







1 **Molecular Identification of *Rickettsia* Species in Ticks Infesting Cattle, Sheep,**
2 **and Goats from Kerman, Southeastern Iran: Application of Real-Time PCR for**
3 **Epidemiological Surveillance**

4

5 **Shiva Shokooh Saljoughi^a , Ashkan Hajjafari^b , Mohammad Khalili^a *, Hamide**
6 **Arefipoor^a , Fatemeh Nazeri^a , Aliasghar Mozaffari^c **

7

8 *^a Department of Pathobiology, Faculty of Vet.medicine, Shahid Bahonar University of Kerman,*
9 *Iran.*

10 *^b Department of Pathobiology, Faculty of Veterinary Specialized Science, Science, and Research*
11 *Branch, Islamic Azad University, Tehran, Iran.*

12 *^c Department of Clinical Sciences, Faculty of Vet.medicine, Shahid Bahonar University of Kerman,*
13 *Iran.*

14

15 Corresponding author: **Mohammad Khalili**, Department of Pathobiology, Faculty of Vet.
16 medicine, Shahid Bahonar University of Kerman, Iran, mdkhalily@uk.ac.ir

17

18 **Abstract**

19 **Background:** *Rickettsia* spp. represent emerging zoonotic pathogens transmitted by arthropods
20 such as ticks, posing considerable implications for public health and livestock management.
21 Although there is growing acknowledgment of their significance in adjacent areas, data from
22 southeastern Iran are still scarce.

23 **Methods:** One hundred ticks were collected from cattle, sheep, and goats in April to June 2025 in
24 Anbarabad, Kerman Province. Genomic DNA extraction was performed, and samples were
25 analyzed for *Rickettsia* spp. utilizing SYBR Green-based real-time PCR that targets the *gltA* gene.
26 Amplification profiles and melt curve analysis were utilized to confirm the sensitivity and
27 specificity of detection.

28 **Results:** *Rickettsia* DNA was detected in 15% of ticks overall, with infection rates of 16% in ticks
29 from cattle and 14% in ticks from sheep and goats. These findings confirm the presence of
30 *Rickettsia* spp. in mixed-livestock systems in Anbarabad, highlighting the circulation of these
31 pathogens across different host species. The observed infection rates suggest a consistent level of
32 exposure among ticks parasitizing various livestock, reinforcing the need for continued
33 surveillance and control measures in such endemic settings.

34 **Conclusions:** This study represents one of the few molecular examinations of *Rickettsia* species
35 conducted in southeastern Iran, offering essential foundational information for future research
36 endeavors. The discovery of a moderate presence of ticks in both cattle and small ruminants
37 underscores the ongoing risk of pathogen spread within mixed-livestock systems, which may
38 facilitate transmission between species. The use of advanced molecular techniques, such as
39 sequencing, is essential for enhancing species-level resolution, clarifying complex
40 epidemiological patterns, and guiding targeted public health strategies in regions where diseases
41 are prevalent.

42 **Keywords:** Epidemiological surveillance, *gltA*, Real-time PCR, *Rickettsia* spp., Zoonotic risk
43 factors

44

1. Introduction

45 Zoonotic illnesses, which are diseases transmitted by arthropod vectors, are a major concern for public
46 health officials worldwide [1]. Zoonotic diseases arise from a diverse array of microorganisms, including
47 bacteria, viruses, and protozoa, which possess the capability to cross the species barrier that distinguishes
48 humans from animals [2]. Among the zoonoses transmitted by vectors, tick-borne illnesses stand out due
49 to their extensive geographic distribution, intricate ecological dynamics, and their potential to induce severe
50 health issues in both humans and domestic animals. The recent decades have seen a resurgence of tick-
51 borne illnesses, highlighting the critical necessity for enhanced monitoring, diagnostic tools, and preventive
52 measures to mitigate the impact of these diseases on public health and livestock productivity [3].

53 The genus *Rickettsia* comprises Gram-negative, obligate intracellular bacteria that are tiny in size. They are
54 classified under the order *Rickettsiales* [4]. Endothelial cells are often the target of infection by these
55 microbes, which are conveyed to vertebrate hosts by arthropod vectors such as fleas, ticks, and lice. A
56 number of groups, such as the Spotted Fever Group (SFG), the Typhus Group (TG), and the Scrub Typhus
57 Group (STG), have been established within the genus on the basis of the genetic and antigenic traits that
58 determine them [5]. These categories not only signify evolutionary connections but also align with the
59 manifestations of the illness, the involved vectors, and the geographical spread of the disease.

