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# Molecular Detection and Distribution of Rickettsia in Ticks Across the Country of Georgia

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## Abstract

*Rickettsia* spp. are obligate intracellular bacteria transmitted by arthropod vectors and are responsible for a range of potentially severe human infections. In Georgia, information on the geographic distribution and species diversity of *Rickettsia* spp. in ticks remains limited. This study investigated the prevalence and species distribution of *Rickettsia* spp. in ticks collected from four Georgian regions. Between May 2020 and June 2022, 4542 ticks were collected from domestic animals and the environment in Kakheti, Mtskheta-Mtianeti, Samegrelo, and Shida Kartli. Ticks were grouped into 699 pools according to species, sex, collection method, and location, then analysed using genus- and species-specific quantitative PCR assays targeting the 17 kDa antigen gene. *Rickettsial* DNA was detected in 158 of 699 pools, corresponding to an overall minimum infection rate (MIR) of 3.48%. The highest MIR was observed in Shida Kartli (5.13%). Six clinically relevant *Rickettsia* species were targeted using species-specific PCR assays; among these, *Rickettsia raoultii*, *R. slovaca*, *R. aeschlimannii*, and *R. monacensis* were detected in the examined tick samples, whereas *R. massiliae* and *R. conorii* were not detected. Mixed infections were detected in 16 pools. *Dermacentor marginatus* and *Hyalomma marginatum* were the tick species most frequently associated with *Rickettsia* detection, particularly in Kakheti and Shida Kartli, where species diversity was highest. These findings highlight regional variation in *Rickettsia* prevalence and diversity and support the need for continued tick-borne disease surveillance in Georgia.

**Keywords:** *Rickettsia* spp.; tick-borne disease; *Rickettsial* prevalence; tick surveillance; Georgia (country)

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Academic Editor: Romain Pigeault

Received: 14 July 2026
Revised: 12 August 2026
Accepted: 24 August 2026
Published: 29 September 2026

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## 1. Introduction

*Rickettsia* bacteria are obligate intracellular pathogens that cause a range of *Rickettsial* diseases [1]. These Gram-negative, rod-shaped organisms belong to a group of vector-borne pathogens, meaning that they are transmitted through the bites of arthropods such as ticks, fleas, and lice. The diseases they cause can be both acute and chronic, affecting people across the globe [2]. *Rickettsia* species are categorized into two groups based on the diseases they cause, including the typhus group and spotted fever group [3]. Owing to their small size, persistence in infected hosts, environmental stability, and ability to be transmitted by aerosols, *Rickettsia* is considered a potential bioweapon [4]. Additionally, climate change has contributed to the expansion of tick populations, resulting in an increased risk of

tick-borne *Rickettsia* infections worldwide. Moreover, changes in host animal movement patterns and advances in diagnostic methods may also influence the observed increase in reported cases [5].

Although *Rickettsial* infections often share similar symptoms, their epidemiology and the specific species involved can differ depending on the region. The clinical spectrum of these diseases ranges from mild symptoms to severe, life-threatening conditions, with complications such as vascular damage, neurological symptoms, and organ failure [3]. In severe cases, *Rickettsial* infections can lead to necrosis and even limb gangrene [6]. Ticks are the primary vectors of spotted fever group *Rickettsia* (SFGR) pathogens, and their host preferences strongly influence the epidemiology and geographic distribution of tick-borne infections worldwide. Several tick genera are important vectors of SFGR, including *Rhipicephalus* (including *R. sanguineus* and *R. bursa*), *Dermacentor* (including *D. marginatus* and *D. reticulatus*), *Ixodes* (including *I. ricinus* and *I. scapularis*), *Amblyomma* (including *A. variegatum* and *A. americanum*), *Hyalomma* (including *H. marginatum* and *H. anatolicum*), and *Haemaphysalis* (including *H. longicornis* and *H. punctata*). These genera parasitize a wide range of vertebrate hosts, including livestock, wildlife, rodents, and birds, which contribute to the maintenance of tick populations and pathogen circulation. Although humans are generally considered incidental hosts, ticks belonging to these genera may also bite humans and contribute to zoonotic transmission. These ticks transmit a variety of SFGR species, including *R. conorii*, *R. massiliae*, *R. slovaca*, *R. rickettsii*, *R. helvetica*, *R. monacensis*, *R. africae*, *R. parkeri*, *R. aeschlimannii*, *R. sibirica*, and *R. heilongjiangensis*. Understanding these tick–host associations is essential for assessing tick-borne *Rickettsia* risk and informing prevention strategies [7].

Humans are incidental hosts, meaning that they do not typically contribute to the transmission cycle of these bacteria. Instead, *Rickettsia* spp. are maintained in nature by animal hosts such as rodents, with arthropod vectors playing a key role in spreading the bacteria to humans [8], and infection occurs when a person is bitten by an infected tick or when infected secretions from a tick come into contact with broken skin or mucous membranes [9]. Infection occurs when a person is bitten by an infected tick or when infected secretions from a tick come into contact with broken skin or mucous membranes [9].

