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Ehrlichia muris in *Ixodes cookei* Ticks, Northeastern United States, 2016–2017

Technical Appendix

Technical Appendix Table 1. Primers and probes used in this study

Target gene	Application	Type	Sequences (5′→3′)	Tm (C)	Reference
16S	Tick species PCR and confirmation	Forward	TGCTGTAGTATTTTGACTATACAAAGG	55	This paper
		Reverse	ATCCTAATCCAACATCGAGGTC		
ITS	*Ixodes scapularis* identification	Forward	TGCGTTTTCTTTGAGCAAATGCACGAG	60	This paper
		Reverse	GTACGGGATTTTCCACAAACGGTATCCA		
		Probe	FAM-TGCGCTTAACCAGTCCTCCTCCTCCTACGA-BHQ		
ITS	*Ixodes pacificus* identification	Forward	CTCGGAGCAAGTACGGAGGTAG	60	This paper
		Reverse	TTTCCACAAAACGGTCGCCATC		
		Probe	Cy5-CTGAGCCAAGTCCTCTTCCTACCCGGTTTG-BHQ		
ITS	*Amblyomma americanum* identification	Forward	CGACCGCCGCAGGAAGG	60	This paper
		Reverse	CGTTTCTCGCAGCAGTTCGG		
		Probe	FAM-CCCGCTGGCCCGCGTACGTGT-BHQ		
ITS	*Dermacentor variabilis* identification	Forward	CTGAAGATTCTTTGCGAGGAGCGG	60	(*1*)
		Reverse	GCGTCAGCTCGGCCAAC		
		Probe	FAM-AGAAGGGCGTGCCCCGAAAGCGG-BHQ		
gltA	*E. muris* PCR and confirmation	Forward	TACAGATTTCTCAAGAATATACA (outer) TGGCATGTTTTTCTGCCTTA (inner)	50	(*2*)
		Reverse	AATGCAATGTTTTCTAATTCTAC (outer) TGACCAAAACCCATTAATCTTG (inner)		
groEL	*E. muris* PCR and confirmation	Forward	GGATCCATTGGCTCTTGCTA (outer) AAGGGATTCAAAGAATTGGATG (inner)	50	(*2*)
		Reverse	CCACCAACCTTTAAGACAGCA (outer) CCACCAACCTTTAAGACAGCA (inner)		
P13	EMLA detection	Forward	TACCTAATTCTTCTCAAGAGATTCAGTTG	60	This paper
		Reverse	ATGATGATACTGCGAACAACTATAAGAG		
		Probe	Cy5-ATATTGATAAAAGAGTCAGTGTTGATCCGTATGAGTTAGGGTT-BHQ		
glpQ	*Borrelia miyamotoi* detection	Forward	GACATAGTTCTAACAAAGGACAATATTCC	60	(*3*)
		Reverse	TCCGTTTTCTCTAGCTCGATTGG		
		Probe	HEX-TGCACGACCCAGAAATTGACACAACCACAA-BHQ		
ospA	*Borrelia burgdorferi Sensu Lato* detection	Forward	ATAGGTCTAATATTAGCCTTAATAGCAT	60	This paper
		Reverse	AGATCGTACTTGCCGTCTT		
		Probe	FAM-aagc+Aaa+Atgtt+Agc+Agccttga-BHQ (LNA)		
Tubulin	*Babesia* detection	Forward	GATTTGGAACCTGGCACCATG	60	(*4*)
		Reverse	AATGACCCTTAGCCCAATTATTTCC		
		Probe	FAM-ATCTGGCCCATACGGTGAATTGTTTCGC-BHQ		
MSP-2	*Anaplasma* detection	Forward	ATGGAAGGTAGTGTTGGTTATGGTATT	60	(*4*)
		Reverse	TTGGTCTTGAAGCGCTCGTA		
		Probe	HEX-TGGTGCCAGGGTTGAGCTTGAGATTG-BHQ		

*We use species-specific taqman qPCR identified *Amblyomma americanum* (243), *Dermacentor variabilis* (271), *Ixodes pacificus* (215), and *Ixodes scapularis* (7800). A fragment of 16S mtDNA was used to identify EMLA-positive *Ixodes scapularis* ticks (2), *Ixodes angustus* (14), *Ixodes cookei* (22), *Ixodes ricinus* (5), *Ixodes spinipalpis* (19) and *Dermacentor occidentalis* (3). The rest 114 ticks were identified by morphological characters or marked as unidentifiable because of poor sample conditions.

Technical Appendix Table 2. DNA sequences used in this study

Organism	Gene	Sample or strain	GenBank accession no.*
Ixodes scapularis	16s rRNA	20170522–362	MG242324
Ixodes scapularis	16s rRNA	20170605–176	MG242325
Ixodes cookei	16s rRNA	20170731–52	MG242326
Ixodes cookei	16s rRNA	20170807–147	MG242327
Ixodes cookei	16s rRNA	20170918–13	MG242328
E. muris	gltA	20170522–362	MG242314
E. muris	gltA	20170605–176	MG242315
E. muris	gltA	20170731–52	MG242316
E. muris	gltA	20170807–147	MG242317
E. muris	gltA	20170918–13	MG242318
E. muris	groEL	20170522–362	MG242319
E. muris	groEL	20170605–176	MG242320
E. muris	groEL	20170731–52	MG242321
E. muris	groEL	20170807–147	MG242322
E. muris	groEL	20170918–13	MG242323
E. muris	gltA	WI22	HQ660494
E. muris	groEL	WI22	HQ660492
E. muris	gltA	WI975	HQ660497
E. muris	groEL	WI975	HQ660493
E. muris	gltA	EmCRT	LANU00000000
E. muris	groEL	EmCRT	LANU00000000
E. muris	gltA	AS145	CP006917
E. muris	groEL	AS145	CP006917
E. muris	groEL	Est7979	KU535864
E. muris	groEL	Nov-Ip300	GU358687
E. muris	groEL	RUS/Nov14–2526-Ipr	KX980049
E. muris	groEL	Est1709	KU535861
E. muris	groEL	IRK1/Warsaw	KF312362
Ehrlichia spp.	gltA	HF	NZ_CP007474
Ehrlichia spp.	groEL	HF	NZ_CP007474
E. ovata	groEL	Shizuoka	DQ672553
E. chaffeensis	gltA	Jax	CP007475
E. chaffeensis	groEL	Jax	CP007475
E. chaffeensis	gltA	Arkansas	CP000236
E. chaffeensis	groEL	Arkansas	CP000236
E. canis	gltA	Jake	NC_007354
E. canis	groEL	Jake	NC_007354
E. ewingii	gltA	Panola Mountain	DQ365879
E. ewingii	groEL		AF195273
E. ruminantium	gltA	Welgevonden	NC_005295
E. ruminantium	groEL	Welgevonden	NC_005295

*GenBank accession numbers MG242314 to MG242328 are new sequences in this study.

Technical Appendix Table 3. EMLA positive ticks in this study

Tick ID#	Tick species	Tick stage	Source	Location
20170522–362	*Ixodes scapularis*	Adult	Human	Laporte, MN 56461
20170605–176	*Ixodes scapularis*	Adult	Human	Eleva, WI 54738
20170731–52	*Ixodes cookei*	Adult	Human	Holden, ME 04429
20170807–147	*Ixodes cookei*	Adult	Human	Salamanca, NY 14779
20170918–13	*Ixodes cookei*	Adult	Human	Littleton, ME 04730

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