

Ehrlichia and lone star ticks: Pathogen prevalence in counties of varying ehrlichiosis incidence

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Background

Ehrlichiosis is a nationally reportable vector-borne disease (VBD). Its incidence has been increasing over the past several decades. Three species of *Ehrlichia* bacteria are known to cause human disease:

- *Ehrlichia chaffeensis* – first described 1987; most commonly reported human disease agent.
- *Ehrlichia ewingii* – first described 1999.
- *Ehrlichia-muris Like Agent (E. muris euclairensis)* – first described 2011; vectored by the black-legged tick (*Ixodes scapularis*).¹ Not found in Tennessee.

- Signs & Symptoms:
- 1-2 weeks after tick bite agent.
 - fever, headaches, fatigue, muscle aches, and gastrointestinal manifestations
 - *E. chaffeensis* ehrlichiosis case fatality rate ~3%; no deaths from *E. ewingii* and EML agent infections have been reported

Novel Panola Mountain Ehrlichia (PME) species – first described in 2006 from an infected goat in Georgia, USA; potential human pathogenicity.²

Transmission



E. chaffeensis and *E. ewingii* are transmitted by the lone star tick, *Amblyomma americanum*, the most common human biting tick in the southeastern U.S.

White-tailed deer are a main host for all three lone star tick life stages and are thought to be an important natural reservoir of *Ehrlichia*.

While all life stages are known to feed on humans, only adult and nymphal *A. americanum* ticks are known *E. chaffeensis* vectors. Absence of transovarial transmission makes *Ehrlichia* transmission less focal and more widespread.¹

