

**Additional file 1: Table S1** Primers used for validation of microfluidic real-time PCR results

Species	Target gene	Forward primer	Reverse primer	Reference
<i>Babesia/Theileria</i>	18S rRNA	GAGGTAGTGACAAGAAATAACAATA	TCTTCGATCCCCTAACTTTC	[1]
<i>Anaplasma/ Ehrlichia/ Rickettsia</i> spp.	16S rRNA	GAACGAACGCTGGCGGCAAGC	AGTAYCGRACCAGATAGC CGC	[2]
	Citrate synthase	GGGGGCCTGCTCACGGCGG	ATTGCAAAAAGTACAGTGAAC	[3]
<i>Hepatozoon</i> spp.	18S rRNA	ATACATGAGCAAAATCTCAAC	CTTATTATTCCATGCTGCAG	[4]
<i>Bartonella</i> spp.	Citrate synthase	GGGGACCAGCTCATGGTGG	AATGCAAAAAGAACAGTAAACA	[5]

**References**

1. Gubbels J, De Vos A, Van der Weide M, Viseras J, Schouls L, De Vries E, et al. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. J Clin Microbio. 1999;37:1782-9.
2. Rar VA, Fomenko NV, Dobrotvorsky AK, Livanova NN, Rudakova SA, Fedorov EG, et al. Tickborne pathogen detection, western Siberia, Russia. Emerg Infect Dis. 2005;11:1708.
3. Regnery RL, Spruill CL, Plikaytis B. Genotypic identification of *Rickettsiae* and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol. 1991;173:1576-89.
4. de Azevedo Gomes L, Moraes PHG, do Nascimento LdCS, O'Dwyer LH, Nunes MRT, Rossi AdRP, et al. Molecular analysis reveals the diversity of *Hepatozoon* species naturally infecting domestic dogs in a northern region of Brazil. Ticks Tick Borne Dis. 2016;7:1061-6.
5. Norman A, Regnery R, Jameson P, Greene C, Krause D. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995;33:1797-803.