



OPEN County-level surveillance for the American dog tick (*Dermacentor variabilis*) and *Rickettsia* species in Kentucky

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Spotted fever group (SFG) rickettsiosis includes a group of illnesses with similar symptoms caused by *Rickettsia* species bacteria. While *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever (RMSF) is a well-known pathogen, other SFG *Rickettsia* spp. can infect ticks and occasionally contribute to human disease. We conducted county-level surveillance of *Dermacentor variabilis* ticks in Kentucky to characterize the distribution and diversity of SFG *Rickettsia*. Ticks were collected from the environment from 2019 to 2024 by dragging a white cloth and through our Kentucky Tick Submission Program. In total, 2,023 *D. variabilis* ticks were collected from 114 counties. *Rickettsia* species were detected in 1% of *Dermacentor variabilis* ticks, including *R. montanensis* (0.47%), *R. amblyommatis* (0.13%), and *R. parkeri* (0.13%). These findings indicate that, while SFG *Rickettsia* are present at low levels in *D. variabilis* ticks in Kentucky, continued surveillance in counties with reported SFG rickettsiosis cases is essential to improve clinical recognition and guide public health efforts to monitor and prevent SFG rickettsiosis.

Keywords *Rickettsia*, *Dermacentor variabilis*, Tick surveillance, Spotted fever group

Spotted fever group (SFG) rickettsioses are a group of illnesses caused by infection with *Rickettsia* spp. *Rickettsia* is a genus of obligate intracellular bacteria that depend on the ability to invade, grow, and replicate inside living eukaryotic host cells for survival. As obligate intracellular bacteria, these pathogens cannot survive or spread independently in the environment; therefore, they rely on vectors such as fleas, lice, mites, and ticks to transport them from one host to another^{1–4}. Although most *Rickettsia* spp. are non-pathogenic to vertebrates, some can cause significant diseases such as boutonneuse fever, rickettsialpox, Rocky Mountain spotted fever, and *R. parkeri* rickettsiosis. Rickettsial diseases range from mild illnesses to severe, life-threatening infections⁵.

Dermacentor variabilis, the American dog tick, is a primary vector of several spotted fever group (SFG) *Rickettsia* species in North America, including *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF). Other ticks, such as *D. andersoni* (Rocky Mountain wood tick), *Rhipicephalus sanguineus* (brown dog tick), and occasionally *Amblyomma americanum* (lone star tick), have also been implicated in transmission of SFG rickettsia^{6–8}. *Dermacentor variabilis*, found throughout the eastern and central United States and southern Canada, is the primary vector of *R. rickettsii* in North America⁹. However, the presence of *R. rickettsii* in this tick has been historically low, even in areas where the disease is endemic¹⁰. Despite Tennessee's high rate of RMSF, *R. rickettsii* was not detected in *D. variabilis*, *A. americanum*, or *Ixodes scapularis* ticks¹¹. Besides *R. rickettsii*, other rickettsial species, such as *R. parkeri*, are linked to human zoonoses¹². Clinical serological tests cannot differentiate between *Rickettsia* infections in humans, making it difficult to determine whether RMSF or another rickettsial agent is present. Identifying *Rickettsia* species requires targeted molecular tests that are often unavailable in most clinics due to resource limitations, lack of standardization, and limited specialized training¹³. The limited data on detecting *R. rickettsii* in ticks, combined with the clinical difficulty in distinguishing *Rickettsia* infections, raises the question of whether other rickettsiae may be responsible for past cases classified as SFG rickettsiosis.

In addition to *R. rickettsii*, other SFG *Rickettsia* species can cause illness in humans, although their clinical presentations and public health significance differ. *Rickettsia parkeri*, transmitted primarily by *Amblyomma*

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maculatum and occasionally detected in other ticks, causes an illness similar to RMSF but typically milder, often presenting with fever, headache, rash, and eschar formation at the bite site.^{14–16} *Rickettsia montanensis* has been detected in *Dermacentor* ticks throughout the United States, but its pathogenicity in humans is not well-established, and infections may be asymptomatic or underreported.¹⁷ *Rickettsia amblyommatis*, commonly found in *Amblyomma americanum*, is widespread in eastern North America; while historically considered non-pathogenic, recent evidence suggests it may occasionally cause mild febrile illness with rash^{18,19}. The presence of multiple SFG *Rickettsia* species in Kentucky underscores the need for species-specific surveillance to accurately assess human health risk.

