

Table S1

PCR primers and thermocycling conditions used in this study.

Target region	Primers	PCR conditions	Amplicon size (base pairs)	Reference
16S for ticks	Forward: 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') Reverse: 16S-1 (5'-CCGGTCTGAACTCAGATCAAGT-3')	Initial denaturation: 95 °C, 4min 35 cycles of Denaturation: 95 °C, 1min Annealing: 50 °C, 1min Extension: 68 °C, 1min Final extension: 68 °C, 10min	460	[25]
cox1 for ticks	Forward: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') Reverse: HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')	Initial denaturation: 95 °C, 5min 40 cycles of Denaturation: 95 °C, 45sec Annealing 50 °C, 45sec Extension: 72 °C, 1min Final extension: 72 °C, 5min	710	[40]
ITS-2 for ticks	Forward: RIB-8 (5'-GTCGTAGTCCGCCGTC-3') Reverse: RIB-11 (5'-GAGTACGACGCCCTACC-3')	Initial denaturation: 94 °C, 5min 35 cycles of Denaturation: 94 °C, 30sec Annealing: 62 °C, 15sec Extension: 72 °C, 15sec Final extension: 72 °C, 5min	280	[41]
18S for piroplasms	Forward: (5'-GAGGTAGTGACAAGAAATAACAATA-3') Reverse: (5'-TCTTCGATCCCCTAACTTTC-3')	Initial denaturation: 95 °C, 5min 35 cycles of Denaturation: 95 °C, 30sec Annealing: 57 °C, 20 sec Extension: 72 °C, 1 min Final extension: 72 °C, 5min	500	[42]

Table S2

List of primers and probes used in this study for identification of microorganisms and ticks.

Species	Target	Name	Sequence	Length (base pair)
<i>Anaplasma marginale</i>	<i>msp1</i>	An_ma_msp1_F	CAGGCTTCAAGCGTACAGTG	85
		An_ma_msp1_R	GATATCTGTGCCTGGCCTTC	
		An_ma_msp1_P	ATGAAAGCCTGGAGATGTTAGACCGAG	
<i>Anaplasma platys</i>	<i>groEL</i>	An_pla_groEL_F	TTCTGCCGATCCTTGAAAACG	75
		An_pla_groEL_R	CTTCTCCTTCTACATCCTCAG	
		An_pla_groEL_P	TTGCTAGATCCGGCAGGCCTCTGC	
<i>Anaplasma ovis</i>	<i>msp4</i>	An_ov_msp4_F	TCATTGACATGCGTGAGTCA	92
		An_ov_msp4_R	TTTGCTGGCGCACTCACATC	
		An_ov_msp4_P	AGCAGAGAGACCTCGTATGTTAGAGGC	
<i>Anaplasma centrale</i>	<i>groEL</i>	An_cen_groEL_F	AGCTGCCCTGCTATACACG	79
		An_cen_groEL_R	GATGTTGATGCCCAATTGCTC	
		An_cen_groEL_P	CTTGCATCTCTAGACGAGGTAAAGGGG	
<i>Anaplasma phagocytophilum</i>	<i>msp2</i>	An_ph_msp2_F	GCTATGGAAGGCAGTGTGG	77
		An_ph_msp2_R	GTCTTGAAGCGCTCGTAACC	
		An_ph_msp2_P	AATCTCAAGCTCAACCCTGGCACCAC	
<i>Anaplasma bovis</i>	<i>groEL</i>	An_bo_groEL_F	CTGATAGCTACAGTTAAGCCC	73
		An_bo_groEL_R	AGGTGCTGTTGGATGTACTGCTGGACC	
		An_bo_groEL_P	TCATTGACATGCGTGAGTCA	
<i>Anaplasma spp.</i>	16S rRNA	Ana_spp_16S_F	CTTAGGGTTGTAAAACCTTTTCAG	160
		Ana_spp_16S_R	CTTTAACTTACCAAACCGCCTAC	
		Ana_spp_16S_P	ATGCCCTTTACGCCCAATAATTCCGAACA	
Apicomplexa	18S rRNA	Apic_18S_F	TGAACGAGGAATGCCTAGTATG	104
		Apic_18S_R	CACCGGATCACTCGATCGG	
		Apic_18S_S	TAGGAGCGACGGGCGGTGTGTAC	
<i>Babesia divergens</i>	<i>hsp70</i>	Bab_di_hsp70_F	CTCATTTGGTGACGCCGCTA	83
		Bab_di_hsp70_R	CTCCTCCCGATAAGCCTCTT	
		Bab_di_hsp70_P	AGAACCAGGAGGCCCGTAACCCAGA	