60 *Rickettsial* infections, which are commonly known as rickettsioses, are zoonotic diseases that are capable
61 of causing a wide variety of clinical symptoms in humans. These symptoms may vary from minor illnesses
62 similar to the flu to difficult situations that are potentially fatal [6]. It is particularly well-known that the
63 Spotted Fever Group is responsible for generating high fever, rash, lymphadenopathy, and, in rare instances,
64 vascular problems. *Rickettsioses* have the potential to cause severe morbidity and death if they are not
65 effectively diagnosed and treated in a timely manner. When it comes to animals, particularly cattle and
66 small ruminants, these illnesses often go undetected, which creates a concealed hazard to public health
67 owing to the possibility of zoonotic transmission [7].

68 Brown dog ticks, also known as *Rhipicephalus sanguineus sensu lato*, are a species of tick that may be
69 found all over the globe, including in both tropical and temperate locations. In spite of the fact that it is
70 most often seen in dogs, it is capable of infecting a wide variety of animals, including humans [8]. In
71 addition, *R. sanguineus* is capable of transmitting some infections, including *Ehrlichia canis*, *Babesia*
72 *vogeli*, and *Rickettsia conorii* [9].

73 The pathogenesis of *Rickettsia* begins with the invasion of endothelial cells by bacteria, which is then
74 followed by the multiplication of the bacteria inside the cells themselves and their transfer via the circulation
75 [10]. This process causes vasculitis and damage to blood vessels that are tiny to medium in size, which

76 contributes to the systemic symptoms that are noticed in hosts who are infected. Despite the fact that vertical
77 transmission among tick populations and exposure to contaminated arthropod excrement have also been
78 identified, the most common mode of transmission is via the bite of infected ticks [11]. The management
79 and monitoring of rickettsial illnesses are extremely difficult to accomplish due to the intricacy of the
80 interaction between the host, the vector, and the pathogen [6].

81 The unclear early symptoms of *rickettsial* infection, which often seem to be similar to those of other febrile
82 illnesses, make it challenging to identify the infection [12]. There are diagnostic methods that are not
83 sensitive enough for early diagnosis, such as serological tests, bacterial cultures, and immunofluorescence.
84 These methods need specialized laboratory facilities. A further limitation of these approaches is that they
85 are often more beneficial during the convalescence period; hence, their use is restricted to circumstances
86 that demand a prompt diagnosis. It is of the utmost importance to make use of molecular methods such as
87 Real-Time Polymerase Chain Reaction (RT-PCR) since these techniques are sensitive, specific, and may
88 offer results in a short period of time. In situations when there is a low pathogen load, RT-PCR is able to
89 correctly identify *Rickettsia* DNA regardless of the kind of sample or the location of the sample. The
90 assessment of zoonotic hazards is very necessary in rural regions because of the close relationship that
91 exists between people and animals, as well as the prevalence of tick infections. In this study, *Rickettsia*
92 species will be characterized by real-time PCR to identify *Rickettsia* species from ticks that are caught on
93 cattle, sheep, and goats in Anbarabad in the country of Kerman.

94

95 **2. Methods and Materials**

96 **2.1. Collection of ticks and study area**

97 Anbarabad, a site targeting cattle, sheep, and goats, is located in Kerman Province, southeast Iran. During
98 April to June 2025, when tick activity is highest in the region, ticks were collected from naturally
99 infested, untreated animals. A sterile forceps was used to carefully remove individual ticks from their host
100 and place them immediately in microtubes containing 70% ethanol using sterile forceps. 100 ticks were
101 collected and stored at -20°C until molecular analysis (50 from cattle, 50 from sheep and goats).

102 **2.2. Tick sterilization and washing**

103 All tick specimens were surface sterilized to ensure that external contamination was not present. Two
104 washes of sterile phosphate-buffered saline (PBS) were applied after immersion in 70% ethanol for five

105 minutes. We constructed a laminar flow cabinet under aseptic conditions for air drying ticks using sterile
106 filter paper.

107 **2.3. Extraction of DNA**

108 Genomic DNA was extracted from whole ticks by homogenizing and digesting them enzymatically. After
109 grinding each tick, proteinase K was introduced into the lysate buffer. This method ensured the complete
110 lysing of the ticks. The purification of DNA was conducted after its extraction through the phenol-
111 chloroform method, followed by precipitation using ethanol. Following the resuspension of the DNA
112 pellet in 30 μ L of TE buffer, it was subsequently stored at -20°C and allowed to freeze. To assess the
113 concentration and purity of DNA, a NanoDrop spectrophotometer was employed.