Uncertainty in diagnoses is especially concerning in regions like Kentucky, where high rates of human SFG rickettsiosis have been reported for years²⁰. However, a lack of statewide and long-term data on tick-borne pathogens limits understanding of *Rickettsia* species in Kentucky. Collecting data through tick and tick-borne pathogen surveillance is crucial for monitoring the spread of vectors and pathogens over time and provides valuable information for managing tick-borne diseases. This study aimed to conduct a multi-year, statewide survey for *D. variabilis* infected with *Rickettsia* spp. in Kentucky. Knowing the distribution of *Rickettsia* spp. is vital for identifying high-risk areas and can help guide efforts to reduce exposure.

Methods

Tick collection

Ticks were collected between January 2019 and December 2024 using two methods: active drag sampling and passive submissions from the public. Drag sampling took place across various counties in Kentucky. At each site, a 1 m x 1 m light-colored drag cloth was pulled along the ground while walking, and the cloth was checked every 10 m for ticks. Sampling locations included hiking trails, state wildlife management areas, state and city parks, and other public lands. Data recorded for each drag sample included the date, location, and habitat type (forest, brush, grassland, or combinations). Tick submissions were received from veterinary offices, public health departments, county extension offices, and Kentucky residents. Each submission required an information form with the submitter's contact details, collection date, county, and, if applicable, host type, and habitat type. Only submissions collected within Kentucky with complete date and county-level location data were processed. Samples collected outside Kentucky or lacking date and/or county-level location information were not included in our analysis. Both ticks collected through active and passive methods were preserved in 70% ethanol and stored at –20 °C until morphological identification at the University of Kentucky using standard keys²¹.

DNA extraction and pathogen detection

For DNA extraction, individual ticks were bead-beaten with 2.0 mm Zirconia beads from BioSpec Products in a TissueMiner (MP Biomedicals) at a speed of 0.6 m/s for three consecutive cycles of 40 s each to ensure proper lysis. DNA extraction was performed using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's instructions. To determine the presence or absence of pathogens, adults and nymphs were separated for testing, and adults were tested in pools of up to five. The presence of *Rickettsia* spp. in *D. variabilis* was detected using primers targeting the *ompB* gene (ATAACCCAAGACTCAAACCTTTGGTA) (GCAGTGTTACCGGGATTGCT)²². Positive DNA controls for *R. rickettsii* were obtained from the Centers for Disease Control and Prevention, Rickettsial Isolate Repository Collection. Negative controls used included non-target DNA and no DNA (nuclease-free water). Ten-microliter reactions contained 5 µL SYBR Green Master Mix (Bio-Rad), 2 µL extracted DNA, primers at a final concentration of 0.25 µM each, with nuclease-free water added to 10 µL. Reactions were run at 95 °C for 3 min, followed by 35 cycles of 95 °C for 15 s and 61 °C for 30 s. Positive samples identified by SYBR Green screening PCR were confirmed using a conventional PCR targeting the *ompA* gene (ATGGCGAATATTTCTCCAAA) (GTTCCGTTAATGGCAGCATCT)^{23,24}.

Positive amplicons from conventional PCR were purified using the QIAquick PCR Purification Kit (Qiagen) and Sanger sequenced by Azenta Life Sciences (formerly Genewiz). Sequences were analyzed using BLASTn against the NCBI nonredundant nucleotide (nt) database, and all samples demonstrated >99% nucleotide identity to *Rickettsia* reference sequences, supporting assignment to the corresponding *Rickettsia* species. Minimum infection rate (MIR) was determined by dividing the number of positive pools by the number of total ticks tested and multiplying by 100.