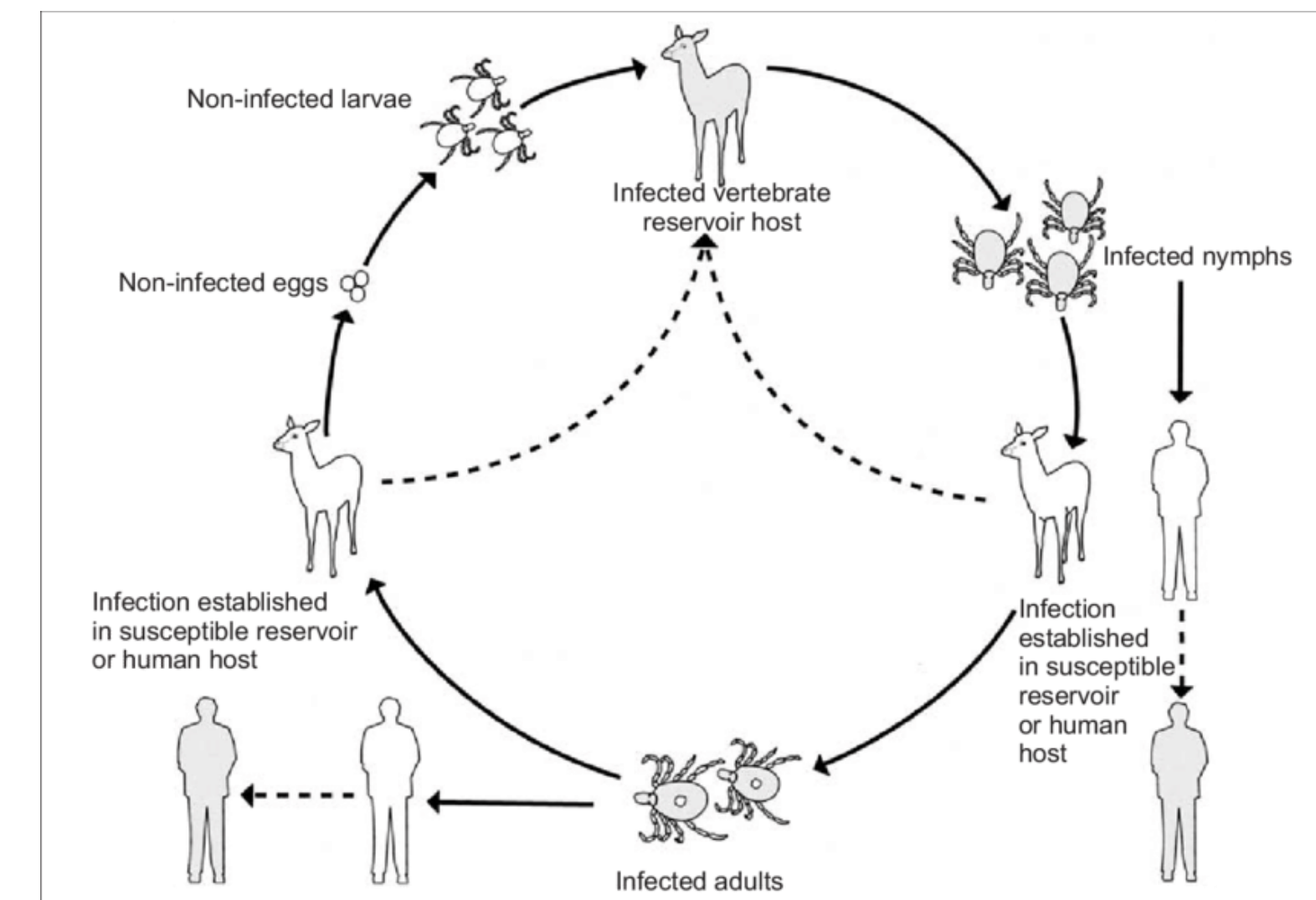


Figure 1. *Ehrlichia chaffeensis* transmission cycle.³

Tennessee: 2nd highest VBD case burden
 111 cases in 2021
 ~50% of confirmed and probable cases hospitalized in 2021⁴

