

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

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DOI: <https://doi.org/10.3201/eid2406.171755>

*Ehrlichia muris* is an agent of human ehrlichiosis. To determine its geographic spread in the United States, during 2016–2017, we tested 8,760 ticks from 45 states. A distinct clade of *E. muris* found in 3 *Ixodes cookei* ticks from the northeastern United States suggests transmission by these ticks in this region.

*Ehrlichia muris* was originally isolated from a mouse in Japan in 1983 (1). In 2009 in the United States, an *E. muris*-like agent (EMLA) was identified as a causative agent of human ehrlichiosis for 3 symptomatic patients in Wisconsin and 1 in Minnesota (2). A retroanalysis of 760 *Ixodes scapularis* ticks collected from 1992 through 1997 in Wisconsin revealed an EMLA infection rate of 0.94%, indicating presence of this pathogen in the upper midwestern region since at least the mid-1990s (3). Another study found this infection in 69 patients from 5 states from 2007 through 2013, although all patients had probably been exposed to the ticks in Minnesota or Wisconsin (4). In 2017, the *E. muris*-like isolates from the upper midwestern United States were proposed as a taxonomically distinct subspecies, *E. muris* subsp. *eaucloirensis* (5).

*E. muris* is thought to be transmitted by *Haemaphysalis flava* ticks in Japan, by *I. persulcatus* ticks in eastern Europe, and by *I. ricinus* ticks in western Europe (5). Detection of the bacterium in nymphal and adult stages of *I. scapularis* ticks (2,5,6) and in white-footed mice (*Peromyscus leucopus*) suggests that the primary vectors and reservoir hosts of Lyme borreliosis play a major role in the enzootic transmission cycle of *E. muris* in the United States. However, despite the broad distribution of *I. scapularis* ticks and *P. leucopus* mice in North America, to our knowledge, *E. muris* has not been reported outside of Wisconsin and Minnesota (2,7).

To evaluate the geographic distribution of *E. muris* from May 30, 2016, through October 1, 2017, we used a Taqman real-time PCR to test 8,760 ticks for EMLA, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *B. miyamotoi*, *B. mayonii*, and *Babesia microti*. The EMLA test is a modified version of a multiplex Taqman assay and targets the p13 gene (8). The human-biting ticks used for this study were submitted to the TickReport public testing