Results

Tick collection

Between January 2019 and December 2024, we collected and received 2,023 *D. variabilis* ticks from 114 counties (Fig. 1). Of these, 857 ticks were collected from 81 counties through active surveillance. All ticks collected using active surveillance were adults. By contrast, 1,158 adult (99%), eight nymphal (0.7%), and one larval (0.09%) *D. variabilis* ticks were received from 99 counties through passive surveillance. Ticks were detected in 66 counties using both surveillance methods (Fig. 1). *Dermacentor variabilis* were collected from cat, cow, dog, horse, and human hosts. Submissions that failed to include the host type were classified as "Unknown." This species was active from March to October, with activity peaking each year in May and June (Fig. 2).

Pathogen detection

One thousand four hundred and eighty-four ticks were suitable for testing and either tested individually or pooled by collection date and life stage. *Rickettsia* species was detected in 15 (MIR = 1.01%) *D. variabilis* samples from 14 counties in Kentucky (Fig. 3). Seven positive samples were collected through active surveillance, and eight through passive surveillance. The MIR was 1.7% for pooled ticks and 0.6% for individual ticks. Sanger sequencing of positive amplicons confirmed the presence of *Rickettsia* spp., including seven *R. montanensis*

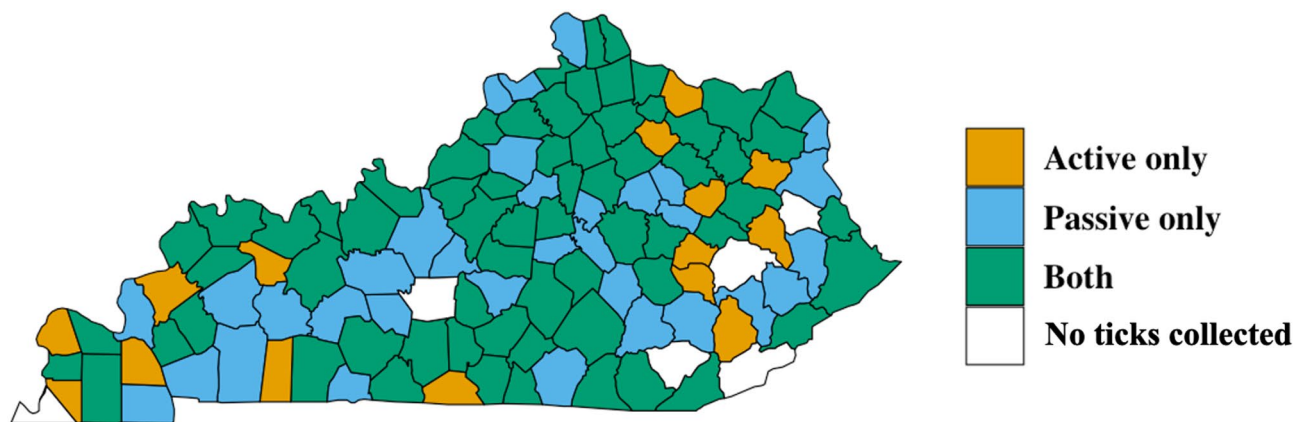


Fig. 1. Kentucky county status for *Dermacentor variabilis* at the end of the study period. Adult, nymphal, and larval *D. variabilis* ticks were collected between January 2019 and December 2024 using active surveillance and passive surveillance. Counties where ticks were collected through active surveillance (orange) and passive surveillance (blue) are shown. Counties where ticks were collected by both active and passive surveillance (green) are also highlighted. Counties with no ticks collected are shown in white.

