

Supplementary table 1: Primers pairs used in this study.

Organisms	Target gene	Primer sequence (5' → 3')	Fragment size (bp)	Tm (°C)	References
Ticks	16S rRNA	F:CTGCTCAATGATTTTTTAAATTGCTGTGG R:CCGGTCTGAACTCAGATCAAGT	456	48 54	1
<i>Nairoviridae</i>	S Segment	F:TCTCAAAGAAACACGTGCCGC R:GTCCTTCCTCCACTTGW	400	55	2
<i>Flaviviridae</i>	NS5	F:TGYRTBTAYAACATGATGG R:GTGTCCCAICCNCGNTRTC	269-272	50	3
	NS5	F:GTGTCCCAGCCGGCGGTGTCATCAGC R:AACATGATGGGRAARAGRGARAA	252	53	4
		F:GTGTCCCAGCCGGCGGTGTCATCAGC R:AARGGHAGYMCDGCHATHGGT	220	54	
Tick-borne Encephalitis virus	3' non-coding region	F:GGGCGGTTCTTGTTCTCC R:ACACATCACCTCCTTGTCAGACT Probe: TGAGCCACCATCACCCAGACACA	67	60	5
<i>Phenuiviridae</i>¹	L Segment ¹	F:GGCTACTTCAARAAYAARGANGA R:TGIAGIATSCCYTGCATCAT	1000	50	6-8
		Fragment 1 F:CAGCATGGIGGICTIAGAGAGAT R:TGIAGIATSCCYTGCATCAT	514	55	
		Fragment 2 F:GGCTACTTCAARAAYAARGANGA R:CTCTCTCAGICCCICCRTGYTG	507	50	
<i>Anaplasma/Ehrlichia/Neoehrlichia</i> spp.	16S rRNA	F: GGTACCYACAGAAGAAGTCC R: TAGCACTCATCGTTTACAGC	345	55	9
	<i>groESL</i>	F:AITGGGCTGGTAITGAAAT R:CCICCIIGGIACIAIACCTTC	1350	48	10
		F:ATWGCWAARGAAGCATAGTC R:CTCAACAGCAGCTCTAGTAGC	1297	55	
	<i>gltA</i> ¹	F :ATGTCTACTGCTGCTTGTGA	1098	50	11-12

Organisms	Target gene	Primer sequence (5'→ 3')	Fragment size (bp)	Tm (°C)	References
Neoehrlichia spp.	groEL ²	R :ATGACCAGTATATAACTGACGTG	1030	50	13-14
		F :ATGTCTACTGCTGCTTGTGA			
		R :GAGCAGACCAACCAGATGTTC			
		F :GAAGTATAGTTTAGTATTTTTGTC	1275	49	
		R :TTAACTTCTACTTCGCTTG			
		F :GAAGTATAGTTTAGTATTTTTGTC	510	49	
		R :ACATCACGTTTCATAGAA			
		F :AAAGGAATTAGTATTAGAATCTTT	567	49	
	R :CTTCCATTTTAACTGCTAA				
	F :AATATAGCAAGATCAGGTAGAC	461	49		
R :TTAACTTCTACTTCGCTTG					
16S rRNA	F: GTGGCAGACGGGTGAGTAAT	1053	60	15	
R: TGCAGCACCTGTGTAAGGTC					
Borrelia spp.	flaB	F: TAATACGTCAGCCATAAATGC	750	56	16
		R: GCTCTTTGATCAGTTATCATTC			
	flaB (Lyme group)	F: AARGAATTGGCAGTTCAATC	497	52	17
		R: GCATTTTCWATTTTAGCAAGTGATG			
		F:ACATATTCAGATGCAGACAGAGGTTCTA	389	55	
		R:GAAGGTGCTGTAGCAGGTGCTGGCTGT			
	glpQ (relapsing fever)	F:ATGGGTTCAAACAAAAAGTCACC	920	70 Touchdown 60	18
		R:CATTACTGTGTCAGTAAAATCTGTAAATATACCA TCTAC			
		F:ATGGGTTCAAACAAAAAGTCACC	700	53	19
		R:CCAGGGTCCAATTCCATCAGAATATTGTGCAAC			
Spotted Fever group Rickettsia spp.	ompA	F:ATGGCGAATATTTCTCCAAAA	631	46	20-21
		R:GTTCCGTTAATGGCAGCATCT			
		F:ATGGCGAATATTTCTCCAAAA	532	48	
		R:AGTGCAGCATTGCTCCCCCT			
	ompB	F:AAACAATAATCAAGGTACTGT	811	55	22
		R:TACTTCCGGTTACAGCAAAGT			