Ehrlichiosis Incidence Rate by County, 2011-2021

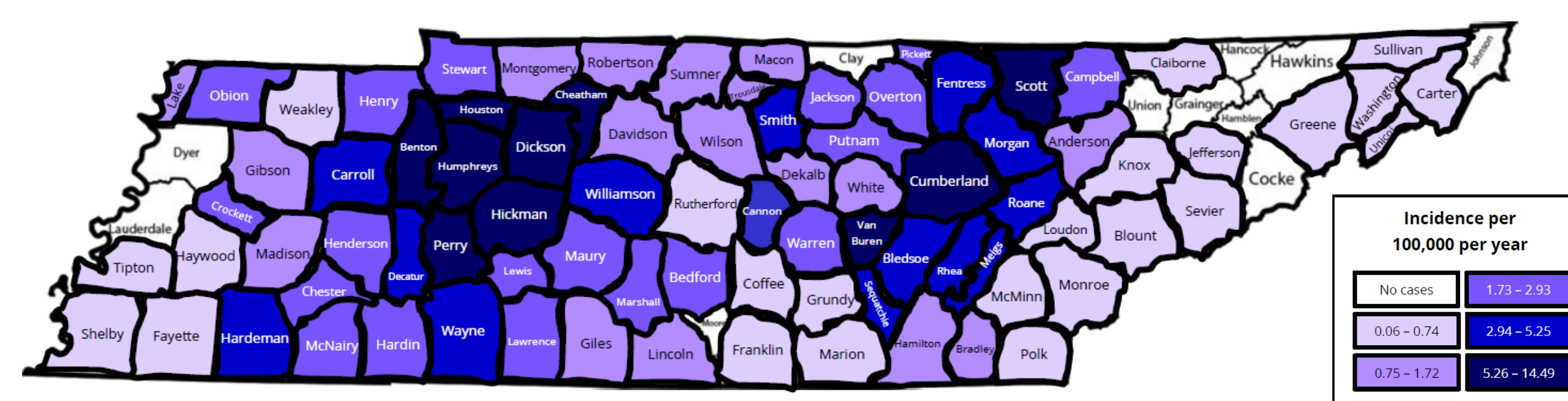


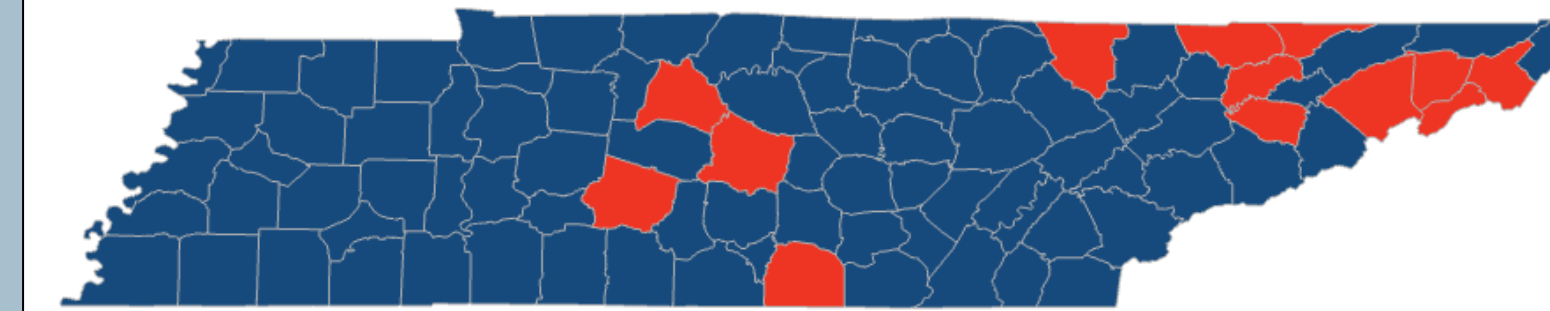
Figure 2. Average annual reported ehrlichiosis incidence varies widely across the state; however, tick-borne diseases are generally underrecognized and cases are reported by a patient's county of residence. The movement and interaction of ticks, animal hosts, bacteria, and humans make it challenging to estimate disease risk from case surveillance alone.

How can active tick and pathogen surveillance supplement disease data to enhance our understanding of disease burden and risk?

Methods

Field

- Between June and August 2021
- 13 counties; 2 sites per county
- Each site sampled 2-3 times

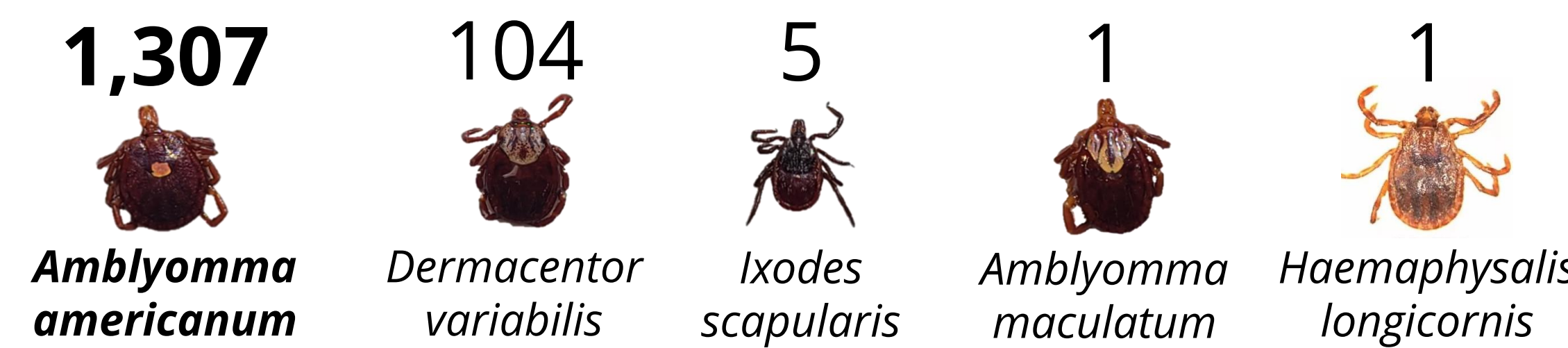


Active surveillance – field collections
 1m² cloth drag
 750m transects – split between forest and trail environments
 Ticks stored alive or in ethanol
 Samples stored at -80°C at lab



Figure 3. Examples of tick dragging and collection. Photos courtesy of Josefina Ramos Frias, Public Health Department in Hidalgo State, México.