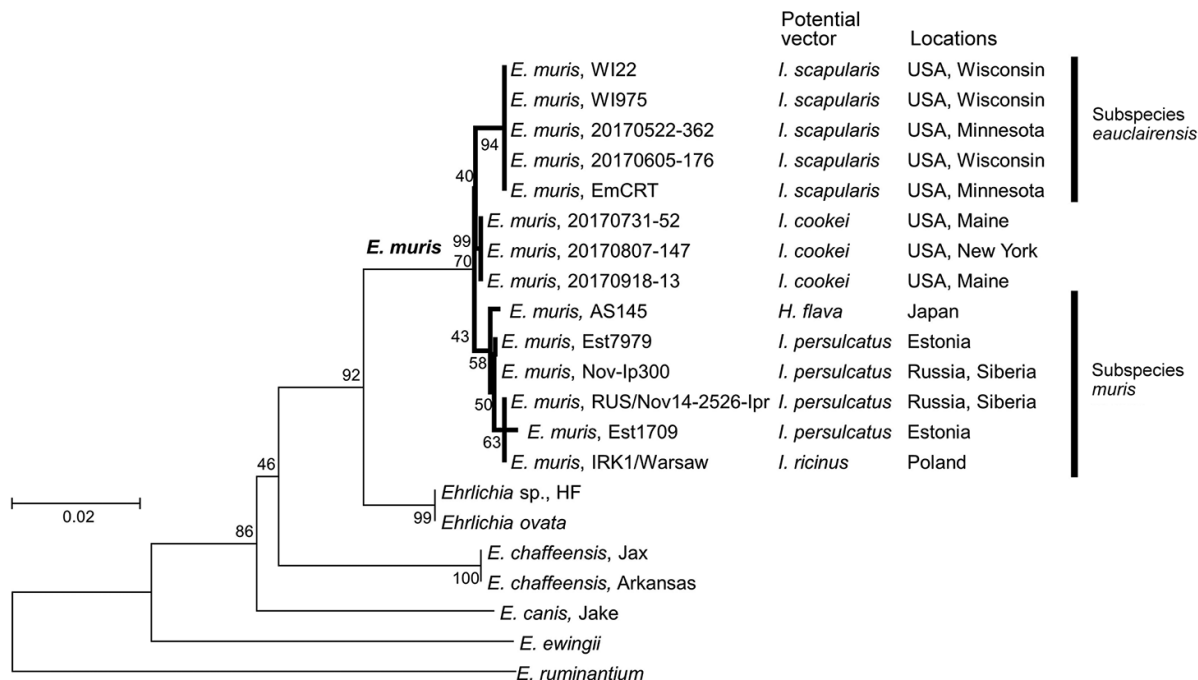
program (<https://www.tickreport.com>) at the University of Massachusetts (Amherst, MA, USA). We confirmed EMLA positivity of the samples by amplifying and sequencing the EMLA citrate synthase (*gltA*) and heat shock protein (*groEL*) genes (3). We confirmed the species of EMLA-positive ticks by amplifying and sequencing a partial tick 16S rRNA gene (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/6/17-1755-Techapp1.pdf>). We received 8,760 ticks from 45 states: 243 *Amblyomma americanum*, 2 *A. maculatum*, 7 *Amblyomma* spp., 6 *Dermacentor andersoni*, 3 *D. occidentalis*, 271 *D. variabilis*, 45 *Dermacentor* spp., 14 *I. angustus*, 22 *I. cookei*, 215 *I. pacificus*, 5 *I. ricinus*, 7,800 *I. scapularis*, 19 *I. spinipalpis*, 47 *Ixodes* spp., and 7 *Rhipicephalus sanguineus*; 54 ticks were unidentifiable.

We found DNA specific for EMLA in only 2 species of *Ixodes* tick: *I. scapularis* and *I. cookei*. The overall prevalence of EMLA was very low. Only 5 (0.057%) ticks were positive for *E. muris*-specific DNA. Although we tested 7,800 *I. scapularis* ticks from 33 states in the northeastern, midwestern, and southeastern regions, we found only 2 (2/7,800, 0.026%) EMLA-positive *I. scapularis* ticks (1 from Laporte, MN, and 1 from Eleva, WI). These 2 ticks were co-infected with *B. burgdorferi* s. l. and *B. microti*. However, no DNA from *B. miyamotoi*, *B. mayonii*, or *A. phagocytophilum* was detected in these 2 ticks.

The prevalence of EMLA in *I. cookei* ticks was much higher than that in *I. scapularis* ticks. Of the 22 *I. cookei* ticks tested, 3 (3/22, 13.64%) were positive for EMLA (from Holden, ME; Littleton, ME; and Salamanca, NY). Co-infections were not detected in these 3 ticks.

To determine the identity of these EMLA isolates, we examined *gltA* and *groEL* gene sequences of isolates from the 2 *I. scapularis* ticks and found them to be identical. Phylogenetic analysis showed that they clustered with *E. muris* subsp. *eaucloirensis*. The *gltA* and *groEL* gene sequences from the 3 *I. cookei* ticks were also identical but formed a new clade between *E. muris* subsp. *eaucloirensis* and subsp. *muris* (Figure).

