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

Guang Xu, Patrick Pearson, Stephen M. Rich

Author affiliation: University of Massachusetts–Amherst, Amherst, Massachusetts, USA

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. *eauclairensis* (*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. *eauclairensis*. The *gltA* and *groEL* gene sequences from the 3 *I. cookei* ticks were also identical but formed a new clade between *E. muris* subsp. *eauclairensis* 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

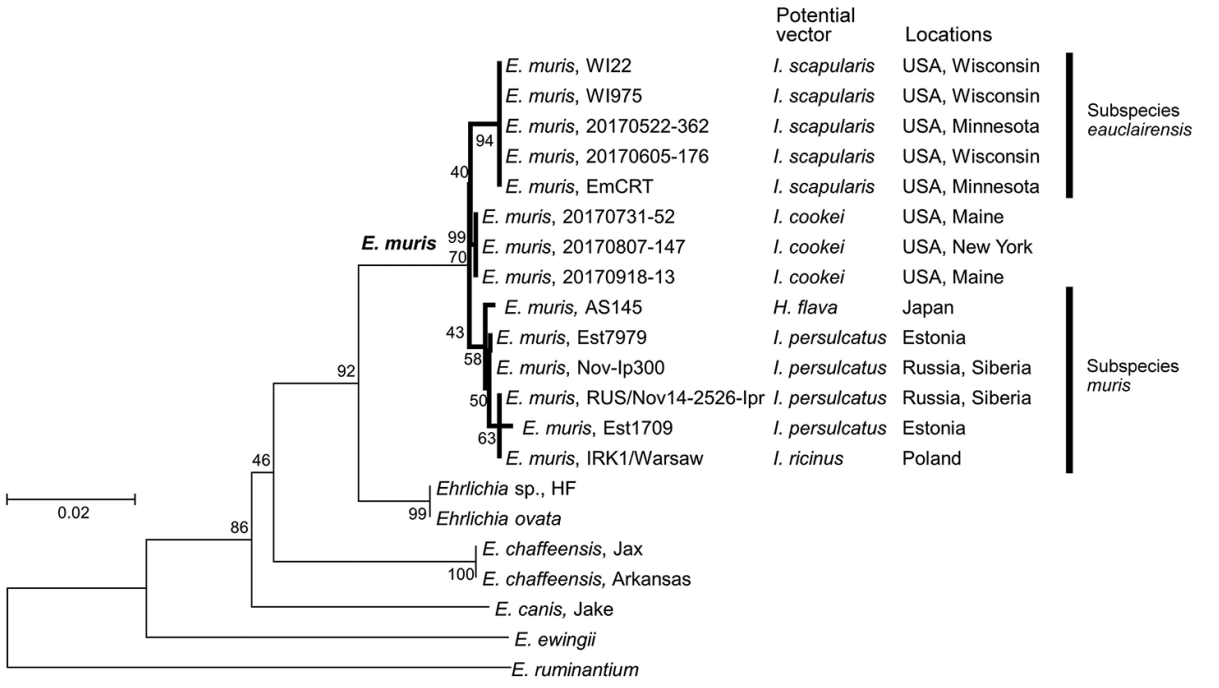

Figure. Phylogenetic tree of *Ehrlichia* citrate synthase (*gltA*) and heat shock protein (*groEL*) genes constructed by the maximum-likelihood method of MEGA6 software (http://www.megasoftware.net). The total length of 2 concatenated genes is 1,045 bp. Hasegawa-Kishino-Yano with invariable sites was 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.

About the Author

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.