<i>Babesia caballi</i>	<i>Rap1</i>	Ba_cab_rap1_F Ba_cab_rap1_R Ba_cab_rap1_P	GTTGTTCGGCTGGGGCATC CAGGCGACTGACGCTGTGT TCTGTCCCGATGTCAAGGGGCAGGT	94
<i>Babesia canis</i>	18S rRNA	Ba_ca_RNA18S_F Ba_ca_RNA18S_R Ba_ca_RNA18S_P	TGGCCGTTCTTAGTTGGTGG AGAAGCAACCGAAACTCAAATA ACCGGCACTAGTTAGCAGGTTAAGGTC	104
<i>Babesia microti</i>	<i>CCTeta</i>	Bab_mi_CCTeta_F Bab_mi_CCTeta_R Bab_mi_CCTeta_P	ACAATGGATTTTCCCAGCAAAA GCGACATTTCCGGCAACTTATATA TACTCTGGTGCAATGAGCGTATGGGTA	145
<i>Babesia bovis</i>	<i>CCTeta</i>	Ba_bo_CCTeta_F Ba_bo_CCTeta_R Ba_bo_CCTeta_P	GCCAAGTAGTGGTAGACTGTA GCTCCGTCATTGGTTATGGTA TAAAGACAACACTGGGTCCGCGTGG	100
<i>Babesia bigemina</i>	18S rRNA	Ba_big_RNA18S_F Ba_big_RNA18S_R Ba_big_RNA18S_P	ATTCCGTTAACGAACGAGACC TTCCCCACGCTTGAAGCA CAGGAGTCCCTCTAAGAAGCAAACGAG	99
<i>Babesia ovis</i>	18S rRNA	Ba_ov_RNA18S_F Ba_ov_RNA18S_R Ba_ov_RNA18S_P	TCTGTGATGCCCTTAGATGTC GCTGGTTACCCGCGCCTT TCGGAGCGGGGTCAACTCGATGCAT	92
<i>Babesia vulpes</i>	COX1	Bab_vulpes_F Bab_vulpes_R Bab_vulpes_P	ACT TCC AGT GTT AAC AGC AGC GGA TGT CCG AAG ACC CAG AA CTT CTG CAT GTT GCT GGC TGA TAG GCA	129
<i>Bartonella henselae</i>	<i>pap31</i>	Bar_he_pap31_F Bar_he_pap31_R Bar_he_pap31_P	CCGCTGATCGCATTATGCCT AGCGATTCTGCATCATCTGCT ATGTTGCTGGTGGTGTTCCTATGCAC	107
<i>Bartonella spp.</i>	<i>ssrA</i>	Bart_spp_ssrA_F Bart_spp_ssrA_R Bart_spp_ssrA_P	CGTTATCGGGCTAAATGAGTAG ACCCCGCTTAAACCTGCGA TTGCAAATGACAACTATGCGGAAGCACGTC	118
<i>Borrelia burgdorferi sensu stricto</i>	<i>rpoB</i>	Bo_bu_rpoB_F Bo_bu_rpoB_R Bo_bu_rpoB_P	GCTTACTCACAAAAGGCGTCTT GCACATCTTACTTCAAATCCT AATGCTCTTGGACCAGGAGGACTTTCA	83
<i>Borrelia garinii</i>	<i>rpoB</i>	Bo_ga_rpoB_F Bo_ga_rpoB_R Bo_ga_rpoB_P	TGGCCGAACCTTACCCACAAAA ACATCTCTTACTTCAAATCCTGC TCTATCTCTTGAAAGTCCCCCTGGTCC	88