Organisms	Target gene	Primer sequence (5' → 3')	Fragment size (bp)	Tm (°C)	References
	<i>sca4</i> ³	F: ATGAGTAAAGACGGTAACCT R AAGCTATTGCGTCATCTCCG	928	50	23
<i>Francisella</i> spp.	<i>tul4</i>	F :ATGGCGAGTGATACTGCTTG R :GCATCATCAGAGCCACCTAA	250	56	24
<i>Babesia/ Theileria/ Hepatozoon</i> spp.	18S rRNA	F:GACACAGGGAGGTAGTGACAAG R:CTAAGAATTTACCTCTGACAGT	400	51	25
	18S rRNA	F:GGCTCATTACAACAGTTATAG R:CCCAAAGACTTTGATTTCTCTC	~930	58	26
		F:CCGTGCTAATTGTAGGGCTAATAC R:GGACTACGACGGTATCTGATCG	~800	62	
	18S rRNA ³	F: GCGCAAATTACCCAATT R: TAAGGTGCTGAAGGAGTCGTTTAT	660	60	27-28

F: Forward; R: Reverse; bp: base pairs; Tm: melting temperature; N= A/C/G/T; Y= C/T; K: G/T; W:A/T; R: G/A;B: C/G/T; S:G/C; H: A/C/T;

¹:Seminested PCR assays using primers previously designed for single targets (Fragment 1⁷ and Fragment 2⁸).; ²: *Ehrlichia canis* and *Alloccryptoplasma* were also detected with these assay; ³: PCR for the *Hepatozoon* spp. detection, performed only with selected samples

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Supplementary table 2. Maximum Identities of the nucleotide sequences obtained in this study comparing to public sequences from GenBank.

Organisms	Species	Target gene	No. of sequences	GenBank accession no.	Identities			
					bp	%	GenBank accession no.	QC
Tick	<i>Haemaphysalis concinna</i>	16S rRNA	1	PX998615	403-404/404	100	MK463620	100
Viruses	<i>Mivirus boleense</i>	S. nucleoprotein	1	PZ012206	402/421	95.5	MN829806*	98
		S. nucleoprotein	1	PZ012207	394/421	93.6	MN829806*	98
		S. nucleoprotein	1	PZ012208	396/421	94.1	MN829806*	98
	Tick-Wolf <i>Hudivirus</i> IR39; <i>Hudivirus luzmelaense</i>	L Segment (Fragment 1)	1	PZ012213	359/379	77.8	MT129680*	100
		L Segment (Fragment 2)	1	PZ012212	388/572	67.8	MT129681	100
	<i>Ixovirus norvergiae</i>	L Segment (Fragment 2)	1	PZ012209	459/460	99.8	OR645567	100
			1	PZ012210	458/460	99.6	OR645567	100
			1	PZ012211	459/460	99.8	OR645567	100
	Tick-Wolf <i>Ixovirus</i> IH8; <i>Ixovirus borragani</i>	L Segment (Fragment 1)	1	PZ012215	373/475	78.53	PQ150396*	100
		L Segment (Fragment 2)	1	PZ012214	333/454	73.4	NC_055432	100
	Tick-Wolf <i>Ixovirus</i> IH9; <i>Ixovirus beronensis</i>	L Segment (Fragment 1)	1	PZ012217	470/475	99.0	PQ150398	100
		L Segment (Fragment 2)	1	PZ012216	319/443	72.0	KX184200	98
	Tick-Wolf <i>Uukuvirus</i> RB11; <i>Uukuvirus lebanense</i>	L Segment (Fragment 1)	1	PZ012219	474/478	97.3	MK216299*	100
		L Segment (Fragment 2)	1	PZ012218	236/242	97.5	MG764523*	100
	Tick-Wolf <i>Uukuvirus</i> RB12; <i>Uukuvirus lebanense</i>	L Segment (Fragment 1)	1	PZ012221	476/487	97.8	LC374275*	100
		L Segment (Fragment 2)	1	PZ012220	378/397	95.2	MG764523*	100