114 **2.4. Real-Time PCR for *Rickettsia* spp. Identification**

115 *Rickettsia* species were detected using a SYBR Green-based real-time PCR assay targeting the citrate
116 synthase gene (*gltA*) (Table 1). 20 μ L of SYBR Green Master Mix (BioFACT, South Korea), 0.3 μ L of
117 forward and reverse primers (10 pmol/ μ L), 2.5 μ L of extracted DNA, and 6.9 μ L of nuclease-free water
118 were used in the reaction. Following an initial denaturation at 95°C for 5 minutes, 40 cycles of
119 denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30
120 seconds were performed. To assess the specificity of the amplification, a melt curve analysis was
121 conducted at the end of the run.

122

123 **Table 1.** Primers used for amplification of the *gltA* gene in *Rickettsia* spp.

124 Primer Name	Sequence (5'→3')	Target Gene	Amplicon
125 SizePanRick-f	ATAGGACAACCGTTTATTT	citrate synthase (<i>gltA</i>)	70 bp
126 PanRick-R	CAAACATCATATGCAGAAA	citrate synthase (<i>gltA</i>)	70 bp

127

128 **2.5. Positive and Negative Controls**

129 In each qPCR experiment, *Rickettsia* DNA was utilized as a positive control, while nuclease-free water
130 acted as a no-template control to verify the validity of the assay. The specificity of detection was
131 evaluated by analyzing the amplification profiles of the test samples alongside the melting temperatures
132 (T_m) of the positive control.

133 2.6. Examination of Data

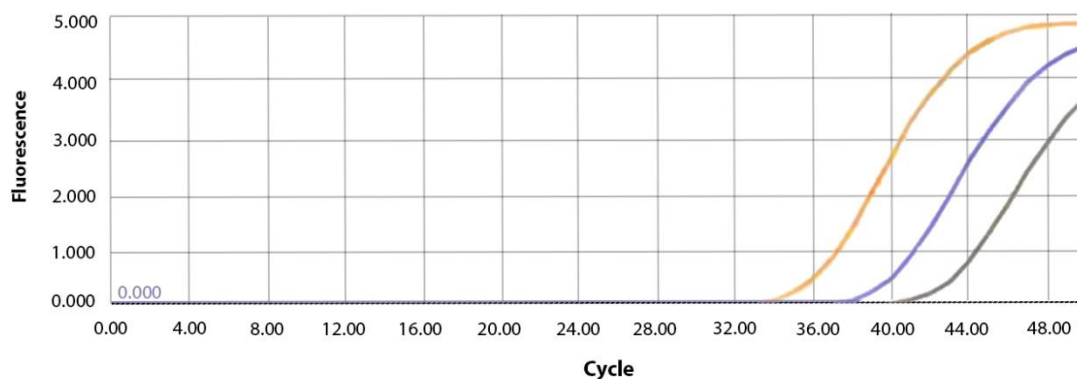
134 Analyzing amplification curves and melting curves was performed using the proprietary software
135 provided by the qPCR platform. A positive sample is determined by its quantification cycle (C_q) value
136 and a single peak in its melt curve matching the positive control. The prevalence of *Rickettsia* species for
137 each host species and geographical location was determined by comparing the number of positive samples
138 to the total number of samples.

139

140 3. Results

141 Validation of real-time PCR assays for *Rickettsia* species targeting the *gltA* gene was conducted. The
142 validation process involved conducting serial dilutions of positive control DNA, ranging from 10⁻¹ to 10⁻³.
143 The consistent amplification of the test yielded average quantification cycles (C_q) of 34.28, 38.74, and
144 40.93 for each dilution level, indicating that the assay exhibited a suitable degree of sensitivity (Figure 1).
145 The absence of an amplified product in the template-free control indicates that contamination was not
146 present. All positive dilutions exhibited a melting peak, and all replicates demonstrated consistent melting
147 temperatures (T_m) (Figure 2).

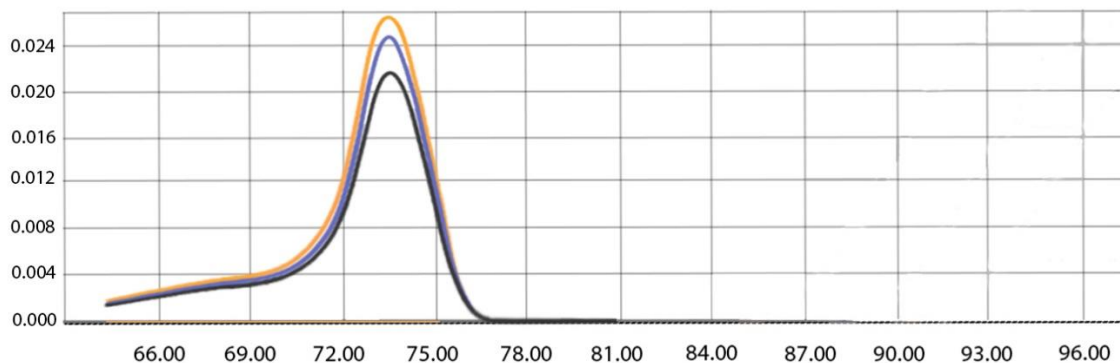
148



149

150 **Figure 1: Amplification curves for serial dilutions of *Rickettsia* positive control DNA using SYBR**
151 **Green-based qPCR.**

