienced sharply rising winter temperatures at the end of January (data from the Hungarian Meteorological Society). Under such conditions species of the spring tick season (such as *D. marginatus* and *H. inermis*) were shown to have their peak activity 1-2 month earlier, in February-March (Hornok, 2009). On the other hand, the seasonality of tick species in the old sample group corresponded to their known activities, except the occurrence of adult *D. reticulatus* during mid-summer on one occasion (the latest presence of questing adults was reported in April: Hornok, 2009).

To our knowledge, this is the first report of screening brucellae in ixodid ticks in Europe, and it yielded negative results. Formerly it was demonstrated by both molecular and culture methods that *D. marginatus* can transmit *Brucella melitensis* and *Br. abortus* transovarially to larvae with high efficacy (entailing 40.9% prevalence: Wang et al., 2018). These brucellae are associated with ruminants and can cause infection in humans (Christopher et al., 2010). A third zoonotic pathogen, *Br. suis* (biovar 2) is known to be highly prevalent in wild boars across Europe including Hungary (Kreizinger et al., 2014). Thus, PCR negativity of ticks analyzed in the present study suggest that tick-borne transmission of brucellae from wild boars towards humans is unlikely in Hungary.

In Hungary, wild boars are known to be infected with *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis and tick-borne fever of ruminants, with a prevalence rate of 39.2% (Hornok et al., 2018). Based on literature data, wild boars are hosts of human pathogenic *A. phagocytophilum* variant (Michalik et al., 2012; Hrazdilová et al., 2021). Most (79%: 15 of 19) *A. phagocytophilum* PCR positive specimens of wild boar ticks in this study belonged to *I. ricinus*, the main vector of this bacterium species in Europe (Stuen et al., 2013). In one case, all five *I. ricinus* nymphs collected from the same wild boar were PCR positive for a zoonotic genotype of *A. phagocytophilum*. It is very unlikely that all these PCR positive ticks attached to the same host individual after having become infected earlier (in the larval stage from known hosts of the zoonotic ecotype, which are medium to large size mammals: Jahfari et al., 2014), suggesting that these ticks had access to *A. phagocytophilum* from their wild boar host, with high efficacy. This confirms previous results (Silaghi et al., 2014) prior to which it was not known whether ticks can become infected with *A. phagocytophilum* from wild boars or not (de la Fuente et al., 2012), because pigs are able to control the infection and have short duration of bacteraemia (Galindo et al., 2012).

In this study, *A. phagocytophilum* was also identified in a female *D. reticulatus*. While there are literature data, which suggest that this tick species could act as a secondary vector (Ben and Lozynskyi, 2019), the significantly lower (0.6%) prevalence in *D. reticulatus* than in *I. ricinus* (16.7%) argues against a substantial epidemiological role of this tick species in *A. phagocytophilum* infection. In addition, *A. phagocytophilum* was present in three *H. concinna* nymphs (10.3%), in line with previous reports on the carrier state and high prevalence of *A. phagocytophilum* among questing individuals of this tick species (Rybářová and Široký, 2017).

Concerning piroplasms, *B. canis* DNA was demonstrated here in *D. reticulatus*. Since wild boars are not known to be susceptible to this piroplasm, PCR positive ticks collected from wild boars in this study almost certainly became infected in one of their previous generations from canids. In addition, a zoonotic species, *B.* cf. *crassa* was identified in *H. concinna* from a wild boar. To the best of our knowledge, this is the first finding of this piroplasm in any tick species in Europe. While the association of this babesia and closely related genotypes (Rar et al., 2014) with *H. concinna* has been proposed based on phylogenetic clustering (Strasek-Smrdel et al., 2020), the presence of its DNA in this tick species from a wild boar is an important supportive evidence. The close geographic position of our sampling site and the city where this piroplasm was recently reported from a human patient in Slovenia (Strasek-Smrdel et al., 2020) near the southwestern border of Hungary suggest that this may be an endemic focus for the zoonotic *B.* cf. *crassa*.

In conclusion, wild boars may import at least five tick species from peri-urban areas into cities/villages in Hungary. As shown here, wild boars support the adults of all these tick species as "reproduction hosts" (Li et al., 2012). Since they are known to reside permanently even in densely populated areas (including the capital city) in the evaluated region, particularly in forested habitats and those with dense vegetation (Csókás et al., 2020), they may contribute to the maintenance of diverse urban tick populations. This is particularly important in the context of such urban areas, where other wild living large mammals are seldom available. In addition, wild boars may transport ticks infected with tick-borne pathogens with high veterinary and/or medical importance, as exemplified by *A. phagocytophilum, B. canis* and *B.* cf. *crassa*.

CRediT authorship contribution statement

Sándor Hornok: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Sándor Szekeres:** Methodology, Software. **Gábor Horváth:** Data curation, Investigation. **Nóra Takács:** Methodology, Visualization, Investigation. **Katinka Bekő:** . **Jenő Kontschán:** Methodology, Software. **Miklós Gyuranecz:** Methodology, Visualization, Investigation. **Barnabás Tóth:** Data curation, Investigation. **Attila D. Sándor:** Data curation, Investigation. **Alexandra Juhász:** Data curation, Investigation. **Relja Beck:** Data curation, Investigation. **Róbert Farkas:** Conceptualization, Supervision, Writing – review &

editing.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2022.101936.

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