References

1. Wen B, Rikihisa Y, Mott J, Fuerst PA, Kawahara M, Suto C. *Ehrlichia muris* sp. nov., identified on the basis of 16S rRNA base sequences and serological, morphological, and biological characteristics. Int J Syst Bacteriol. 1995;45:250–4. http://dx.doi.org/10.1099/00207713-45-2-250
2. Pritt BS, Sloan LM, Johnson DKH, Munderloh UG, Paskewitz SM, McElroy KM, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. N Engl J Med. 2011;365:422–9. http://dx.doi.org/10.1056/NEJMoa1010493
3. Telford Iii SR, Goethert HK, Cunningham JA. Prevalence of *Ehrlichia muris* in Wisconsin deer ticks collected during the mid 1990s. Open Microbiol J. 2011;5:18–20. http://dx.doi.org/10.2174/1874285801105010018
4. Johnson DKH, Schiffman EK, Davis JP, Neitzel DF, Sloan LM, Nicholson WL, et al. Human infection with *Ehrlichia muris*–like pathogen, United States, 2007–2013. Emerg Infect Dis. 2015;21:1794–9. http://dx.doi.org/10.3201/eid2110.150143
5. Pritt BS, Allerdice MEJ, Sloan LM, Paddock CD, Munderloh UG, Rikihisa Y, et al. Proposal to reclassify *Ehrlichia muris* as *Ehrlichia muris* subsp. *muris* subsp. nov. and description of *Ehrlichia muris* subsp. *eauclairensis* subsp. nov., a newly recognized tick-borne pathogen of humans. Int J Syst Evol Microbiol. 2017;67:2121–6. http://dx.doi.org/10.1099/ijsem.0.001896
6. Stromdahl E, Hamer S, Jenkins S, Sloan L, Williamson P, Foster E, et al. Comparison of phenology and pathogen prevalence, including infection with the *Ehrlichia muris*–like (EML) agent, of *Ixodes scapularis* removed from soldiers in the midwestern and the northeastern United States over a 15 year period (1997–2012). Parasit Vectors. 2014;7:553. http://dx.doi.org/10.1186/s13071-014-0553-z
7. Wormser GP, Pritt B. Update and commentary on four emerging tick-borne infections*: Ehrlichia muris*-like agent, *Borrelia miyamotoi*, deer tick virus, Heartland virus, and whether ticks play a role in transmission of *Bartonella henselae*. Infect Dis Clin North Am. 2015;29:371–81. http://dx.doi.org/10.1016/j.idc.2015.02.009
8. Allerdice MEJ, Pritt BS, Sloan LM, Paddock CD, Karpathy SE. A real-time PCR assay for detection of the *Ehrlichia muris*–like agent, a newly recognized pathogen of humans in the upper Midwestern United States. Ticks Tick Borne Dis. 2016;7:146–9. http://dx.doi.org/10.1016/j.ttbdis.2015.10.004
9. Rand PW, Lacombe EH, Dearborn R, Cahill B, Elias S, Lubelczyk CB, et al. Passive surveillance in Maine, an area emergent for tick-borne diseases. J Med Entomol. 2007;44:1118–29. http://dx.doi.org/10.1093/jmedent/44.6.1118

Address for correspondence: Guang Xu, University of Massachusetts–Amherst, Microbiology Fernald Hall, Rm B1, 270 Stockbridge Rd, Amherst, MA 01003, USA; email: gxu@umass.edu

Article DOI: https://doi.org/10.3201/eid2406.171755

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

References

1. Shone SM, Dillon HJ, Hom SS, Delgado N. A novel real-time PCR assay for the speciation of medically important ticks. Vector Borne Zoonotic Dis. 2006;6:152–60. PubMed http://dx.doi.org/10.1089/vbz.2006.6.152

2. Telford Iii SR, Goethert HK, Cunningham JA. Prevalence of *Ehrlichia muris* in Wisconsin Deer Ticks Collected During the Mid 1990s. Open Microbiol J. 2011;5:18–20. PubMed http://dx.doi.org/10.2174/1874285801105010018

3. Krause PJ, Schwab J, Narasimhan S, Brancato J, Xu G, Rich SM. Hard Tick Relapsing Fever Caused by Borrelia miyamotoi in a Child. Pediatr Infect Dis J. 2016;35:1352–4. PubMed http://dx.doi.org/10.1097/INF.0000000000001330

4. Xu G, Mather TN, Hollingsworth CS, Rich SM. Passive Surveillance of *Ixodes scapularis* (Say), Their Biting Activity, and Associated Pathogens in Massachusetts. Vector Borne Zoonotic Dis. 2016;16:520–7. PubMed http://dx.doi.org/10.1089/vbz.2015.1912