Several spotted fever group (SFG) *Rickettsia* species are recognized human pathogens and have been reported in ticks across Europe and Asia, including *Rickettsia conorii*, *R. slovaca*, *R. raoultii*, *R. aeschlimannii*, *R. massiliae*, *R. helvetica*, and *R. monacensis*. Their distribution is closely associated with the geographic distribution of their tick vectors and ecological conditions [10]. Because Georgia is located at the crossroads of Eastern Europe and Western Asia, surveillance of *Rickettsia* species in tick populations is important for monitoring the circulation of pathogenic species and assessing potential public health risks.

In Georgia, *Rickettsial* diseases are spread primarily by ticks, although there are limited data on this issue [11]. A study from 2014 detected *Rickettsia* across all regions of Georgia, with 33% of collected tick samples testing positive (a total of 1000 single-species tick pools representing 23 species collected from eight regions were analyzed) [12]. A more recent survey conducted from 2012–2016 across eight regions identified nine *Rickettsia* species among twelve tick species, including several human-associated spotted fever group species such as *R. aeschlimannii*, *R. slovaca*, *R. raoultii*, *R. monacensis*, and *R. conorii* [11]. This highlights the need for further research in this area.

The aim of this study was to determine the prevalence and distribution of *Rickettsia* species in ticks from various regions of Georgia via the use of molecular methods to provide updated epidemiological data. As Georgia occupies a strategic position between Europe and Asia, updated surveillance of tick populations is essential for monitoring the circulation

of pathogenic *Rickettsia* species, improving knowledge of their distribution, and providing baseline data to support vector surveillance and public health risk assessment.

## 2. Materials and Methods

### *2.1. Tick Samples*

We collected a total of 4542 individual ticks from livestock and tick drags/flags from 30 sites across four regions of Georgia: Kakheti, Mtskheta-Mtianeti, Samegrelo, and Shida Kartli. Detailed data on livestock breeds or pasture characteristics were not recorded during field collections. The collection of ticks from domestic animals was exempt from the animal care protocol, as per the Institutional Animal Care and Use Committee (IACUC) of the Georgia National Center for Disease Control and Public Health and the U.S. Defense Threat Reduction Agency. Ticks were identified on the basis of their morphological characteristics via a key for ixodid ticks in Georgia [13]. Detailed information regarding the developmental stage of individual ticks was not available from the original field records. Each sample was documented in terms of species, sex, collection site (GPS coordinates), method, and time and then grouped into 699 DNA pools (1–14 ticks). Tick pooling was performed according to the internal protocol of the organisation. Ticks collected from the same GPS location and of the same species were pooled together. Well-fed ticks were pooled in groups of 3–5, whereas unfed or partially fed ticks were pooled in groups of up to 14. This approach ensured efficient processing while preserving species- and location-specific information for molecular analysis.

### *2.2. Tick Database*

A database was created for the 699 pooled tick sample. To estimate the prevalence of *Rickettsia* spp. in different regions, we calculated the minimum infection rate (MIR) for pooled samples. The MIR was determined by dividing the number of positive pools by the total number of ticks in all pools and multiplying by 100.

### *2.3. Tick Processing*

We homogenized tick samples (either individual or pooled) using a bead mill (Biospec Products Inc., Bartlesville, OK, USA) with 2.5 mm stainless steel beads (MO BIO Laboratories, Carlsbad, CA, USA) in 750 µL of grinding solution (Eagle's Minimum Essential Medium (EMEM) cell culture media with 10% bovine serum; Sigma-Aldrich, St. Louis, MO, USA). DNA extraction was performed using the Qiagen DNA Kit and the MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems™ by Thermo Fisher Scientific, Waltham, MA, USA). The extraction was done using the KingFisher Flex automated purification system (MagMaxCoreFlexNoHeat, 96 DW), and DNA was stored at −20 °C until polymerase chain reaction (PCR) analysis.

### *2.4. Initial qPCR Assays*

A genus-specific qPCR assay targeting the Rick17-kDa gene was used to screen all tick DNA samples for *Rickettsia* spp. DNA. Amplification was performed on a Bio-Rad CFX96™ real-time PCR system using previously published primers, and probe sets with minor modifications [14]. Each reaction was run in a 20 µL volume and included 5 µL of DNA template. Cycling conditions consisted of initial denaturation at 95 °C for 5 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 35 s. Samples with a Ct value values less than 32 were considered weak positives and excluded from downstream analysis. This cutoff was based on validation of assay sensitivity in previous studies and internal optimization, where Ct $\geq$ 32 often corresponds to low DNA quantity insufficient for reliable species identification. Each qPCR run included positive controls (*Rickettsia* DNA) and negative

controls (nuclease-free water) to monitor amplification efficiency and contamination. Genus-positive samples were subsequently subjected to species-specific PCR assays targeting six clinically relevant *Rickettsia* species (*R. raoultii*, *R. massiliae*, *R. slovaca*, *R. conorii*, *R. aeschlimannii*, and *R. monacensis*) [10,11]. These species were selected based on previous reports identifying them as the most frequently detected and epidemiologically relevant spotted fever group *Rickettsia* species in Georgia and neighboring regions. The diagnostic approach was based on species-specific PCR targeting predefined species rather than sequencing-based characterization; therefore, species not included in the assay panel could not be detected.

