

Table S1 Prevalence of *Babesia* spp. in questing *Haemaphysalis concinna* per site in 2011–2013

		2011		2012		2013		Fisher's exact test	Total	
Site		% (pos/ex)	95% CI	% (pos/ex)	95% CI	% (pos/ex)	95% CI		% (pos/ex)	95% CI
Bratislava	Nymphs	33.3 (3/9)	11.1–66.7	0.0 (0/3)	–	4.3 (1/23)	0.0–13.0	0.073	11.4 (4/35)	2.9–22.9
	Females	0.0 (0/4)	–	0.0 (0/1)	–	25.0 (1/4)	0.0–75.0	1.000	11.1 (1/9)	0.0–33.3
	Males	0.0 (0/7)	–	–	–	0.0 (0/5)	–	–	0.0 (0/12)	–
	Adults total	0.0 (0/11)	–	0.0 (0/1)	–	11.1 (1/9)	0.0–33.3	0.476	4.8 (1/21)	0.0–14.3
	Total	15.0 (3/20)	0.0–34.9	0.0 (0/4)	–	6.3 (2/32)	0.0–15.6	0.565	8.9 (5/56)	1.8–17.9
Fúgelka	Nymphs	0.0 (0/16)	–	0.0 (0/4)	–	0.0 (0/4)	–	–	0.0 (0/24)	–
	Females	0.0 (0/3)	–	0.0 (0/1)	–	0.0 (0/2)	–	–	0.0 (0/6)	–
	Males	33.3 (1/3)	0.0–100.0	0.0 (0/1)	–	0.0 (0/1)	–	1.000	20.0 (1/5)	0.0–60.0
	Adults total	16.7 (1/6)	0.0–50.0	0.0 (0/2)	–	0.0 (0/3)	–	1.000	9.1 (1/11)	0.0–27.3
	Total	4.5 (1/22)	0.0–13.6	0.0 (0/6)	–	0.0 (0/7)	–	1.000	2.9 (1/35)	0.0–8.6
Total		9.5 (4/42)	2.4–19.0	0.0 (0/10)	–	5.1 (2/39)	0.0–12.8	0.715	6.6 (6/91)	2.2–12.1

(pos/ex), number of positive/number of examined; 95% CI, confidence interval

Table S2 Occurrence of *Babesia* spp. in questing *Ixodes ricinus* in Bratislava and Fúgelka

	Bratislava % (pos Ba/pos total)	Fúgelka % (pos Fu/pos total)
<i>Babesia microti</i>	27.3 (12/44)	72.7 (32/44)
<i>Babesia venatorum</i>	53.8 (14/26)	46.2 (12/26)
other <i>Babesia</i> spp.	87.5 (7/8)	12.5 (1/8)

pos, positive; Ba, Bratislava; Fu, Fúgelka

Table S3 Occurrence of *Babesia* spp. in questing *Ixodes ricinus* in 2011–2013

	2011 % (pos 2011/pos total)	2012 % (pos 2012/pos total)	2013 % (pos 2013/pos total)
<i>Babesia microti</i>	50.0 (22/44)	38.6 (17/44)	11.4 (5/44)
<i>Babesia venatorum</i>	57.7 (15/26)	7.7 (2/26)	34.6 (9/26)
other <i>Babesia</i> spp.	62.5 (5/8)	0.0 (0/8)	37.5 (3/8)

pos, positive

Table S4 Occurrence of *Babesia* spp. in questing *Ixodes ricinus* males, females, and nymphs

	Males % (pos M/pos total)	Females % (pos F/pos total)	Nymphs % (pos N/pos total)
<i>Babesia microti</i>	4.5 (2/44)	9.1 (4/44)	86.4 (38/44)
<i>Babesia venatorum</i>	30.8 (8/26)	7.7 (2/26)	61.5 (16/26)
other <i>Babesia</i> spp.	37.5 (3/8)	0.0 (0/8)	62.5 (5/8)

pos, positive; M, males; F, females; N, nymphs

Table S5 Variables remaining in the best selected model for *Babesia microti* prevalence in rodents

	B	S.E.	Wald	df	P	Exp(B)
Genus			37.250	2	< 0.001	
Genus (1)	−4.142	0.742	31.163	1	< 0.001	0.016
Genus (2)	−5.755	1.194	23.217	1	< 0.001	0.003
Gender (1)	2.345	0.813	8.312	1	0.004	10.431
Constant	−1.438	0.656	4.801	1	0.028	0.237

Categorical variables codings: Genus (1), Mice, Genus (2), *Myodes*; Gender (1), Males; variable removed by backward method was site; B, parameter estimate; S.E., standard error; Wald, Wald statistic = test of significance of the regression coefficient; P, significance level; Exp(B), odds ratio for parameter B

Table S6 Occurrence of *Babesia*, *Candidatus N. mikurensis* and *Anaplasma phagocytophilum* in questing *Ixodes ricinus*

