



Supplemental 1. Primers and thermal cycle conditions for amplifying genes used in the molecular identification of *Haemaphysalis longicornis* ticks and potential animal-associated pathogens.

Target (Reference)	Gene (band size)	Primers 5'-3' (10 µM concentration)	Cycling Conditions
Tick DNA [29,30]	16S ribosomal DNA (~455bp)	16S_F- 5' TTA AAT TGC TGT RGT ATT 16S_R- 5' CCG GTC TGA ACT CAS AWC	initial denaturation (94°C for 5 min) <ul style="list-style-type: none"> 5 cycles of 94°C for 30s, 49°C for 30s, 68°C for 60s; 5 cycles of 94°C for 30s, 47°C for 30s, and 68°C for 60s; 5 cycles of 94°C for 30s, 45°C for 30s, and 68°C for 60s; 25 cycles of 94°C for 30s, 43°C for 30s, and 68°C for 60s; final extension (68°C for 5min)
	cytochrome oxidase I (~820bp)	COX1_F- 5' GGA ACA ATA TAT TTA ATT TTT GG COX1_R- 5' ATC TAT CCC TAC TGT AAA TAT ATG	initial denaturation (95°C for 10 min); <ul style="list-style-type: none"> 40 cycles of 95°C for 30s, 55°C for 60s, and 72°C for 60s final extension (72°C for 7 min)
<i>Anaplasma</i> and <i>Ehrlichia</i> DNA [32]	<i>groEL</i> (~664 bp)	<u>Primary Reaction</u> Gro607F- 5' GAA GAT GCW GTW GGW TGT ACK GC Gro1294R- 5' AGM GCT TCW CCT TCW ACR TCY TC	initial denaturation (95°C for 15 min); <ul style="list-style-type: none"> 40 cycles of 95°C for 30s, 58°C for 30s, and 72°C for 30s final extension (72°C for 3 min)
	(~315 bp)	<u>Nested Reaction</u> Gro677F- 5' ATT ACT CAG AGT GCT TCT CAR TG Gro1121R- 5' TGC ATA CCR TCA GTY TTT TCA AC	initial denaturation (95°C for 15 min); <ul style="list-style-type: none"> 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s final extension (72°C for 3 min)
<i>Babesia</i> DNA [33]	(~1600 bp)	<u>Primary Reaction</u> <i>Bab18s_1F</i> 5'- AAG CCA TGC ATG TCT AAG TAT AAG CTT TT <i>Bab18s_1R</i> 5'- CTT CTC CTT CCT TTA AGT GAT AAG GTT CAC	initial denaturation (94°C for 10 min); <ul style="list-style-type: none"> 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s final extension (72°C for 5 min)
	(~390 bp)	<u>Nested Reaction</u> <i>Bab18S2_437-461.F</i> 5'- AAT CCT GAC ACA GGG AGG TAG TGA C -3' <i>Bab18S2_898-873.R</i> 5'- CTA AGA ATT TCA CCT CTG ACA GT -3'	initial denaturation (94°C for 10 min); <ul style="list-style-type: none"> 35 cycles of 94°C for 30s, 65°C for 30s, and 72°C for 30s final extension (72°C for 5 min)
<i>Anaplasma marginale</i> and <i>Theileria orientalis</i> DNA [8]	qPCR	<u><i>Theileria orientalis</i></u> Forward primer 5' – GCA AAC AAG GAT TTG CAC GC Reverse primer 5' – TGT GAG ACT CAA TGC GCC TAG A Universal probe 5' – NED-TCG ACA AGT TCT CAC CAC MGB-NFQ <u><i>Anaplasma marginale</i></u> Forward primer 5' – TTG GCA AGG CAG CAG CTT	Initial denature (95°C for 10 min); 45 cycles of 95°C for 15s, 60°C for 60s

Reverse primer 5' –
TTC CGC GAG CAT GTG CAT

Supplement 2. Amplified genetic sequences from *Haemaphysalis longicornis* ticks, confirming their identification.

>16S sequences for all of the Tennessee ticks were 100% identical to *H. longicornis* GenBank sequences (MK439888, KX083342, KX450293, KU986714, KP324925, KJ710084, AB819205, JX051073, JX051071, JX051070, JX051069, JX051066, JX051064, MW602986, JF979373, KR259991)

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TATGTAAAAAATACTCTAGGGATAACAGCGTAATAATTTTAGATAGATCTTATAGAAAAAATAGTTTGCGA
CCTCGATGTTGGATTAGGATACTTGTTTAATGAAGAAGTtAAATAAAGAAG
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>COX-1 sequences for all of the Tennessee ticks were 100% identical to *H. longicornis* GenBank sequences (MK439888, MF6668880)

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TAAGAATTTTAATTCTGAATAGAACTAGGGCAACCTGGTACATTAATTGGAAATGATCAAATCTATAATGTA
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