### *2.5. Data Analysis*

The data wereusing Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), and then analysed the data by R version 4.3.1 [15], using R studio. Species-specific findings by region and tick vector were visualized via the R statistical programming environment (Figure 1: *R_Slovaca* status by Tick species and Region; Figure 2: *R_raoultii* status by Tick species and Region; Figure 3: *R_monacensis* status by Tick species and Region; Figure 4: *R_massiliae* status by Tick species and Region; Figure 5: R_conorii status by Tick species and Region; Figure 6: *R_aeschlimannii* status by Tick species and Region). For these figures, percentages represent the proportion of positive tick pools among the total number of analyzed pools for each *Rickettsia* species, tick species and region. These pool-level percentages were reported separately from the MIR presented in Table 2. The 95% confidence intervals (CI) for the MIR were calculated using the normal approximation method, where the standard error of the proportion of positive pools per total ticks tested was multiplied by 1.96 and added to/subtracted from the MIR to derive the upper and lower bounds.

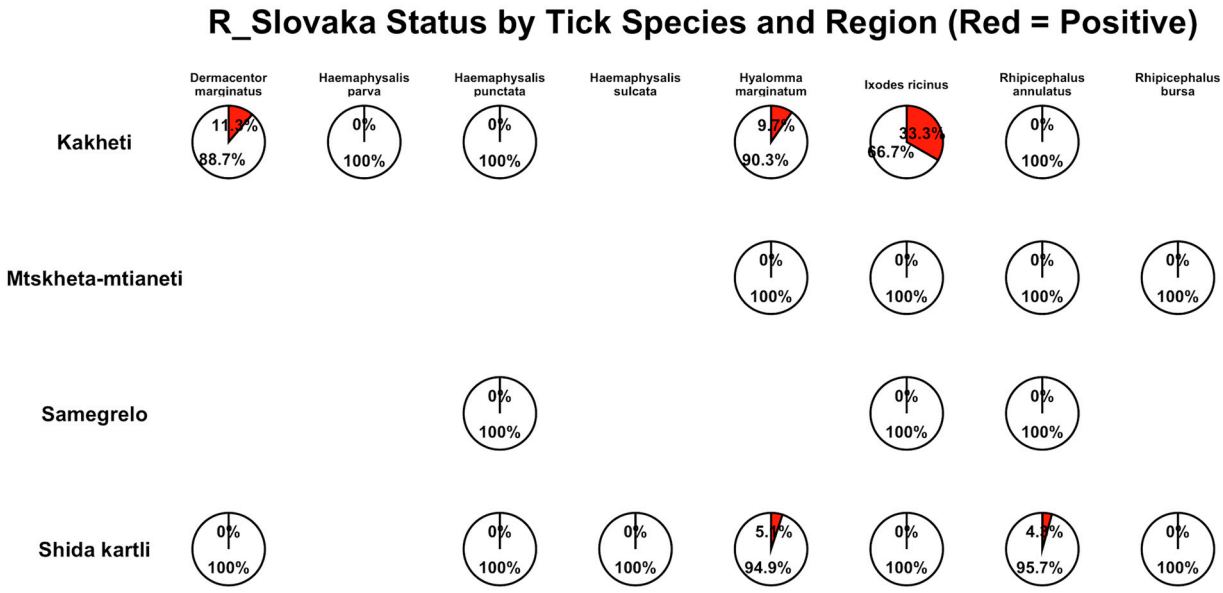



**Figure 1.** Percentage of analyzed tick pools positive for *R. slovaca* by tick species and region.

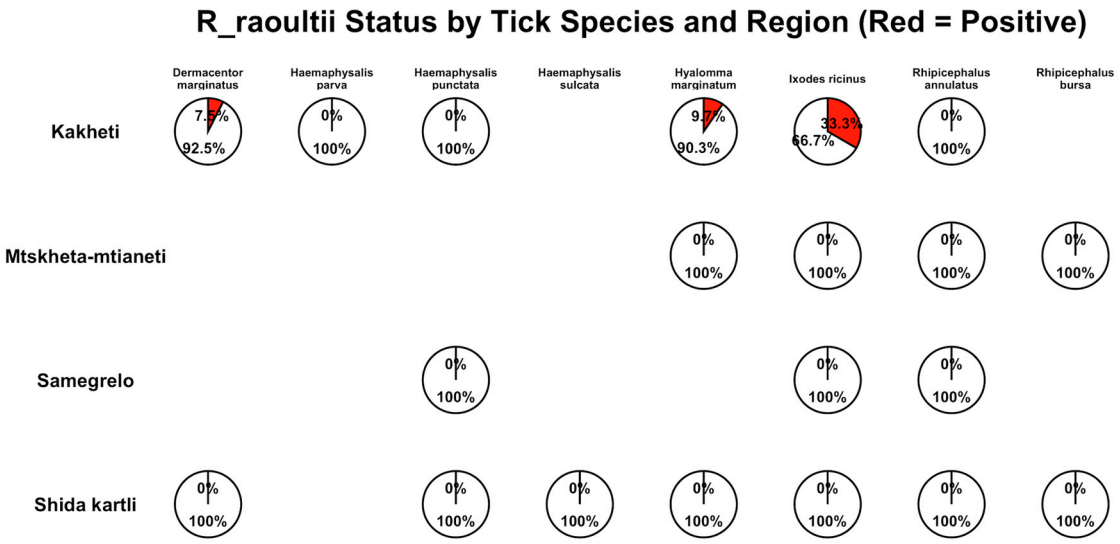



**Figure 2.** Percentage of analyzed tick pools positive for *R. raoultii* by tick species and region.

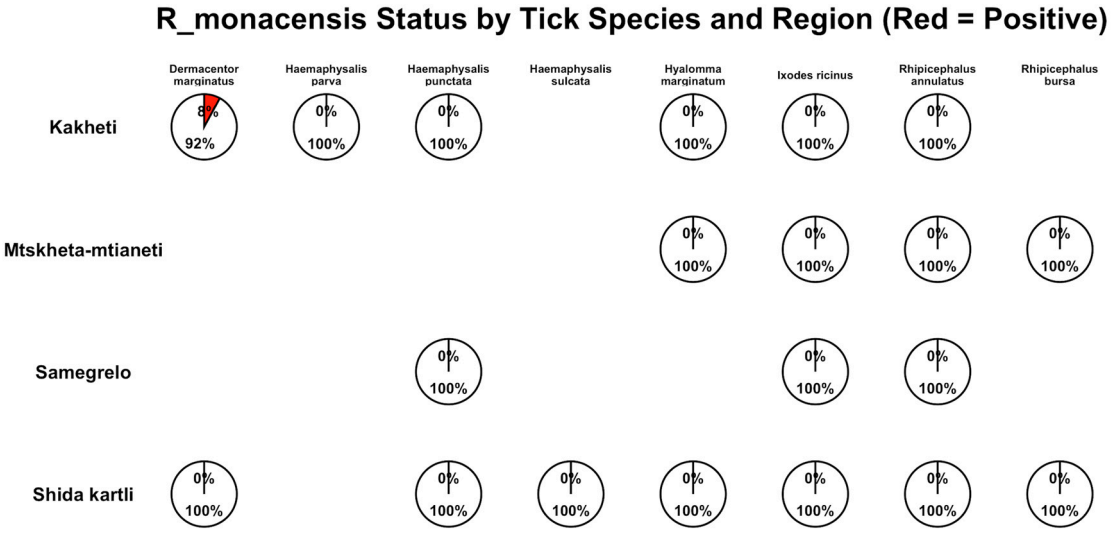



**Figure 3.** Percentage of analyzed tick pools positive for *R. monacensis* by tick species and region.

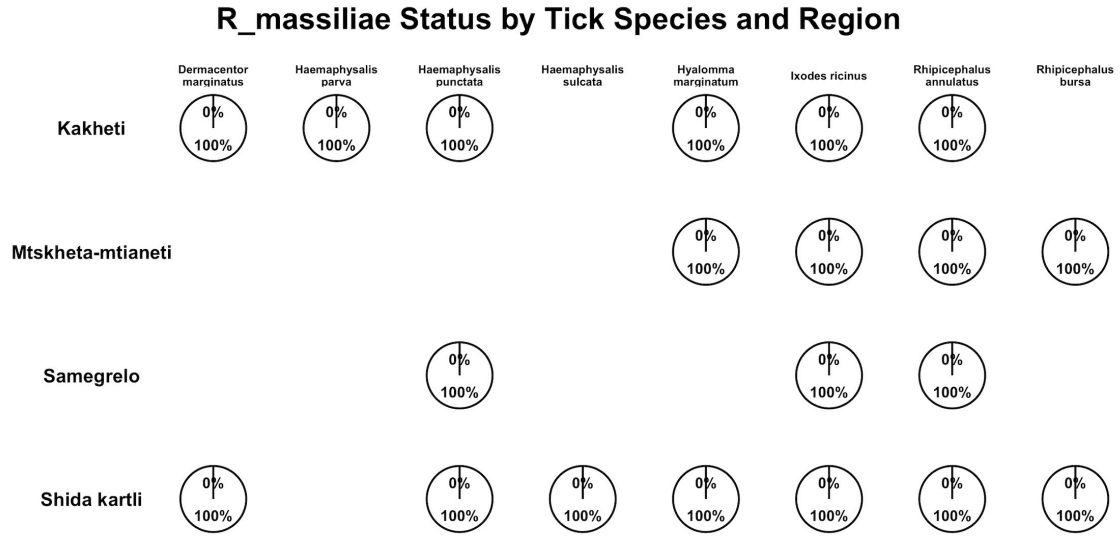



**Figure 4.** Percentage of analyzed tick pools positive for *R. massiliae* by tick species and region.

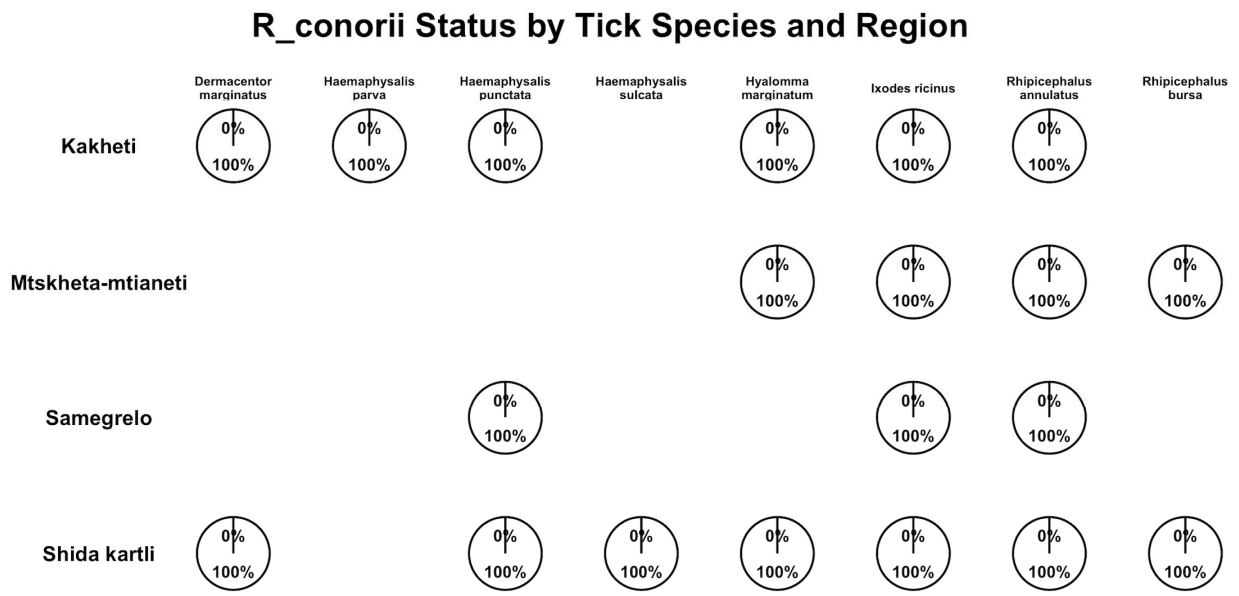



**Figure 5.** Percentage of analyzed tick pools positive for *R. conorii* by tick species and region.

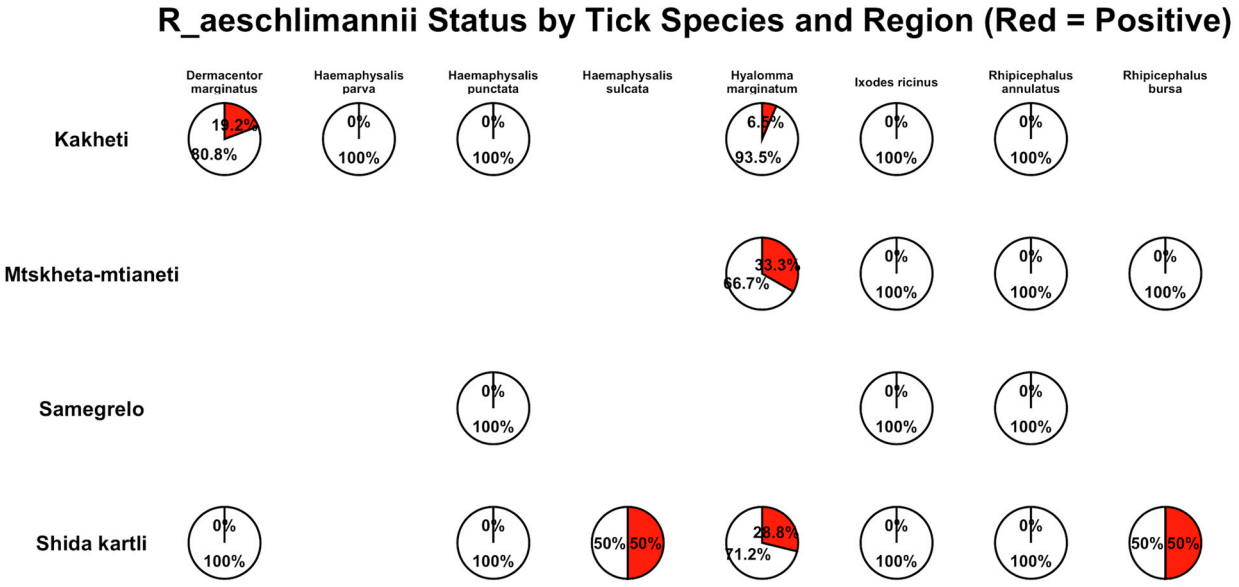



**Figure 6.** Percentage of analyzed tick pools positive for *R. aeschlimannii* by tick species and region.

## 3. Results

A total of 4542 ticks were collected from four regions of Georgia (Table 1). Overall, the most frequently identified tick species were *Rhipicephalus annulatus* (*n* = 2097, 46.2%) and *Dermacentor marginatus* (*n* = 1673, 36.8%). The distribution of species varied by region. *Dermacentor marginatus* was found almost exclusively in Kakheti (68.1%), whereas *Hyalomma marginatum* was predominantly collected in Kakheti (10.8%), with smaller proportions in Shida Kartli and Mtskheta-Mtianeti. *Ixodes ricinus* was found mainly in Shida Kartli (13.4%) and Samegrelo (46.4%). *Haemaphysalis punctata* appeared across Kakheti, Shida Kartli, and Samegrelo. Rare tick species such as *Haemaphysalis sulcata* and *H. parva* were confined to single regions (Table 1).