152 Figure 2 illustrates the fluorescence amplification curves corresponding to a 10-fold serial dilution of
153 positive control DNA, demonstrating assay sensitivity and dynamic range. The consistent increase in C_q
154 values across dilutions confirms the linearity of the reaction and optimization of qPCR conditions.



156

157 **Figure 2: Melt curve analysis showing specificity of amplification in positive and negative samples.**

158 Distinct single-melt peaks were observed in positive control samples, with no amplification peaks detected
 159 in negative controls. This confirms the analytical specificity of the SYBR Green-based qPCR assay
 160 targeting the *gltA* gene of *Rickettsia* spp.

161

162 3.1. Identification of *Rickettsia* DNA in Tick Samples from Cattle

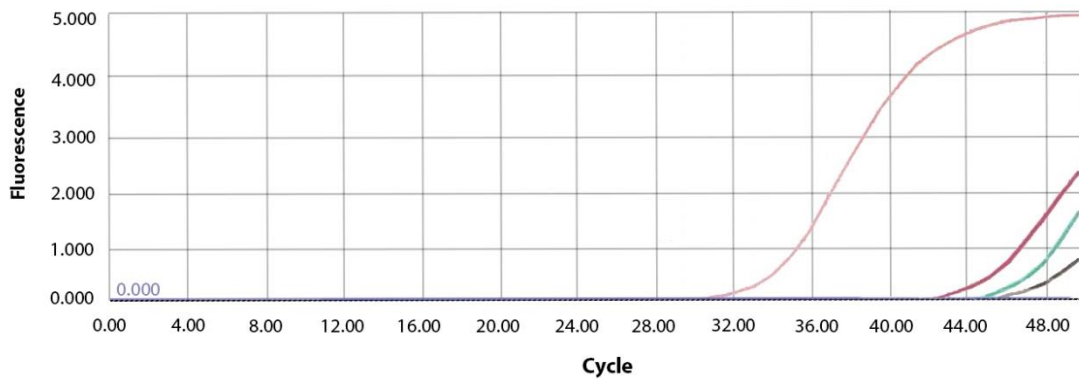
163 Among the total fifty samples, 8 were positive for *Rickettsia* spp amplification, accounting for 16%. A
 164 positive control result was consistent with the Cq values and melting peaks. A total of 84% of the samples
 165 were classified as negative due to nonspecific melt profiles or no amplification (Table 2, Figure 3, and
 166 Figure 4).

167

168 **Table 2.** Frequency of positive and negative samples for *Rickettsia* spp. among 50 cattle tested specimens

169 Categories	Positive	Negative
170 Count (Total = 50)	8	42
171 Frequency (%)	16%	84%

172

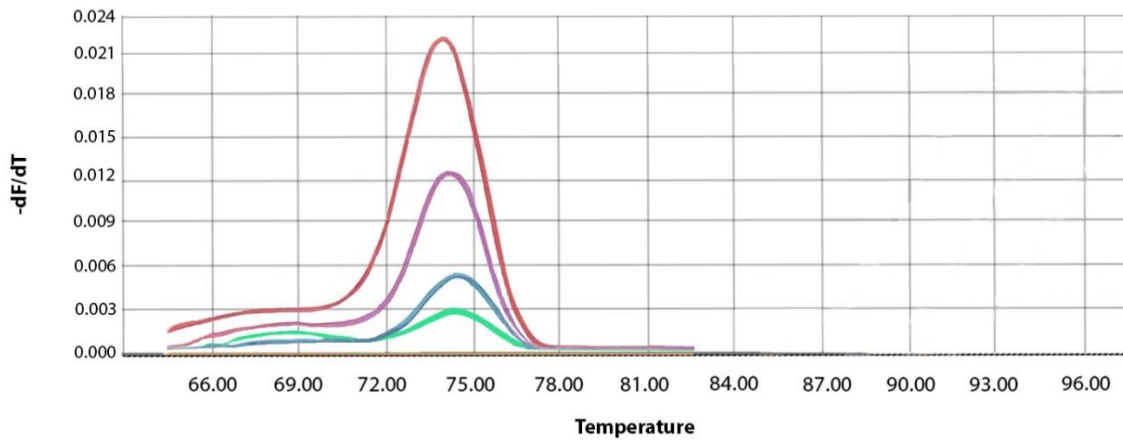


173

174 **Figure 3: qPCR amplification curves of tick samples from sheep and goats.**

175 Eight positive samples showed distinct Cq signals; the remaining samples were negative with flat baseline
 176 curves.