<i>Borrelia afzelii</i>	<i>fla</i>	Bo_af_flA_F	GGAGCAAATCAAGATGAAGCAAT	116
		Bo_af_flA_R	TGAGCACCCCTCTTGAACAGG	
		Bo_af_flA_P	TGCAGCCTGAGCAGCTTGAAGCTCC	
<i>Borrelia valaisiana</i>	<i>ospA</i>	Bo_va_ospA_F	ACTCACAAATGACAGATGCTGAA	135
		Bo_va_ospA_R	GCTTGCTTAAAGTAACAGTACCT	
		Bo_va_ospA_P	TCCGCCTACAAGATTTCTGGAAGCTT	
<i>Borrelia miyamotoi</i>	<i>glpQ</i>	B_miya_glpQ_F	CACGACCCAGAAATTGACACA	94
		B_miya_glpQ_R	GTGTGAAGTCAGTGGCGTAAT	
		B_miya_glpQ_P	TCGTCCGTTTTCTCTAGCTCGATTGGG	
<i>Borrelia spielmanii</i>	<i>fla</i>	Bo_spi_flA_F	ATCTATTTTCTGGTGAGGGAGC	71
		Bo_spi_flA_R	TCCTTCTGTGAGCACCTTC	
		Bo_spi_flA_P	TTGAACAGGCGCAGTCTGAGCAGCTT	
<i>Borrelia lusitaniae</i>	<i>rpoB</i>	Bo_lus_rpoB_F	CGAACTTACTCATAAAAGGCGTC	87
		Bo_lus_rpoB_R	TGGACGTCTCTTACTTCAAATCC	
		Bo_lus_rpoB_P	TTAATGCTCTCGGGCCTGGGGGACT	
<i>Borrelia bissettii</i>	<i>rpoB</i>	Bo_bi_rpoB_F	GCAACCAGTCAGCTTTACAG	118
		Bo_bi_rpoB_R	CAAATCCTGCCCTATCCCTTG	
		Bo_bi_rpoB_P	AAAGTCCTCCCGGCCCAAGAGCATTAA	
<i>Borrelia bavariensis</i>	<i>pyrG</i>	Bo_bavar_pyrG_F	GTC TTT GGT TCA TGT TGG AGC	141
		Bo_bavar_pyrG_R	CCC TCA TAT CCT TTG CCT CC	
		Bo_bavar_pyrG_P	AAA GCC TCC GGG AAC AAT AAT GCC GTC AA	
<i>Borrelia mayonii</i>	<i>fla</i>	Bo_mayo_flA_F	ACA CAC CAT CAT CAC TTT CAG G	173
		Bo_mayo_flA_R	TTG AGC ACC TTC TTG AAC AGG	
		Bo_mayo_flA_P	CGC AAC CTG AGC AGT TTG AGT TCC CT	
<i>Borrelia</i> spp.	23S rRNA	Bo_bu_sl_23S_F	GAGTCTTAAAAGGGCGATTTAGT	73
		Bo_bu_sl_23S_R	CTTCAGCCTGGCCATAAATAG	
		Bo_bu_sl_23S_P	AGATGTGGTAGACCCGAAGCCGAGT	
<i>Candidatus</i> <i>Neoehrlichia mikurensis</i>	<i>groEL</i>	Neo_mik_groEL_F	AGAGACATCATTTCGATTTTGGGA	96
		Neo_mik_groEL_R	TTCCGGTGTACCATAAAGGCTT	
		Neo_mik_groEL_P	AGATGCTGTTGGATGTACTGCTGGACC	
<i>Coxiella burnetii</i>	<i>idc</i>	Co_bu_idc_F	AGGCCCGTCCGTTATTTTACG	74
		Co_bu_idc_R	CGGAAAATCACCATATTCACCTT	
		Co_bu_idc_P	TTCAGGCGTTTTGACCGGGCTTGGC	