**Table 1.** Geographical distribution of tick species collected from four regions in Georgia.

| Tick Species | Overall, n[a] (Column %) | Region, n[a] (Column %) | | | |
|---|---|---|---|---|---|
| | | Kakheti | Shida Kartli | Mtsketa-Mtianeti | Samegrelo |
| *Dermacentor marginatus* | 1673 (36.8) | 1669 (68.1) | 4 (0,5) | 0 (0) | 0 (0) |
| *Hyalomma marginatum* | 335 (7.4) | 265 (10.8) | 59 (7.2) | 11 (1.3) | 0 (0) |
| *Haemaphysalis punctata* | 198 (4.3) | 146 (6.0) | 25 (3.06) | 0 (0) | 27 (6.4) |
| *Haemaphysalis sulcata* | 7 (0.1) | 0 (0) | 7 (0.86) | 0 (0) | 0 (0) |
| *Haemaphysalis parva* | 1 (0.02) | 1 (0.04) | 0 (0) | 0 (0) | 0 (0) |
| *Ixodes ricinus* | 224 (4.9) | 8 (0.3) | 110 (13.4) | 2 (0.2) | 104 (46.4) |
| *Rhipicephalus annulatus* | 2097 (46.2) | 362 (14.8) | 609 (74.4) | 836 (98.1) | 290 (24.7) |
| *Rhipicephalus bursa* | 7 (0.1) | 0 (0) | 4 (0.5) | 3 (0.4) | 0 (0) |
| Total | 4542 (100) | 2451 (100) | 818 (100) | 852 (100) | 421 (100) |

n[a]—Total number of ticks collected.

Among the 4542 collected ticks, 699 pools were tested for *Rickettsia* spp., and 158 pools tested positive, resulting in an overall minimum infection rate (MIR) of 3.48% (Table 2). MIR varied by region; the highest MIR was observed in Shida Kartli (5.13%), followed by Kakheti (4.32%) and Samegrelo (2.14%). Mtskheta-Mtianeti had the lowest MIR (0.12%), with only one positive pool detected.

**Table 2.** Minimum infection rate of Rickettsia-positive tick samples by region.

| Region | No. of Pools | Total Ticks Tested | Positive Pools | MIR [a] (%, 95%CI) |
|---|---|---|---|---|
| Kakheti | 363 | 2451 | 106 | 4.3 (3.52 to 5.13) |
| Shida Kartli | 154 | 818 | 42 | 5.1 (3.62 to 6.65) |
| Mtskheta-Mtianeti | 96 | 852 | 1 | 0.1 (0.0 to 0.35) |
| Samegrelo | 86 | 421 | 9 | 2.1 (0.76 to 3.52) |
| Total | 699 | 4542 | 158 | 3.5 (2.95 to 4.01) |

[a]—MIR, minimum infection rate, defined as the number of positive tick pools divided by the total number of ticks in the pools multiplied by 100.

Four *Rickettsia* species were identified among the positive pools: *R. aeschlimannii* (*n* = 62), *R. slovaca* (*n* = 19), *R. monacensis* (*n* = 16), and *R. raoultii* (*n* = 5). Although *R. massiliae* and *R. conorii* were included in the species-specific PCR panel, they were not detected in any positive pools (Table 3). The remaining 40 genus-positive pools could not be assigned to a species using the species-specific PCR assays employed in this study. Mixed infections involving combinations of *R. slovaca* with either *R. aeschlimannii* (*n* = 1) or *R. raoultii* (*n* = 15) were observed in 16 pools. Kakheti presented the highest species diversity, with all six species and both types of mixed infections detected. Shida Kartli had 38 *Rickettsia*-positive pools, mainly involving *R. aeschlimannii* and *R. slovaca*, whereas only isolated detections occurred in Samegrelo and Mtskheta-Mtianeti. *Dermacentor marginatus* and *Hyalomma marginatum* accounted for the majority of *Rickettsia*-positive detections in this study, particularly *R. slovaca* and *R. aeschlimannii*. *Dermacentor marginatus* accounted for most detections in Kakheti, whereas *H. marginatum* was associated with *R. aeschlimannii* detections in both Kakheti and Shida Kartli. Other tick species, including *Ixodes ricinus*, *Rhipicephalus annulatus*, and *Haemaphysalis* spp., accounted for a smaller proportion of *Rickettsia*-positive detections (Table 3).