177



178

179 **Figure 4: Melt curve analysis of selected positive tick samples from cattle.**

180 Single, sharp melt peaks were observed in all positive samples, confirming the specificity of amplification
 181 and absence of primer-dimer or nonspecific products.

182

183 **3.2. Detection of *Rickettsia* DNA in Tick Samples from Sheep and Goats**

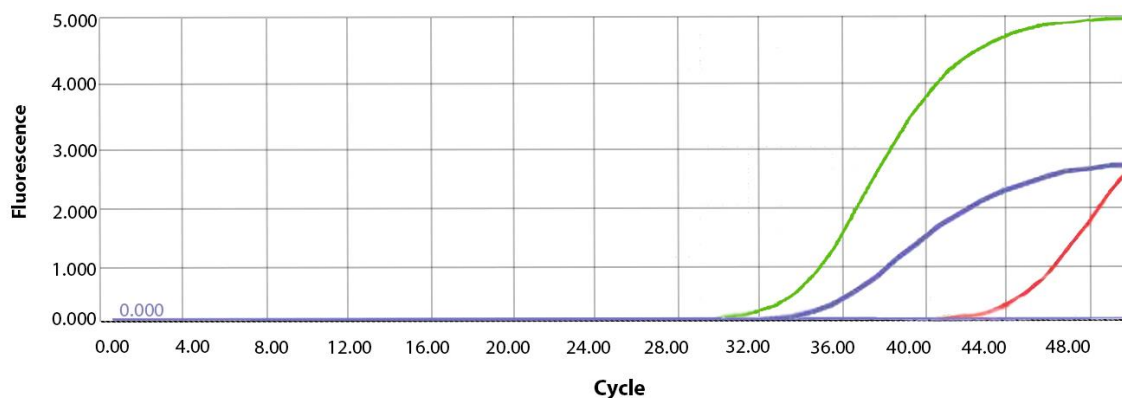
184 A total of 50 tick samples obtained from sheep and goats in Anbarabad County revealed the presence of
 185 seven *Rickettsia* DNA sequences. Seven samples are presented, which constitute fourteen percent of the
 186 entire total. The profiles of the melting curves indicated that the amplification was indeed specific.
 187 Furthermore, the amplification curves obtained from these samples exhibited exponential growth phases
 188 that corresponded with those of the positive control. Regarding the remaining 43 samples, which account
 189 for 86% of the total, there was no observed amplification signal or generic melting behavior (Table 3, Figure
 190 5, and Figure 6).

191

192 **Table 2.** Frequency of positive and negative samples for *Rickettsia* spp. among 50 Sheep and goat
 193 specimens

194 Categories	Positive	Negative
195 Count (Total = 50)	7	43
196 Frequency (%)	14%	86%

197

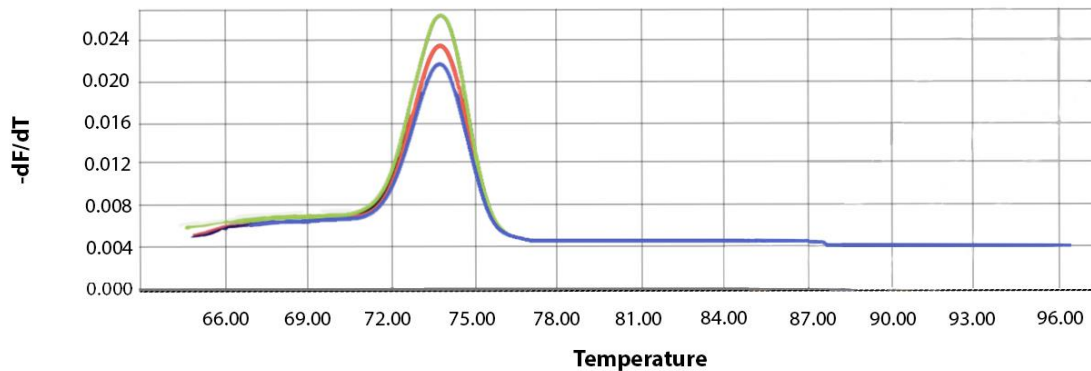


198

199 **Figure 5: qPCR amplification curves of tick samples from sheep and goats.**

200 Seven positive samples showed distinct Cq signals; the remaining samples were negative with flat
 201 baseline curves.