	Bratislava % (pos Ba/pos total)	Fúgelka % (pos Fu/pos total)
<i>Candidatus N. mikurensis</i>	34.5 (20/58)	65.5 (38/58)
<i>Anaplasma phagocytophilum</i>	74.7 (145/194)	25.3 (49/194)
<i>Babesia</i> spp.	37.7 (23/61)	62.3 (38/61)

pos, positive; Ba, Bratislava; Fu, Fúgelka

Table S7 Accession numbers of Apicomplexa 18S rRNA gene sequences

Species	Source	Name of the isolate (number of analysed samples with identical sequences)	BP	Accession numbers
<i>Babesia microti</i>	questing <i>I. ricinus</i> nymph from Bratislava	N233B (11)	474	KU362887
	questing <i>I. ricinus</i> nymph from Fúgelka	N178F (27)	474	KU550676
	questing <i>I. ricinus</i> female from Fúgelka	F43F (4)	474	KU362888
	questing <i>I. ricinus</i> male from Bratislava	M51B (1)	474	KU550677
	questing <i>I. ricinus</i> male from Fúgelka	M8F (1)	474	KU550678
	rodent-attached <i>I. ricinus</i> larva from Bratislava	L275HB (5)	474	KU362889
	rodent-attached <i>I. ricinus</i> larva from Fúgelka	L27HF (6)	474	KU550679
	rodent-attached <i>I. ricinus</i> nymph from Bratislava	N263HB (8)	474	KU362890
	rodent-attached <i>I. ricinus</i> nymph from Fúgelka	N1HF (1)	474	KU550680
	rodent-attached <i>I. ricinus</i> female from Fúgelka	F2HF (1)	474	KU362891
	rodent-attached <i>H. concinna</i> larva from Fúgelka	L126HF (2)	474	KU362892
	rodent-attached <i>H. concinna</i> female from Bratislava	F262HB (1)	474	KU362893
	rodent-attached <i>H. concinna</i> female from Fúgelka	F1HF (1)	474	KU550681
	<i>Apodemus flavicollis</i> from Bratislava	AF122B (4 spleens, 3 blood, 3 lungs, 2 skin biopsies from ear)	474	KU362894
	<i>Apodemus flavicollis</i> from Fúgelka	AF63F (3 spleens, 3 blood, 2 lungs, 1 skin biopsy from ear)	474	KU550682
<i>Babesia venatorum</i>	<i>Microtus arvalis</i> from Fúgelka	MA20F (6 spleens, 6 blood, 5 lungs, 6 skin biopsies from ear)	474	KU362895
	<i>Myodes glareolus</i> from Fúgelka	MG15F from blood (1)	474	KU362896
	questing <i>I. ricinus</i> nymph from Bratislava	N176B (7)	447	KU362897
	questing <i>I. ricinus</i> nymph from Fúgelka	N497F (9)	447	KU550683
	questing <i>I. ricinus</i> female from Bratislava	F56B (1)	447	KU550684
	questing <i>I. ricinus</i> female from Fúgelka	F1F (1)	447	KU362898
	questing <i>I. ricinus</i> male from Bratislava	M54B (6)	447	KU550685
	questing <i>I. ricinus</i> male from Fúgelka	M89F (2)	447	KU550686
<i>Babesia sp.</i>	rodent-attached <i>I. ricinus</i> larva from Bratislava	L8HB (1)	447	KU362899
	rodent-attached <i>I. ricinus</i> nymph from Bratislava	N253HB (1)	447	KU362900
	questing <i>I. ricinus</i> nymph from Fúgelka	N41F (1)	447	KU362901
<i>Babesia canis</i>	questing <i>I. ricinus</i> male from Bratislava	M340B (1)	447	KU362902
	rodent-attached <i>I. ricinus</i> larva from Bratislava	L302HB (1)	447	KU362903
<i>Babesia odocoilei</i>	questing <i>I. ricinus</i> nymph from Bratislava	N30B (3)	447	KU362904
	questing <i>I. ricinus</i> male from Bratislava	M398B (2)	447	KU362905
<i>Babesia odocoilei</i>	questing <i>I. ricinus</i> nymph from Bratislava	N149B (1)	446	KU550687

<i>Babesia</i> sp. 2 (Eurasia)	questing <i>H. concinna</i> male from Fúgelka	M58F (1)	436	KU550688
	rodent-attached <i>H. concinna</i> larva from Bratislava	L189HB (1)	436	KU550689
<i>Babesia</i> sp. 1 (Eurasia)	questing <i>H. concinna</i> nymph from Bratislava	N141B (4)	436	KU550690
	questing <i>H. concinna</i> female from Bratislava	F117B (1)	436	KU550691
	rodent-attached <i>H. concinna</i> larva from Bratislava	L433HB (1)	436	KU550692
	rodent-attached <i>H. concinna</i> larva from Fúgelka	L244HF (1)	436	KU550693
	rodent-attached <i>H. concinna</i> larva from Bratislava	L355HB (2)	436	KU550694
<i>Theileria</i> sp.	questing <i>H. concinna</i> nymph from Bratislava	N419B (2)	467	KU550695
	rodent-attached <i>H. concinna</i> female from Fúgelka	F4HF (1)	467	KU550696
<i>Sarcocystis</i> sp.	<i>Microtus arvalis</i> from Fúgelka	MA128F skin biopsy from ear (4)	509	KU550697
	<i>Myodes glareolus</i> from Fúgelka	MG156 skin biopsy from ear (1)	501	KU550698
	<i>Myodes glareolus</i> from Fúgelka	MG223 skin biopsy from ear (1)	495	KU550699