	IS1111	Co_bu_IS111_F	TGGAGGAGCGAACCATTGGT	86
		Co_bu_IS111_R	CATACGGTTTGACGTGCTGC	
		Co_bu_IS111_P	ATCGGACGTTTATGGGGATGGGTATCC	
<i>Ehrlichia canis</i>	<i>dsb</i>	Eh_ca_dsb_F	AATACTTGGTGAGTCTTCACTCA	110
		Eh_ca_dsb_R	GTTGCTTGTAATGTAGTGCTGC	
		Eh_ca_dsb_P	AAGTTGCCCAAGCAGCACTAGCTGTAC	
<i>Ehrlichia</i> spp.	16S rRNA	Neo_mik_16S_R	GCAACGCGAAAAACCTTACCA	98
		Neo_mik_16S_R	AGCCATGCAGCACCTGTGT	
		Neo_mik_16S_P	AAGGTCCAGCCAAACTGACTCTTCCG	
<i>Francisella tularensis</i>	<i>tul4</i>	Fr_tu_tul4_F	ACCCACAAGGAAGTGTAAGATTA	76
		Fr_tu_tul4_R	GTAATTGGGAAGCTTGTATCATG	
		Fr_tu_tul4_P	AATGGCAGGCTCCAGAAGGTTCTAAGT	
	<i>fopA</i>	Fr_tu_fopA_F	GGCAAATCTAGCAGGTCAAGC	91
		Fr_tu_fopA_R	CAACACTTGCTTGAACATTTCTAG	
		Fr_tu_fopA_P	AACAGGTGCTTGGGATGTGGGTGGTG	
<i>Hepatozoon</i> spp.	18S rRNA	Hepa_spp_18S_F	ATTGGCTTACCGTGGCAGTG	175
		Hepa_spp_18S_R	AAAGCATTTTAACTGCCTTGTATTG	
		Hepa_spp_18S_S	ACGGTTAACGGGGGATTAGGGTTTCGAT	
<i>Rickettsia conorii</i>	23S-5S ITS	Ri_co_ITS_F	CTCACAAAGTTATCAGGTTAAATAG	118
		Ri_co_ITS_R	CGATACTCAGCAAATAATTCTCG	
		Ri_co_ITS_P	CTGGATATCGTGGCAGGGCTACAGTAT	
<i>Rickettsia slovaca</i>	23S-5S ITS	Ri_slo_ITS_F	GTATCTACTCACAAAGTTATCAGG	138
		Ri_slo_ITS_R	CTTAACTTTTACTACAATACTCAGC	
		Ri_slo_ITS_P	TAATTTTCGCTGGATATCGTGGCAGGG	
<i>Rickettsia massiliae</i>	23S-5S ITS	Ri_ma_ITS_F	GTTATTGCATCACTAATGTTATACTG	128
		Ri_ma_ITS_R	GTTAATGTTGTTGCACGACTCAA	
		Ri_ma_ITS_P	TAGCCCCGCCACGATATCTAGCAAAAA	
<i>Rickettsia helvetica</i>	23S-5S ITS	Ri_he_ITS_F	AGAACCGTAGCGTACACTTAG	79
		Ri_he_ITS_R	GAAAACCCTACTTCTAGGGGT	
		Ri_he_ITS_P	TACGTGAGGATTTGAGTACCGGATCGA	
<i>Rickettsia felis</i>	<i>orfB</i>	Ri_fel_orfB_F	ACCCTTTTCGTAACGCTTTGC	163
		Ri_fel_orfB_R	TATACTTAATGCTGGGCTAAACC	
		Ri_fel_orfB_P	AGGGAAACCTGGACTCCATATTCAAAAGAG	
<i>Rickettsia aeschlimannii</i>	ITS	Rick_aesch_ITS_F	CTCACAAAGTTATCAGGTTAAATAG	134