202



203

204 **Figure 6: Melt curve analysis of selected positive tick samples from sheep and goats.**

205 Single, sharp melt peaks were observed in all positive samples, confirming the specificity of amplification
 206 and absence of primer-dimer or nonspecific products.

207

208 **4. Discussion and Conclusion**

209 A total of one hundred tick specimens were collected from cattle, sheep, and goats in Anbarabad County,
 210 which is located in the southeast of Iran. The purpose of this research was to determine the prevalence of
 211 *Rickettsia* species. We were able to identify *Rickettsia* DNA in ticks from cattle as well as small ruminants
 212 by using a real-time PCR test that was based on SYBR Green and targeted the *gltA* gene. It was found that
 213 out of the fifty ticks that were collected from cattle, eight samples (16%) were positive, whereas forty-two
 214 samples (84%) were negative. Ticks taken from sheep and goats (n = 50) generated 7 positives (14%),
 215 whereas 43 negatives (86%). The validation of all favorable findings was achieved through melt curve
 216 analysis, which demonstrated that specific single-peak profiles matched those of the positive control. The
 217 identification of *Rickettsia* DNA in ticks gathered from cattle and small ruminants suggests that the
 218 pathogen is actively circulating in the area, highlighting the importance of implementing monitoring
 219 systems that include multiple species. Notably, the variation in infection rates among the host groups,
 220 recorded at 14% and 16%, was quite minimal.

221 Molecular research conducted in Iran indicates that the incidence of *Rickettsia* species varies significantly
 222 from one region to another. Approximately fifty percent of the cases are reported from Khuzestan, with *R.*
 223 *aeschlimannii*, *R. massiliae*, and *R. conorii* frequently identified during the infection. The disease exhibits
 224 a moderate incidence in northern provinces, with Golestan at 29.7%, and both Mazandaran and Gilan

225 provinces around 25%, as documented by Ghasemi et al. [13] in 2022. The presence of ecological
226 conditions conducive to tick vectors may account for this phenomenon. The study presented by Ghavami
227 et al. in 2024 [14] indicates that the prevalence rate in Zanzan stands at 6.44 percent. The total prevalence
228 observed in this research is 15% in Anbarabad, with 16% in cattle ticks and 14% in small ruminant ticks.
229 This aligns effectively with mid-range endemic areas, providing a sound estimate within the local
230 epidemiological context.

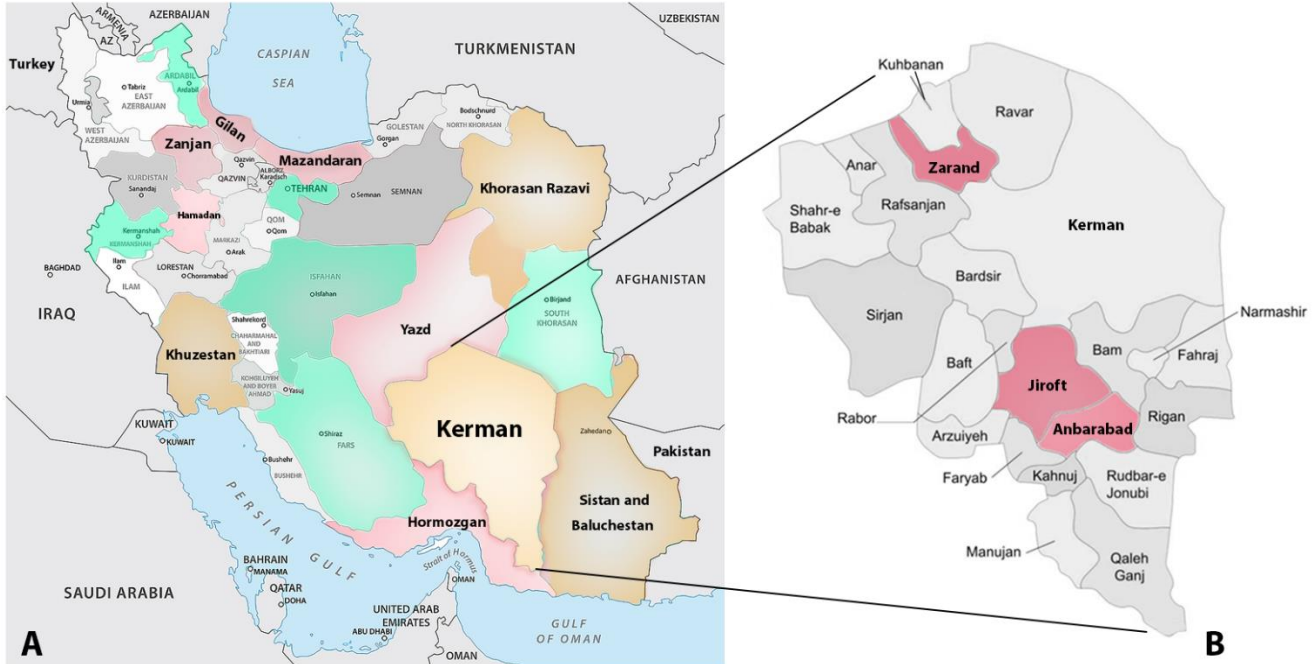
231 There is a relatively high frequency of this condition in Pakistan and Turkey, both of which are
232 geographically close to Iran, and the topic has been extensively researched. Ghafar et al. (2021) [15],
233 reported that Pakistan has 54 different species of ticks, the majority of which are *Haemaphysalis*,
234 *Hyalomma*, and *Rhipicephalus*. In the summer months, crossbred young calves exhibit elevated parasite
235 loads compared to older cattle. The connection between these strains and Turkish isolates suggests a
236 likelihood of their dissemination beyond national boundaries. In Turkey, a prevalence of up to 25 percent
237 has been recorded, with *R. aeschlimannii*, *R. slovaca*, and *R. raoultii* detected in ticks from both livestock
238 and wildlife [16]. These findings highlight the significant epidemiological relevance of Pakistan and Turkey
239 for Iran. In both countries, there is a genetic and epidemiological connection, which contributes to the
240 occurrence of this disease. Both countries are prone to the disease, and there may be a genetic link between
241 them that explains its occurrence.