**Table 3.** Distribution of Rickettsia species in different tick species and regions.

| Region | Ticks | *R. slovaka* | *R. aeschlimannii* | *R. raoultii* | *R. monacensis* | *R. massiliae* | *R. conorii* | *R. slovaka & R. aeschlimannii* | *R. slovaka & R. raoultii* | Not Identified | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kakheti | Total | 14 | 43 | 5 | 16 | | | | 15 | 13 | 106 |
| | *Dermacentor marginatus* | 12 | 41 | 3 | 16 | | | | 13 | 12 | 97 |
| | *Hyalomma marginatum* | 1 | 2 | 1 | | | | | 2 | | 6 |
| | *Rhipicephalus annulatus* | | | | | | | | | 1 | 1 |
| | *Ixodes ricinus* | 1 | | 1 | | | | | | | 2 |
| | Total | | 1 | | | | | | | | 1 |
| Mtsketa-Mtianeti | *Hyalomma marginatum* | | 1 | | | | | | | | 1 |
| Shida Kartli | Total | 5 | 18 | | | | | 1 | | 18 | 38 |
| | *Haemaphysalis sulcata* | | 1 | | | | | | | | 1 |
| | *Haemaphysalis punctata* | | | | | | | | | 2 | 2 |
| | *Hyalomma marginatum* | 2 | 16 | | | | | 1 | | 4 | 23 |
| | *Dermacentor marginatus* | | | | | | | | | 2 | 2 |
| | *Ixodes ricinus* | | | | | | | | | 1 | 1 |
| | *Rhipicephalus annulatus* | 3 | | | | | | | | 9 | 9 |
| | *Rhipicephalus bursa* | | 1 | | | | | | | | |
| Samegrelo | Total | | | | | | | | | 9 | 9 |
| | *Ixodes Ricinus* | | | | | | | | | 8 | 8 |
| | *Rhipicephalus annulatus* | | | | | | | | | 1 | 1 |
| Total | | 19 | 62 | 5 | 16 | | | 1 | 15 | 40 | 158 |

*Rickettsia slovaca* was primarily detected in Kakheti, notably in *D. marginatus* (11.3%) and *H. marginatum* (9.7%). A single positive *I. ricinus* pool was also identified. Lower rates were found in Shida Kartli (*H. marginatum*, 5.1%; *R. annulatus*, 4.3%). It was absent in Samegrelo and Mtskheta-Mtianeti (Figure 1). *R. raoultii* appeared only in Kakheti in *D. marginatus* (7.5%) and *H. marginatum* (9.7%). A single positive *I. ricinus* pool was also detected (Figure 2). *R. monacensis* was exclusive to Kakheti and was detected only in *D. marginatus* (8%) (Figure 3). *R. massiliae* and *R. conorii* were not detected in any tick species across all regions (Figures 4 and 5). *R. aeschlimannii* was found in three regions: Kakheti (*D. marginatus* 19.2%, *H. marginatum* 6.5%), Mtskheta-Mtianeti (*H. marginatum* 33.3%), and Shida Kartli (*H. sulcata* 50%.0, *H. marginatum* 28.8%, *R. bursa* 50.0%). No detections occurred in Samegrelo (Figure 6).

## 4. Discussion

Our study provides the most comprehensive and region-specific snapshot to date of *Rickettsia* spp. diversity and prevalence in Georgian tick populations, revealing new patterns of tick–Rickettsia associations and highlighting potential public health concerns.

Our findings align with those of a previous nationwide study, which reported that the six species as the most common. *Rickettsia aeschlimannii* was again the most prevalent and widespread species, predominantly identified in *Hyalomma marginatum* and *Dermacentor marginatus*, especially in Kakheti and Shida Kartli [11]. This pattern is consistent with earlier studies showing that *H. marginatum* frequently carries *R. aeschlimannii* and plays a major role in *Rickettsia* circulation in Georgia [11].

Similar patterns have been reported across the Mediterranean Basin, where *Rickettsia aeschlimannii* has been frequently detected and isolated from *Hyalomma marginatum* and is considered an emerging spotted fever group pathogen in southern Europe, North Africa, and western Asia [16].

*Dermacentor marginatus* and *Hyalomma marginatum* accounted for the majority of *Rickettsia*-positive detections in this study. Previous studies have identified *D. marginatus* as an important vector and reservoir of *R. slovaca*, supported by evidence of transstadial and transovarial maintenance and documented human infections [17–19]. The frequent association of *R. aeschlimannii* with *H. marginatum* reported in previous studies is consistent with the detection of this species in *H. marginatum* ticks in Georgia [16,20]. Therefore, our findings support the established association between these *Rickettsia* species and their commonly reported tick hosts. However, the detection of *Rickettsia* DNA in ticks does not by itself demonstrate vector competence, and our results should be interpreted as evidence of pathogen–tick associations rather than direct proof of transmission. These host–pathogen associations are consistent with broader European patterns of spotted fever group rickettsiae circulation. Similar ecological cycles involving Dermacentor and Hyalomma species have been documented in Spain, France, Italy, and the Balkans [16].

*Rickettsia slovaca* and *R. raoultii* were identified mainly in *D. marginatus* from Kakheti, consistent with their well-established association with *Dermacentor* spp. These species are widely distributed across Europe and are recognized as major agents of tick-borne lymphadenopathy (SENLAT) syndrome [21,22]. Although both species were also detected in *Ixodes ricinus* in the present study, these findings should be interpreted cautiously because of the limited number of analyzed specimens. No clinical investigation was performed in the current study; therefore, the presence of these species in ticks cannot be directly linked to human disease occurrence.

The detection of *Rickettsia monacensis* only in Kakheti contrasts with earlier studies, where it was identified in the western part of Georgia. [11] This regional difference could reflect variations in sampling, changes in tick-host ecology, or shifts in pathogen distribution

over time. Consistent with earlier studies, *R. massiliae* and *R. conorii* were identified in only a small number of samples, reflecting their sporadic occurrence in Georgia [11]. Rickettsia monacensis has been reported widely in Ixodes ricinus across Europe, including Germany, Italy, and Spain, where it is associated with forest ecosystems and wildlife reservoirs [23].