The detection of *E. muris* in *I. scapularis* ticks from the upper midwestern United States corroborates previously reported findings (2,3,6). The detection of a distinct clade of *E. muris* in *I. cookei* ticks from the northeastern United States represents a potential risk to humans or a different enzootic cycle of *E. muris* in the Northeast. As primary vectors of Powassan virus (lineage 1), *I. cookei* ticks are widely distributed in eastern North America and are the second most common species of *Ixodes* ticks found on persons in Maine, USA (9). Further study is warranted with regard to the vector competence of *I. cookei* ticks for transmitting *E. muris*, the natural enzootic cycle of *E. muris*, and the transmission potential of the pathogen to humans in this region. Meanwhile, human ehrlichiosis should be considered as a possible diagnosis for persons who have been



**Figure.** Phylogenetic tree of *Ehrlichia* citrate synthase (*glfA*) and heat shock protein (*groEL*) genes constructed by the maximum-likelihood method of MEGA6 software (<http://www.megasoftware.net>). The total length of 2 concatenated genes is 1,045 bp. Hasegawa-Kishino-Yano with invariable sites was selected as the best model based on Bayesian information criterion scores. Numbers on the branches represent bootstrap support with 500 bootstrap replicates. Scale bar indicates nucleotide substitutions per site.

exposed to *I. scapularis* and *I. cookei* ticks in the upper midwestern and northeastern United States, respectively.

### Acknowledgment

We thank Timothy Daly and Fumiko Ribbe for sample preparation and DNA extractions.

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Dr. Xu is an extension associate professor in the Laboratory of Medical Zoology, Department of Microbiology, University of Massachusetts–Amherst. His research interests include tick and tickborne diseases.