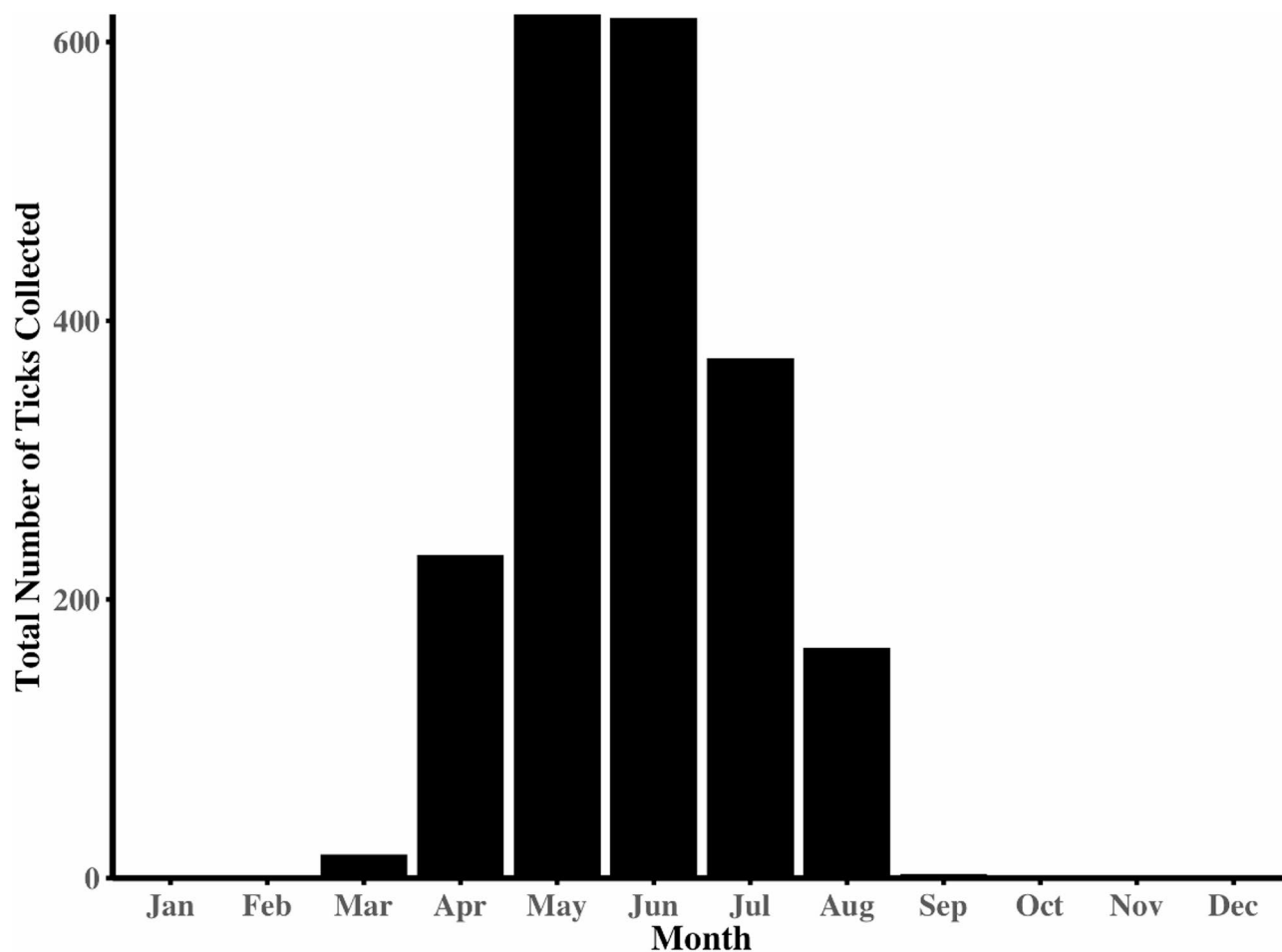


Fig. 2. Seasonality of *Dermacentor variabilis* collected from 2019 to 2024. Adult, nymphal, and larval *D. variabilis* ticks were collected statewide between January 2019 and December 2024 using active drag sampling and passive submissions from the public. Counts represent the total number of ticks collected per month across all years and sampling methods.

- Dermacentor variabilis* collected
- No ticks collected
- Ticks positive for *Rickettsia* spp.