Organisms	Species	Target gene	No. of sequences	GenBank accession no.	Identities			
					bp	%	GenBank accession no.	QC
	Tick-Wolf <i>Uukuvirus</i> RB92; <i>Uukuvirus lebanense</i>	L Segment (Fragment 1)	1	PZ012223	480/487	98.6	LC374275*	100
		L Segment (Fragment 2)	1	PZ012222	448/467	95.9	MG764523*	100
	<i>Uukuvirus tachengense</i>	L Segment (Fragment 1)	1	PZ012224	688/736	93.5	MW561143	100
			1	PZ012225	458/486	94.2	MW561143	100
			1	PZ012226	405/485	83.5	MW561154	100
			1	PZ012227	408/486	84.0	ON684362	100
			1	PZ012228	703/754	93.2	OR327613	100
			1	PZ012229	408/485	94.1	MW561143	100
			1	PZ012230	408/486	83.3	MW561143*	100
			1	PZ012231	406/486	83.6	OR327610*	100
			1	PZ012232	400/425	94.1	PQ900140	100
			1	PZ012233	458/486	94.2	MW561143	100
			1	PZ012234	405/485	83.5	MW561154	100
			1	PZ012235	365/437	83.5	OR327604	100
			1	PZ012236	458/486	94.2	MW561143	100
			1	PZ012237	407/485	83.9	OQ162435	100
			1	PZ012238	449/480	93.5	ON684362	100
			1	PZ012239	403/485	93.1	MW561154	100
			1	PZ012240	700/750	93.3	MW561143	100
			1	PZ012241	459/487	94.3	PQ900140	100
			1	PZ012242	406/485	83.7	MW561143	100
			1	PZ012243	458/486	94.2	MW561143	100
			1	PZ012244	633/679	93.2	OR327613	100
			1	PZ012245	403/486	82.2	OR327611	100
			1	PZ012246	407/485	83.9	MW561143	100

Organisms	Species	Target gene	No. of sequences	GenBank accession no.	Identities			
					bp	%	GenBank accession no.	QC
		L Segment (Fragment 2)	1	PZ012247	438/463	94.3	MT380797	100
			1	PZ012248	437/463	94.4	MT380797	100
			1	PZ012249	408/468	88.3	MT380797	100
			1	PZ012250	407/462	88.1	ON684362	100
			1	PZ012251	437/463	94.4	MT380795	100
			1	PZ012252	408/462	88.3	MT380797	100
			2	PZ012253	410/462	88.8	MT380797	100
			1	PZ012254	431/463	93.1	PV769794	100
			1	PZ012255	407/462	88.1	MT380797	100
			2	PZ012256	406/462	87.9	MT380797	100
			1	PZ012257	365/408	89.5	MW561155	99
			1	PZ012258	436/438	94.6	MT380795	100
			1	PZ012259	409/462	88.5	MT380797	100
			1	PZ012260	435/463	94.0	MT380795	100
			1	PZ012261	433/463	93.5	MT380797	100
			1	PZ012262	434/463	93.4	MT380797	100
			1	PZ012263	408/462	88.3	MT380797	100
Bacteria	<i>Anaplasma phagocytophilum</i>	16S rRNA	1	PX999058	305/305	100	CP035303*	100
		<i>groESL</i>	2	PZ054585	1211/1212	99.9	CP046639*	100
			3	PZ054586	1174/1174	100	CP046639*	100
			2	PZ054587	1204/1208	99.7	CP046639*	100
	<i>Ehrlichia canis</i>	16S rRNA	1	PX999062	816/816	100	CP025749*	100
		<i>gltA</i>	1	PZ054588	967/967	100	CP025749*	100
	<i>Alloccryptoplasma</i> sp.	16S rRNA	1	PX999061	872/872	100	MG924904	100
	<i>Midichloria mitochondrii</i>	16S rRNA	11	PX999059	305/305	100	CP002130*	100
	<i>Wolbachia</i> sp.	16S rRNA	1	PX999060	305/305	100	CP185405*	100

Organisms	Species	Target gene	No. of sequences	GenBank accession no.	Identities			
					bp	%	GenBank accession no.	QC
	<i>Borrelia lusitaniae</i>	<i>flaB</i>	1	PZ054589	334/334	100	CP132461*	100
	<i>Borrelia miyamotoi</i>	<i>glpQ</i>	1	PZ054590	667/667	100	CP044783*	100
	<i>Rickettsia kotlanii</i>	<i>ompA</i>	1	PZ054593	488/489	99.8	DQ243938	100
		<i>ompB</i>	1	PZ054594	765/770	99.4	LC379471	100
		<i>sca4</i>	1	PZ054595	801/804	99.5	KJ619625	100
	<i>Rickettsia raoultii</i>	<i>ompA</i>	30	PZ054591	488/488	100	AH015609	100
		<i>ompB</i>	29	PZ054592	770/770	100	CP010969*	100
	<i>Francisella</i> spp.	<i>tul4</i>	31	PZ019862	208/208	100	GU113085*	100
Protozoa	<i>Babesia caballi</i>	18S rRNA	5	PX998639	867/867	100	KY952233	100
	<i>Babesia capreoli</i>	18S rRNA	3	PX998640	894/897	99.7	DQ866842	100
	<i>Hepatozoon canis</i>	18S rRNA	3	PX998641	622/622	100	PP574309*	100
	<i>Hepatozoon martis</i>	18S rRNA	1	PX998642	429/429	100	PQ144754	100