Tick Collections



All lone star ticks were collected from the four Middle Tennessee counties surveyed

Table 1. *A. americanum* collected

County	Site	Adult female	Adult male	Nymphs	Total
Davidson	Beaman Park	2	2	496	743
	Peeler Park	27	26	190	
Franklin	State Forest	7	8	25	159
	Tims Ford State Park	11	11	97	
Maury	Chickasaw Trace Park	7	2	15	125
	Yanhli Park	7	2	101	
Rutherford	Barfield Crescent Park	8	5	58	280
	Long Hunter State Park	26	23	160	

Laboratory

Processing

- Morphological identification
- Pooled by species, sex, life stage
- ≤ 3 adults, ≤ 5 nymphs
- Homogenization
- Cuticle disrupted with scalpel
- Bead beat with TissueLyser II

Nucleic acid extraction

- QIAGEN RNeasy kit automated with QIACube HT



Molecular detection

- Real-time PCR



Real-time PCR Assay Menu

	<i>A. americanum</i>	<i>D. variabilis</i>	<i>I. scapularis</i>
<i>Rickettsia</i> spp.	✓	✓	
<i>R. rickettsii</i>	✓	✓	
<i>R. parkeri</i> *	✓	✓	
<i>E. chaffeensis</i>	✓		
<i>E. ewingii</i>	✓		
PME	✓		
Heartland virus	✓		
Bourbon virus	✓		
<i>Borrelia</i> spp.			✓
<i>B. burgdorferi</i>			✓
<i>A. phagocytophilum</i>			✓

*Single *A. maculatum* sample tested for *R. parkeri*.

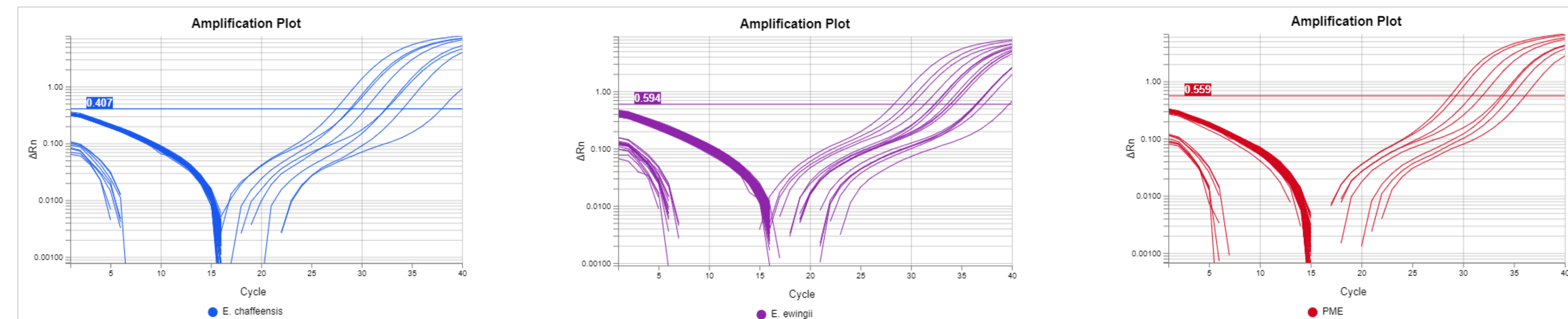
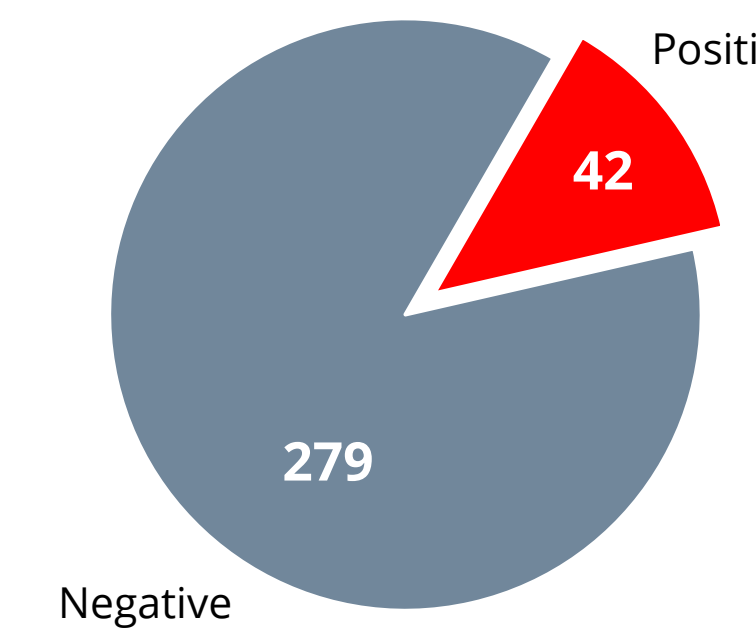