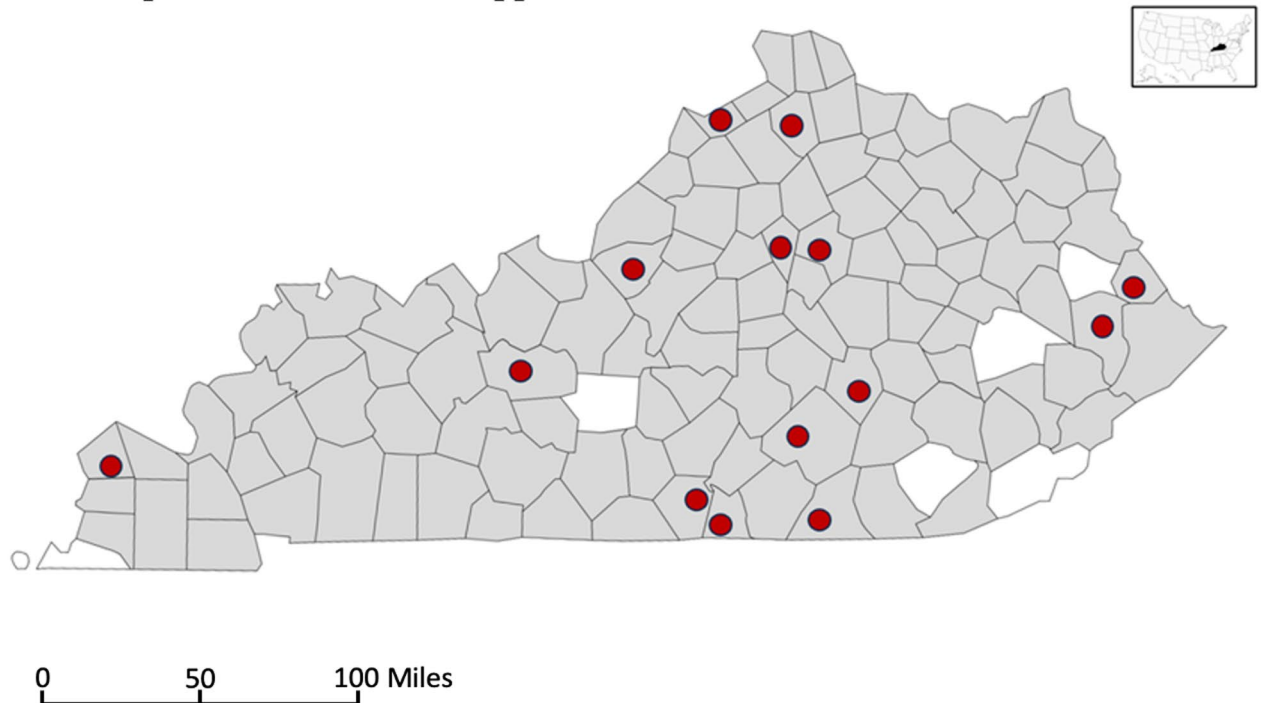


Fig. 3. County-level distribution of *Dermacentor variabilis* in Kentucky and detection of *Rickettsia* species. Adult and nymphal *D. variabilis* ticks were collected between January 2019 and December 2024 using active and passive sampling. Shading of counties indicates that *D. variabilis* were collected from that county, red dots symbolize counties where at least 1 tick or pool tested positive for *Rickettsia* species.

(0.47%), two *R. parkeri* (0.13%), and two *R. amblyommatis* (0.13%) among all ticks screened (GenBank accession numbers PX756887–PX756897). Four PCR-positive samples (0.27%) could not be recovered for sequencing and were therefore classified as *Rickettsia* spp. (Table 1); priming was unsuccessful for one sample and three samples could not be recovered.

Discussion

The primary goal of this study was to conduct a multi-year, statewide survey for *D. variabilis* and test them for *Rickettsia* spp. in Kentucky using active and passive surveillance methods. *Dermacentor variabilis* has been reported in Kentucky since at least the 1940s^{25–30}. The CDC's American dog tick surveillance program reported 21 counties with established populations. We found this species in approximately a third of all samples submitted through the Kentucky Tick Submission Program from various hosts, including common hosts like cats and dogs, livestock, and humans. Our data indicate that *D. variabilis* is widespread across Kentucky, with collections from 114 counties, and was frequently encountered by both humans and animals. This is supported by its common presence in other Kentucky tick collections: it was the most frequently collected species in previous surveys by Fritzen, C. M. et al.,³¹ and the second most collected species after *D. albipictus*²⁹. High populations are also observed in neighboring Tennessee and Virginia^{33,32,11}.