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## 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	<i>Ixodes scapularis</i> identification	Forward	TGCGTTTTCTTTGAGCAAATGCACGAG	60	This paper
		Reverse	GTACGGGATTTCCACAAACGGTATCCA		
ITS	<i>Ixodes pacificus</i> identification	Forward	FAM-TGCGCTTAACCAGTCTCTCTCTACGA-BHQ	60	This paper
		Reverse	CTCGGAGCAAGTACGGAGGTAG		
ITS	<i>Amblyomma americanum</i> identification	Forward	TTCCACAAAACGGTCGCCATC	60	This paper
		Reverse	Cy5-CTGAGCCAAGTCCTTCTACCCGGTTTG-BHQ		
ITS	<i>Dermacentor variabilis</i> identification	Forward	CGACCCGCCGAGGAAGG	60	(1)
		Reverse	CGTTTCTCGCAGCAGTTCGG		
gltA	<i>E. muris</i> PCR and confirmation	Forward	FAM-CCCCTGGCCCCGCTACGTGT-BHQ	50	(2)
		Reverse	CTGAAGATTCTTTGCGAGGAGCGG		
groEL	<i>E. muris</i> PCR and confirmation	Forward	GCGTCAGCTCGGCCAAC	50	(2)
		Reverse	FAM-AGAAGGGCGTGCCCCGAAAGCGG-BHQ		
P13	EMLA detection	Forward	TACAGATTTCTCAAGAAATATACA (outer)	60	This paper
		Reverse	ATGATGATACTGCCAACAATAAGAG		
gIpQ	<i>Borrelia miyamotoi</i> detection	Forward	Cy5-ATATTGATAAAAGAGTCAGTGTTGATCCGTATGAGTTA	60	(3)
		Reverse	GGTT-BHQ		
ospA	<i>Borrelia burgdorferi</i> <i>Sensu Lato</i> detection	Forward	GACATAGTTCTAACAAAGGACAATATTCC	60	This paper
		Reverse	TCCGTTTTCTCTAGCTCGATTGG		
Tubulin	<i>Babesia</i> detection	Forward	HEX-TGCACGACCCAGAAATTGACACAACCACAA-BHQ	60	(4)
		Reverse	ATAGGTCTAATATTAGCCTTAATAGCAT		
MSP-2	<i>Anaplasma</i> detection	Forward	AGATCGTACTTGCCGTCTT	60	(4)
		Reverse	FAM-aagc+Aaa+Atgtt+Agc+Agccttga-BHQ (LNA)		
MSP-2	<i>Anaplasma</i> detection	Forward	GATTTGGAACCTGGCACCATG	60	(4)
		Reverse	AATGACCCTTAGCCCAATTATTTCC		
MSP-2	<i>Anaplasma</i> detection	Forward	FAM-ATCTGGCCCATACGGTGAATTGTTTCGC-BHQ	60	(4)
		Reverse	ATGGAAGGTAGTGTTGGTTATGGTATT		
MSP-2	<i>Anaplasma</i> detection	Forward	TTGGTCTTGAAGCGCTCGTA	60	(4)
		Reverse	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.*
<i>Ixodes scapularis</i>	16s rRNA	20170522–362	MG242324
<i>Ixodes scapularis</i>	16s rRNA	20170605–176	MG242325
<i>Ixodes cookei</i>	16s rRNA	20170731–52	MG242326
<i>Ixodes cookei</i>	16s rRNA	20170807–147	MG242327
<i>Ixodes cookei</i>	16s rRNA	20170918–13	MG242328
<i>E. muris</i>	gltA	20170522–362	MG242314
<i>E. muris</i>	gltA	20170605–176	MG242315
<i>E. muris</i>	gltA	20170731–52	MG242316
<i>E. muris</i>	gltA	20170807–147	MG242317
<i>E. muris</i>	gltA	20170918–13	MG242318
<i>E. muris</i>	groEL	20170522–362	MG242319
<i>E. muris</i>	groEL	20170605–176	MG242320
<i>E. muris</i>	groEL	20170731–52	MG242321
<i>E. muris</i>	groEL	20170807–147	MG242322
<i>E. muris</i>	groEL	20170918–13	MG242323
<i>E. muris</i>	gltA	WI22	HQ660494
<i>E. muris</i>	groEL	WI22	HQ660492
<i>E. muris</i>	gltA	WI975	HQ660497
<i>E. muris</i>	groEL	WI975	HQ660493
<i>E. muris</i>	gltA	EmCRT	LANU00000000
<i>E. muris</i>	groEL	EmCRT	LANU00000000
<i>E. muris</i>	gltA	AS145	CP006917
<i>E. muris</i>	groEL	AS145	CP006917
<i>E. muris</i>	groEL	Est7979	KU535864
<i>E. muris</i>	groEL	Nov-lp300	GU358687
<i>E. muris</i>	groEL	RUS/Nov14–2526-lpr	KX980049
<i>E. muris</i>	groEL	Est1709	KU535861
<i>E. muris</i>	groEL	IRK1/Warsaw	KF312362
<i>Ehrlichia spp.</i>	gltA	HF	NZ_CP007474
<i>Ehrlichia spp.</i>	groEL	HF	NZ_CP007474
<i>E. ovata</i>	groEL	Shizuoka	DQ672553
<i>E. chaffeensis</i>	gltA	Jax	CP007475
<i>E. chaffeensis</i>	groEL	Jax	CP007475
<i>E. chaffeensis</i>	gltA	Arkansas	CP000236
<i>E. chaffeensis</i>	groEL	Arkansas	CP000236
<i>E. canis</i>	gltA	Jake	NC_007354
<i>E. canis</i>	groEL	Jake	NC_007354
<i>E. ewingii</i>	gltA	Panola Mountain	DQ365879
<i>E. ewingii</i>	groEL		AF195273
<i>E. ruminantium</i>	gltA	Welgevonden	NC_005295
<i>E. ruminantium</i>	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	<i>Ixodes scapularis</i>	Adult	Human	Laporte, MN 56461
20170605–176	<i>Ixodes scapularis</i>	Adult	Human	Eleva, WI 54738
20170731–52	<i>Ixodes cookei</i>	Adult	Human	Holden, ME 04429
20170807–147	<i>Ixodes cookei</i>	Adult	Human	Salamanca, NY 14779
20170918–13	<i>Ixodes cookei</i>	Adult	Human	Littleton, ME 04730

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