Figure 3. Examples of amplification curves from real-time PCR plate results for each *Ehrlichia* assay.

Ehrlichia Testing Results

A. americanum pools
 n = 321



13% of lone star tick pools were positive for an *Ehrlichia* species

Total pools positive:

- 13 *E. chaffeensis*
- 25 *E. ewingii*
- 14 Panola Mountain *Ehrlichia* sp.

8 pools positive for > 1 species

Discussion & Analysis

Table 2. Ehrlichiosis incidence compared to *A. americanum* *Ehrlichia*-infection rates in four Middle TN counties

County	Ehrlichiosis Incidence Rate [†]	Infection Prevalence [‡]			
		<i>E. chaffeensis</i> + <i>E. ewingii</i>	<i>E. chaffeensis</i>	<i>E. ewingii</i>	PME
Maury	2.67	7.42 (3.45, 13.44)	4.36 (1.59, 9.17)	2.52 (0.63, 6.42)	0
Davidson	1.55	3.29 (2.13, 4.78)	0.68 (0.25, 1.46)	2.35 (1.54, 3.87)	0.96 (0.41, 1.85)
Franklin	0.47	0.63 (0.04, 2.75)	0.63 (0.04, 2.75)	0	0
Rutherford	0.35	1.38 (0.43, 3.17)	0.72 (0.12, 2.21)	1.46 (0.46, 3.36)	2.62 (1.13, 5.01)

[†]Average annual ehrlichiosis IR per 100,000 population from 2011-2021; Tick IP point estimates calculated as the Minimum Infection Rate per 100 ticks tested with 95% confidence interval limits.

- Higher prevalence of *E. ewingii* than *E. chaffeensis* in Davidson and Rutherford counties
- PME was the most prevalent *Ehrlichia* spp. in Rutherford county
- The combined infection prevalence in ticks of *E. chaffeensis* and *E. ewingii* matches the relative disease incidence by county
 - * 16 cases of *E. ewingii* ehrlichiosis reported in TN since 2010; in that period 48% of *E. chaffeensis* cases were classified as "probable"⁵
 - * *E. chaffeensis* and *E. ewingii* infections are clinically and serologically indistinguishable; *E. ewingii* ehrlichiosis surveillance is based on molecular diagnostics⁶
- Our results suggest there are more *E. ewingii* cases than have been reported; a proportion of reported *E. chaffeensis* cases are likely due to *E. ewingii* infection

Providers should consider testing for *E. ewingii* as well as *E. chaffeensis* when ehrlichiosis is suspected.

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