The tick seasonality observed in this study matches previous reports^{27,34}. *Dermacentor variabilis* collections took place during warm months, with peaks in May and June. This seasonal pattern aligns with periods of increased human and animal outdoor activity, which may elevate opportunities for tick encounters and submission through passive surveillance programs. The late spring and summer months seem to pose the highest risk of tick exposure and bites; therefore, increased awareness and prevention efforts should be exercised during this period. Human cases of SFG rickettsiosis are more common in the southern and western regions of Kentucky, with a 2023 incidence rate of 20.77 per million people³⁵. We detected *Rickettsia* spp. in 15 samples from active and passive surveillance. Subsequent species-level characterization of positive samples indicated the presence of *R. montanensis*, *R. parkeri*, and *R. amblyommatis*; *Rickettsia rickettsii* was not detected. Whether or not the disease occurred in the hosts remains unknown. In our study, the overall infection rate of *Rickettsia* spp. in *D. variabilis* was 1%, with higher rates in 2023 and 2024. The higher detection rate in 2023 and 2024 may

County	Collection date	Surveillance type	Collection method	No. ticks	Life stage(s)/Sex	<i>Rickettsia</i> detected
Grayson	19 Jun 2019	Passive	Found on animal	1	Adult female	<i>Rickettsia</i> spp.
Rockcastle	28 Apr 2022	Passive	Found on human	1	Adult female	<i>Rickettsia</i> spp.
Floyd	03 Jun 2022	Passive	Found on human	1	Adult female	<i>Rickettsia</i> spp.
Ballard	07 Jul 2023	Active	Dragging	2	Adult female, adult male	<i>R. amblyommatidis</i>
Clinton	03 Sep 2023	Active	Dragging	1	Adult male	<i>R. montanensis</i>
Pulaski	03 Sep 2023	Passive	Found on human	2	Adult female, adult male	<i>R. montanensis</i>
Martin	01 Aug 2023	Active	Dragging	1	Adult female	<i>R. montanensis</i>
Woodford	19 Aug 2023	Passive	Found on human	1	Adult male	<i>R. montanensis</i>
Carroll	29 Aug 2023	Passive	Found on human	5	Adult females (n=2), adult males (n=3)	<i>R. parkeri</i>
Bullitt	01 Jul 2024	Active	Dragging	3	Adult males (n=3)	<i>Rickettsia</i> spp.
Cumberland	01 Jul 2024	Active	Dragging	1	Adult female	<i>R. montanensis</i>
Fayette	28 Oct 2024	Passive	Found on human	1	Adult male	<i>R. parkeri</i>
Grant	06 May 2024	Passive	Found on dog	1	Adult female	<i>R. montanensis</i>
McCreary	13 Jun 2024	Active	Dragging	5	Adult females (n=3), adult males (n=2)	<i>R. amblyommatidis</i>
Pulaski	25 Jun 2024	Active	Dragging	1	Adult male	<i>R. montanensis</i>

Table 1. Detection of *Rickettsia* spp. in *Dermacentor variabilis* ticks collected in Kentucky, 2019–2024.

This table summarizes tick surveillance across multiple counties, showing collection date, method, life stage, sex and number of ticks, along with detected *Rickettsia* species.

reflect interannual variation in ecological conditions influencing tick-host interactions, pathogen transmission dynamics, or host-seeking behavior. Differences in sample composition and submission rates across years likely contributed to this pattern. Given the overall low prevalence of *Rickettsia* spp. in *D. variabilis*, stochastic effects may disproportionately influence annual infection estimates, underscoring the importance of long-term surveillance to distinguish true temporal trends from sampling variation. However, because *D. variabilis* is abundant and widely distributed, even a low infection rate may result in substantial exposure risk.