242 A variety of *Rickettsia* species are responsible for significant tick-borne diseases, with their prevalence
243 influenced by ecological conditions and the specific species of vectors involved. According to Orkun et al.
244 (2014) [16], the strains of *R. helvetica* most frequently reported in Europe include *R. conorii*, *R. slovaca*,
245 and *R. helvetica*. Depending on the species of tick, infection rates range from 2% to 30% in Europe. Zhang
246 et al. (2024) [17] performed a comprehensive synthesis of more than 65 studies conducted in sub-Saharan
247 Africa, demonstrating that *R. africae* stands out as the most prevalent tick species identified in this area.
248 The occurrence of tick-borne pathogens (TBP) in cattle was documented at 52.2% as per the findings of
249 Tawana et al. (2022) [18]. In contrast Cossu et al. (2024) [19] reported that the prevalence of *Rickettsia* in
250 ticks stood at 18.4%.

251 Several studies conducted in Iran indicate that *Rickettsia* prevalence differs markedly based on host species,
252 geography, and tick genus. Qorbani et al. conducted a survey, which employed both the ELISA method and
253 *gltA*-targeted quantitative polymerase chain reaction (qPCR) method (2024) [20], akin to ours, revealing
254 the presence of *R. aeschlimannii* and *R. conorii* in ticks collected from cattle in Zarand and Jiroft. This
255 validated the regional distribution of these zoonotic agents. Our research builds upon their previous work
256 by presenting host-specific infection rates with a differentiation based on a larger sample size. The current

257 research employed SYBR Green-based real-time PCR, empowering the accurate identification of *Rickettsia*
258 DNA. Furthermore, the amplification profiles demonstrated reliability, exhibiting minimal background
259 noise, and the melt curve validation remained consistent. As a result of its many advantages, this technology
260 makes it ideal for field-based surveillance programs because of its high-throughput screening capabilities
261 and reduced risk of cross-contamination compared to traditional PCR and nested techniques. Although the
262 method does not achieve resolution at the species level without sequencing, it offers a reliable initial step
263 in the pathogen screening process. There have been a number of studies conducted in Iran, such as Ghasemi
264 et al. in 2017 [13], Mostafavi et al. 2022 [21], Ghavami et al. 2024 [14], and Qorbani et al. 2024 [20], that
265 have used sequencing of *ompA* or *gltA* amplicons in order to identify *Rickettsia* to the species level. When
266 it comes to mapping the zoonotic risk of certain *Rickettsia* lineages in Iranian cattle, the incorporation of
267 such genetic confirmation into future investigations will be very necessary.