<i>Rickettsia</i> spp.	gltA	Rick_aesch_ITS_R	CTTAAC TTTTACTACGATACTTAGCA	78
		Rick_aesch_ITS_P	TAATTTTTGCTGGATATCGTGGCGGGG	
		Rick_spp_gltA_F	GTCGCAAATGTTACGGTACTT	
		Rick_spp_gltA_R	TCTTCGTGCATTTCCTTCCATTG	
<i>Theileria</i> spp.	18S rRNA	Rick_spp_gltA_P	TGCAATAGCAAGAACCGTAGGCTGGAT	126
		Th_an_18S_F	GCGGTAATTCCAGCTCCAATA	
		Th_an_18S_R	AAACTCCGTCCGAAAAAAGCC	
		Th_an_18S_P	ACATGCACAGACCCCAGAGGGACAC	
<i>Escherichia coli</i>	eae	eae-F2	CATTGATCAGGATTTTTCTGGTGATA	102
		eae-R	CTCATGCGGAAATAGCCGTTA	
		eae-P	ATAGTCTCGCCAGTATTCGCCACCAATACC	
<i>Rhipicephalus sanguineus</i> sensu lato	ITS-2	Rhi_san_ITS2_F	TTGAACGCTACGGCAAAGCG	110
		Rhi_san_ITS2_R	CCATCACCTCGGTGCAGTC	
		Rhi_san_ITS2_P	ACAAGGGCCGCTCGAAAGGCGAGA	
Tick spp.	16S rRNA	Tick_spp_16S_F	AAATACTCTAGGGATAACAGCGT	99
		Tick_spp_16S_R	TCTTCATCAAACAAGTATCCTAATC	
		Tick_spp_16S_P	CAACATCGAGGTCGCAAACCATTTTGTCTA	

Table S3

Pairwise comparison of 16S nucleotide sequences (aligned over 429 bp) of ticks determined herein. Nucleotide similarity and percentage differences are given above and below the diagonal, respectively.

Tick species	Sequence ID (GenBank accession no.)	191	187	290	355	192	30	116	302	357	298	7	225
<i>Haemaphysalis punctata</i>	191 (MT799944)	-	0.884	0.882	0.884	0.887	0.818	0.829	0.829	0.831	0.801	0.801	0.806
<i>Hs. sulcata</i>	187 (MT799946)	11.6	-	0.985	0.988	0.99	0.818	0.828	0.828	0.83	0.819	0.822	0.826
<i>Hs. sulcata</i>	290 (MT799948)	11.8	1.5	-	0.992	0.995	0.811	0.823	0.823	0.826	0.814	0.81	0.814
<i>Hs. sulcata</i>	355 (MT799949)	11.6	1.2	0.8	-	0.997	0.813	0.826	0.826	0.828	0.817	0.812	0.817
<i>Hs. sulcata</i>	192 (MT799947)	11.3	1	0.5	0.3	-	0.816	0.828	0.828	0.83	0.819	0.814	0.819
<i>Hyalomma anatolicum</i>	30 (MT799950)	18.2	18.2	18.9	18.7	18.4	-	0.861	0.861	0.863	0.847	0.849	0.852
<i>Rhipicephalus microplus</i>	116 (MT799951)	17.1	17.2	17.7	17.4	17.2	13.9	-	0.995	0.997	0.901	0.884	0.887
<i>Rh. microplus</i>	302 (MT799952)	17.1	17.2	17.7	17.4	17.2	13.9	0.5	-	0.997	0.898	0.882	0.884
<i>Rh. microplus</i>	357 (MT799953)	16.9	17	17.4	17.2	17	13.7	0.3	0.3	-	0.901	0.884	0.887
<i>Rh. haemaphysaloides</i>	298 (MT799956)	19.9	18.1	18.6	18.3	18.1	15.3	9.9	10.2	9.9	-	0.931	0.933
<i>Rh. turanicus</i>	7 (MT799954)	19.9	17.8	19	18.8	18.6	15.1	11.6	11.8	11.6	6.9	-	0.992
<i>Rh. turanicus</i>	225 (MT799955)	19.4	17.4	18.6	18.3	18.1	14.8	11.3	11.6	11.3	6.7	0.8	-