Rickettsia rickettsii was not detected in this study. Previous studies, despite smaller sample sizes, have similarly failed to detect this pathogen³¹ or have reported a very low infection rate³⁰. One proposed explanation for this is that *D. variabilis* is a relatively inefficient reservoir of *R. rickettsii*, as the bacterium may reduce tick fitness and lead to reduced survival or transovarial transmission³⁶. In addition, infection prevalence in questing ticks may be influenced by host community composition, with variation in reservoir competence among vertebrate hosts affecting pathogen amplification in tick populations.

It is also important to note that the conventional PCR and sequencing methods used in this study may have limited sensitivity for detecting coinfections within individual ticks. When multiple *Rickettsia* species are present in the same tick, one species may amplify preferentially, potentially masking the presence of others. Consequently, the actual prevalence of mixed infections may be underestimated, and the MIR reported here likely reflects only the dominant species detected in each tick or pool.

In this study, the MIR for pooled ticks (1.7%) was higher than that for individually screened ticks (0.6%). While pooled testing is efficient for large-scale surveillance, it may mask variability among individual ticks and underestimate low-prevalence infections. In contrast, screening ticks individually provides a more precise estimate of infection prevalence, though it requires greater resources. The relatively high incidence of reported SFG rickettsiosis in Kentucky contrasts with the low prevalence of *Rickettsia* spp. detected in *D. variabilis*³⁵. This discrepancy has been widely noted and is thought to reflect limitations in clinical diagnosis, as many cases are reported as RMSF without molecular confirmation of the causative agent. While *R. rickettsii* is traditionally associated with RMSF, it is important to note that other *Rickettsia* species, such as *R. montanensis* and *R. parkeri*, may also contribute to the high number of reported SFG rickettsiosis cases in Kentucky³⁷.

Susceptibility to infection may vary by tick life stage, with immature ticks potentially acquiring and maintaining rickettsiae more efficiently than adults. Vertical transmission and transstadial maintenance may amplify pathogen prevalence across life stages, such that adult infection rates may underestimate the true

ecological burden of rickettsiae³⁸. These dynamics underscore the importance of including juvenile ticks in surveillance programs to more accurately assess the potential for human and animal exposure. Furthermore, other tick species with higher pathogen prevalence or greater human-biting frequency may play a more substantial role in disease transmission. The Lone star tick (*Amblyomma americanum*), which is abundant in Kentucky, has been documented with high infection rates of *R. amblyommatis* and *R. parkeri*³⁹, and, more rarely, *R. rickettsii*^{8,40}. Future studies should prioritize expanded surveillance across multiple tick species and life stages, particularly in areas with elevated SFG rickettsiosis incidence.

Data availability

All data generated during this study are included in the main article and supplementary information files.

Received: 30 September 2025; Accepted: 22 January 2026

Published online: 05 February 2026

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Acknowledgements

This work was supported by the Kentucky Department for Public Health, the Agricultural Safety and Health (ASH) Training Program of the Central Appalachian Regional Education and Research Center (CARERC) traineeship awarded to CWV and ARP, and the National Institute for Occupational Safety and Health (NIOSH) grant awarded to SRP and WS. We want to thank members of the Kentucky Department for Public Health for their help in collecting ticks and establishing the Kentucky Tick Submission program. We also want to thank the Centers for Disease Control and Prevention and the Rickettsial Isolate Repository Collection for providing the materials used as positive controls.

Author contributions

CWV: Data curation, Formal analysis, Project administration, writing review & editing, Investigation JSR: Data curation, Project administration, Writing-review & editing. MCC: Data curation, Writing—review & editing. ARP: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Writing—original draft. WS: Funding acquisition, Resources and Writing—review & editing. SRP: Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Writing—original draft; Writing—review & editing.

Funding

This work was supported by the Kentucky Department for Public Health, the Agricultural Safety and Health (ASH) Training Program of the Central Appalachian Regional Education and Research Center (CARERC) traineeship awarded to CWV and ARP, and the National Institute for Occupational Safety and Health (NIOSH) grant awarded to JSR.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-37422-0>.

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