

Supplementary Materials:

**Table S1.** Primers used in the study for the amplification of piroplasms (*Babesia/Theileria* spp.), specifically, *Babesia bigemina*, *B. bovis* and *Theileria orientalis*.

Organism and Type of PCR	Target Gene	Primer Name	Sequence (5' → 3')	Expected Size (bp)	References
Piroplasms ( <i>Babesia/ Theileria</i> spp.) Nested	18s rRNA	piro18s forward 1	GGTGAAACTGCGAATGGCTC	1600	[29]
		piro18s reverse 1	AAGTGATAAGGTTCACAAAACCTT		
		piro18s forward 2	TGGCTCATTACAACAGTTATA	1500	
		piro18s reverse 2	CGGTCCGAATAATTCACC		
<i>Babesia bigemina</i> Conventional	apical membrane antigen-1	AMA-1 forward	TACTGTGACGAGGACGGATC	211	[30]
		AMA-1 reverse	CCTCAAAAGCAGATTCGAGT		
<i>Babesia bovis</i> Nested	spherical body protein- 4	SBP-4 forward	GAAATCCCTGTTCCAGAG	907	[31]
		SBP-4 reverse	TCGTTGATAACACTGCAA		
		SBP-4 forward	GAAATCCCTGTTCCAGAG	503	
		SBP-4 reverse	TCGTTGATAACACTGCAA		
<i>Theileria orientalis</i> Conventional	major piroplasm surface protein	MPSP forward	CTTTGCCTAGGATACTTCCT	776	[32]
		MPSP reverse	ACGGCAAGTGGTGAGAACT		

**Table S2.** PCR conditions used in the study for the amplification of piroplasms (*Babesia/Theileria* spp.), specifically, *Babesia bigemina*, *B. bovis*, and *Theileria orientalis*.

Target Gene	PCR Condition
18s rRNA	1 <sup>st</sup> round: $\frac{95^{\circ}C}{9\ min} \rightarrow \left[ \frac{94^{\circ}C}{30\ s} - \frac{55^{\circ}C}{30\ s} - \frac{72^{\circ}C}{90\ s} \right] 35x \rightarrow \frac{72^{\circ}C}{7\ min} \rightarrow 10^{\circ}C\ \infty$
	2 <sup>nd</sup> round: $\frac{95^{\circ}C}{9\ min} \rightarrow \left[ \frac{94^{\circ}C}{30\ s} - \frac{53^{\circ}C}{30\ s} - \frac{72^{\circ}C}{90\ s} \right] 35x \rightarrow \frac{72^{\circ}C}{5\ min} \rightarrow 10^{\circ}C\ \infty$
apical membrane antigen-1	$\frac{95^{\circ}C}{5\ min} \rightarrow \left[ \frac{95^{\circ}C}{30\ s} - \frac{68^{\circ}C}{1\ min} - \frac{72^{\circ}C}{1\ min} \right] 35x \rightarrow \frac{72^{\circ}C}{10\ min} \rightarrow 10^{\circ}C\ \infty$
spherical body protein- 4	1 <sup>st</sup> round: $\frac{95^{\circ}C}{5\ min} \rightarrow \left[ \frac{94^{\circ}C}{1\ min} - \frac{55^{\circ}C}{1\ min} - \frac{72^{\circ}C}{1\ min} \right] 35x \rightarrow \frac{72^{\circ}C}{10\ min} \rightarrow 10^{\circ}C\ \infty$
	2 <sup>nd</sup> round: $\frac{95^{\circ}C}{5\ min} \rightarrow \left[ \frac{94^{\circ}C}{1\ min} - \frac{55^{\circ}C}{1\ min} - \frac{72^{\circ}C}{1\ min} \right] 35x \rightarrow \frac{72^{\circ}C}{10\ min} \rightarrow 10^{\circ}C\ \infty$
major piroplasm surface protein	$\frac{94^{\circ}C}{10\ min} \rightarrow \left[ \frac{94^{\circ}C}{1\ min} - \frac{58^{\circ}C}{1\ min} - \frac{72^{\circ}C}{1\ min} \right] 35x \rightarrow \frac{72^{\circ}C}{4\ min} \rightarrow 10^{\circ}C\ \infty$