Table S4

Pairwise comparison of *cox1* nucleotide sequences (aligned over 673 bp) of ticks determined herein. Nucleotide similarity and percentage differences are given above and below the diagonal, respectively.

Tick species	Sequence ID (GenBank accession no.)	30	116	302	7	225	18	298	230	340	191	187	192	355
<i>Hyalomma anatolicum</i>	30 (MT80311)	-	0.823	0.821	0.815	0.826	0.82	0.82	0.824	0.826	0.802	0.808	0.806	0.811
<i>Rhipicephalus microplus</i>	116 (MT800322)	17.7		0.998	0.861	0.87	0.864	0.86	0.855	0.857	0.817	0.818	0.817	0.82
<i>Rh. microplus</i>	302 (MT800323)	17.9	0.2	-	0.86	0.869	0.863	0.858	0.854	0.855	0.818	0.82	0.818	0.821
<i>Rh. turanicus</i>	7 (MT800312)	18.5	13.9	14	-	0.982	0.986	0.893	0.878	0.879	0.829	0.823	0.818	0.818
<i>Rh. turanicus</i>	225 (MT800313)	17.4	13	13.1	1.8	-	0.992	0.895	0.887	0.888	0.841	0.83	0.829	0.826
<i>Rh. turanicus</i>	18 (MT800314)	18	13.6	13.7	1.4	0.8	-	0.888	0.879	0.881	0.835	0.824	0.823	0.82
<i>Rh. haemaphysaloides</i>	298 (MT800315)	18	14	14.2	10.7	10.5	11.2	-	0.924	0.925	0.827	0.833	0.833	0.832
<i>Rh. haemaphysaloides</i>	230 (MT800316)	17.6	14.5	14.6	12.2	11.3	12.1	7.6	-	0.998	0.835	0.827	0.827	0.829
<i>Rh. haemaphysaloides</i>	340 (MT800317)	17.4	14.3	14.5	12.1	11.2	11.9	7.5	0.2	-	0.836	0.829	0.829	0.83
<i>Haemaphysalis punctata</i>	191 (MT800318)	19.8	18.3	18.2	17.1	15.9	16.5	17.3	16.5	16.4	-	0.851	0.848	0.848
<i>Hs. sulcata</i>	187 (MT800319)	19.2	18.2	18	17.7	17	17.6	16.7	17.3	17.1	14.9	-	0.989	0.988
<i>Hs. sulcata</i>	192 (MT800320)	19.4	18.3	18.2	18.2	17.1	17.7	16.7	17.3	17.1	15.2	1.1	-	0.992
<i>Hs. sulcata</i>	355 (MT800321)	18.9	18	17.9	18.2	17.4	18	16.8	17.1	17	15.2	1.2	0.8	-



Table S5

Pairwise comparison of ITS-2 nucleotide sequences (aligned over 273 bp) of ticks belonging to *Rhipicephalus* and *Hyalomma* genera determined herein. Nucleotide similarity and percentage differences are given above and below the diagonal, respectively.

Tick species	Sequence ID (GenBank accession no.)	7	298	302	30
<i>Rhipicephalus turanicus</i>	7 (MT818226)	-	0.972	0.913	0.734
<i>Rh. haemaphysaloides</i>	298 (MT818227)	2.8	-	9.8	0.752
<i>Rh. microplus</i>	302 (MT818223)	8.7	0.902	-	0.72
<i>Hyalomma anatolicum</i>	30 (MT818222)	26.6	24.8	28	-