A key new observation was the presence of mixed infections in 16 pools, particularly those involving *R. slovaca* and *R. raoultii*. Because these findings are based on pooled samples, the source of coinfection cannot be determined, and it remains unclear whether these detections reflect cofeeding, shared vertebrate hosts, or simply multiple infected ticks within a single pool.

In contrast to the previous national survey [11], we did not detect *Candidatus Rickettsia barbariae*, *R. helvetica*, or *R. hoogstraalii*. However, this absence likely reflects methodological differences and the limited range of species-specific assays applied in the current study. An earlier study [11], employed multilocus sequence typing (MLST), which can detect a broader range of *Rickettsia* species, including novel or less common strains. In addition, several *Rickettsia*-positive pools in the present study could not be assigned to a specific species using the applied assays, suggesting that additional *Rickettsia* diversity may be present among Georgian tick populations. Our use of species-specific PCR assays, while targeted and cost-effective, may have limited the detection of species outside the predefined panel. This highlights the importance of incorporating broader molecular approaches, such as MLST or metagenomic sequencing, in future surveillance to better characterize the full diversity of *Rickettsia* species in Georgian ticks.

Regional differences in MIR observed in this study may reflect variation in tick species composition, habitat characteristics, and ecological conditions influencing *Rickettsia* circulation. Similar geographic differences in tick-borne *Rickettsia* prevalence have been reported in Europe and Asia, where pathogen distribution is influenced by vector abundance, vertebrate host availability, and environmental factors [10]. However, detailed ecological parameters, including host abundance, livestock management practices, and microclimatic conditions, were not evaluated in the present study. Future investigations incorporating these factors will help clarify the drivers of regional variation in *Rickettsia* circulation in Georgia.

Overall, the findings demonstrate the circulation of diverse *Rickettsia* spp. in Georgian tick populations, with particularly high species diversity observed in Kakheti in the current dataset. The frequent detection of pathogenic or potentially pathogenic *Rickettsia* species in ticks associated with livestock highlights the importance of continued molecular surveillance, particularly in rural areas where humans and animals share tick habitats.

Several limitations should be acknowledged. First, only four Georgian regions were included, potentially missing other circulating *Rickettsia* spp. in unexamined areas. Second, pooling of tick specimens, while cost-efficient, may underestimate true infection rates and obscure host–pathogen relationships at the individual level. Third, low numbers of some tick species (e.g., *Haemaphysalis sulcata* and *H. parva*) reduce the reliability of MIR estimates for those vectors. Fourth, detailed metadata on tick sex (male/female), life stage (larvae, nymphs, and adults), collection source (free-living versus host-collected), and host animal species were not systematically recorded during field collection. Consequently, stratified analyses of infection according to these ecological variables and host–vector relationships could not be performed. Future surveillance studies should systematically collect these data to enable more comprehensive ecological and epidemiological analyses. In addition, 40 genus-positive pools could not be assigned to a specific *Rickettsia* species using the targeted PCR assays. This likely reflects the limited species coverage of the assay panel and suggests that additional *Rickettsia* species may be circulating in Georgian tick populations. Broader molecular approaches, such as multilocus sequence typing (MLST)

or metagenomic sequencing, would improve species identification in future surveillance studies. Finally, as a cross-sectional study, this investigation provides only a snapshot and does not capture seasonal or annual fluctuations in tick-borne *Rickettsia* activity.

## 5. Conclusions

This study contributes valuable, region-specific data on the prevalence and diversity of *Rickettsia* spp. in Georgian tick populations. The identification of four species, including several with known pathogenicity to humans, highlights ongoing public health risks in regions where ticks, livestock, and wildlife coexist closely. While our findings support previous national data, they also reveal regional variation and new patterns of tick–Rickettsia associations.

Our results emphasize the need for expanded surveillance efforts incorporating broader geographic coverage, longitudinal sampling, and the inclusion of immature tick stages. Future studies using molecular typing and phylogenetic tools will be essential for detecting emerging strains, assessing genetic diversity, and improving the understanding of the ecology and evolution of *Rickettsia* spp. in the South Caucasus. These efforts will enhance risk assessments and support targeted interventions for *Rickettsial* disease prevention in Georgia.

**Author Contributions:** N.B. analysed and interpreted the data and drafted the manuscript. R.S. and E.Z. performed the laboratory work. E.K., D.C., G.C. and S.T. contributed to manuscript review and editing. D.C. also managed the project administration and acquired funding. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Funding—Defence threat reduction agency (HDTRA1-19-1-0042).

**Institutional Review Board Statement:** The collection of ticks from domestic animals was exempt from the animal care protocol, as per the Institutional Animal Care and Use Committee (IACUC) of the Georgia National Center for Disease Control and Public Health and the U.S. Defense Threat Reduction Agency (FP/AP-001/2020, 14 May 2020).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data generated or analysed during this study are included in this published article.

**Acknowledgments:** We wish to acknowledge support from the University of California, San Francisco's International Traineeships in AIDS Prevention Studies (ITAPS), U.S. NIMH, R25MH123256. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The corresponding author thanks Tbilisi State University for providing support during PhD research.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| MIR | Minimum infection rate |
| IACUC | Institutional Animal Care and Use Committee |
| PCR | Polymerase chain reaction |
| SENLAT | Scalp Eschat and Neck Lymph Adenopathy After a Tick Bit) |

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