* among others

QC: Query cover; *Ca.*: *Candidatus*; Fragment 1: PCR from Matsuno et al., 2015 (doi: 10.1128/JVI.02704-14); Fragment 2: PCR from Klimentov et al., 2016 (doi: 10.1016/j.jviromet.2016.02.009).

Supplementary table 3. Maximum Identities of the amino acid sequences from viruses obtained in this study comparing to public sequences from GenBank.

Species	Target gene	No. of sequences	GenBank accession no.	Identities			
				Amino acid	%	GenBank accession no.	QC
<i>Mivirus boleense</i>	S. nucleoprotein	1	YEN91084	137/140	97.9	WHV00509*	100
	S. nucleoprotein	2	YEN91085-6	136/140	97.1	WHV00509*	100
Tick-Wolf <i>Hudivirus</i> IR39; <i>Hudivirus luzmelaense</i>	L Segment (Fragment 1)	1	YEN91091	131/151	82.9	QIJ70046*	100
	L Segment (Fragment 2)	1	YEN91090	130/180	93.3	QIJ70046*	99
<i>Ixovirus norvergiae</i>	L Segment (Fragment 2)	3	YEN91087-9	142/142	100	XNO64830*	100
Tick-Wolf <i>Ixovirus</i> IH8; <i>Ixovirus borragani</i>	L Segment (Fragment 1)	1	YEN91093	130/158	82.3	XEN41026*	100
	L Segment (Fragment 2)	1	YEN91092	139/149	93.3	YAI74784	99
Tick-Wolf <i>Ixovirus</i> IH9; <i>Ixovirus beronensis</i>	L Segment (Fragment 1)	1	YEN91095	157/158	99.4	XEN41024-6	100
	L Segment (Fragment 2)	1	YEN91094	108/147	73.5	YAI74784	98
Tick-Wolf <i>Uukuvirus</i> RB11; <i>Uukuvirus lebanense</i>	L Segment (Fragment 1)	1	YEN91096	161/162	98.4	QBP05510*	100
	L Segment (Fragment 2)	1	YEN91097	80/80	100	AWK68113	100
Tick-Wolf <i>Uukuvirus</i> RB12; <i>Uukuvirus lebanense</i>	L Segment (Fragment 1)	1	YEN91099	160/162	98.8	QBP05510*	100
	L Segment (Fragment 2)	1	YEN91098	131/132	99.2	AWK68113	99
Tick-Wolf <i>Uukuvirus</i> RB92; <i>Uukuvirus lebanense</i>	L Segment (Fragment 1)	1	YEN91101	162/162	100	QBP05510*	100
	L Segment (Fragment 2)	1	YEN91100	154/155	99.4	AWK68113	99
<i>Uukuvirus tachengense</i>	L Segment (Fragment 1)	4	YEN91102; YEN91106; YEN91118; YEN91122	245/245	100	WPO07318*	100
		13	YEN91103-5; YEN91107-9; YEN91116-7; YEN91120; YEN91123-4	162/162	100	WPO07318*	100
		1	YEN91115	162/163	99.4	WPO07318*	100

Species	Target gene	No. of sequences	GenBank accession no.	Identities			
				Amino acid	%	GenBank accession no.	QC
		1	YEN91121	161/162	99.4	WPO07318*	100
		1	YEN91119	161/162	99.4	WPO07318*	100
		1	YEN91110	89/89	100	XNO64841	100
		2	YEN91111; YEN91114	161/162	99.4	WPO07318*	100
	L Segment (Fragment 2)	7	YEN91125-6; YEN91129; YEN91132; YEN91136; YEN91139; YEN91141	154/154	100	WPO07318*	100
		9	YEN91127; YEN91130; YEN91131; YEN91133-4; YEN91138; YEN91142	153/154	99.4	WPO07327*	100
		1	YEN91140	153/154	100	WPO07318*	100
		2	YEN91128; YEN91135	154/154	100	WPO07327*	100

* among others

QC: Query cover; Fragment 1: PCR from Matsuno et al., 2015 (doi: 10.1128/JVI.02704-14); Fragment 2: PCR from Klimentov et al., 2016 (doi: 10.1016/j.jviromet.2016.02.009).