268 *Rickettsia* species and public health detection in livestock-associated ticks are amplified in regions where
269 other high-risk tick-borne diseases, such as Crimean-Congo Hemorrhagic Fever (CCHF), are co-endemic.
270 It has been reported that the CCHF virus has been detected in *Hyalomma* ticks and livestock throughout
271 Iran, including Kerman Province, such as Jiroft. Farhadpour et al. [22] (2016) reported 6.6% and 4.5% in
272 the south of Iran. In contrast, Champour et al. [23] reported the 4 varieties of CCHF species in northeastern
273 Iran, as opposed to southeastern Iran, where Kerman is located, with *Hyalomma dromedarii* as the most
274 predominant detected tick species in camels, suggesting that *Hyalomma* and *Rhipicephalus* may be the main
275 vectors of the CCHF virus. Interestingly, *Rickettsia*'s primary vector is *Rhipicephalus*, known as the brown
276 dog tick. CCHF and *Rickettsia* infection often share similar clinical features like fever, malaise, and
277 headache, making it difficult to differentiate them. Tick-borne diseases are especially prevalent among
278 herders, veterinarians, and abattoir workers, who may be detected using differential molecular diagnostics.
279 The need for multiplex PCR panels that detect *Rickettsia*, CCHFV, and other tick-borne pathogens is
280 particularly acute in southeastern Iran. *Rickettsia* DNA is found in cattle (16%) and small ruminants (14%),
281 possibly due to similar tick species that parasitize them. There may be subtle differences due to different
282 grooming behaviors, immune responses, and preferences for vectors. To demonstrate the similarities
283 between *Rickettsia* and CCHFV, we conducted similar location studies and provided a comparative figure
284 that enabled us to better understand their similarities (Figure 7).



286 **Figure 7: Iranian provinces that have reported *Rickettsia* and Crimean-Congo Hemorrhagic Fever**
 287 **(CCHF) in the past 10 years (2015-2025).**

288 **A:** The provinces highlighted in red represent the regions affected by *Rickettsia*, while the green areas show
 289 the locations of CCHF reports in Iran. Interestingly, the major overlap between them, the orange provinces,
 290 indicates a similar host origin for these two diseases.

291 **B:** The current research in Anbarabad, located in Kerman province, on cattle, sheep, and goats (n=100, red
 292 highlighted), indicates the prevalence of *Rickettsia* in this region. Zarand is also reported to be affected by
 293 *Rickettsia* [24].

294

295 Although the locations of the two groups are separate and have a distance from each other, a common
 296 grazing environment and tick vector likely contributed to the detection of *Rickettsia* DNA in cow ticks
 297 (16%) and small ruminant ticks (14%) in the study area. In regions with tick-borne diseases, integration of
 298 multiplex PCR panels could improve surveillance precision and public health preparedness. Anbarabad has
 299 a warm climate, which favors tick vectors, which contribute to pathogen spread during the long grazing
 300 period. A high-risk ecological zone requires sensitive molecular tools, as well as improved diagnostic
 301 capabilities, such as sequencing, in Iranian livestock systems, to better understand pathogen diversity,
 302 transmission dynamics, and appropriate care and support.

303 In endemic areas such as southeastern Iran, the clinical similarities between spotted fever group *Rickettsia*
304 infections and CCHF create a significant diagnostic challenge. Both pathogens are transmitted by
305 Hyalomma ticks and present with initial clinical manifestations, including acute fever, cephalalgia, myalgia,
306 and general malaise, which complicates differentiation in the absence of laboratory confirmation.
307 *Rickettsial* infections often present with symptoms such as skin rashes, necrosis of tissues, and
308 lymphadenopathy. Symptoms associated with CCHF may often be disregarded or incorrectly diagnosed
309 during the evaluation process. This diagnostic uncertainty can lead to delays in appropriate treatment,
310 unnecessary isolation of patients, or improper care. The prompt identification of rickettsial infections holds
311 significant importance, as opposed to CCHF, these infections exhibit a swift response to antibiotic
312 treatment, with doxycycline being the preferred pharmacological agent and showcasing considerable
313 effectiveness when administered without delay [25]. Consequently, medical practitioners operating in
314 endemic regions ought to uphold a heightened level of vigilance for *Rickettsia* species in individuals
315 exhibiting fever, rash, and a background of tick exposure, especially when CCHF laboratory evaluations
316 are either negative or awaiting results. From a public health perspective, awareness initiatives and updated
317 clinical protocols are crucial to provide medical practitioners, veterinary professionals, and health officials
318 with decision-making tools that include both CCHF and rickettsioses in differential diagnostic assessments.
319 Improving molecular monitoring and integrating multiplex diagnostic systems that can simultaneously
320 detect *Rickettsia*, CCHFV, and multiple tick-borne pathogens will reduce diagnostic uncertainty. The timely
321 initiation of doxycycline in suspected cases, supported by epidemiological and clinical evidence, may
322 prevent complications, reduce mortality rates, and enhance trust in healthcare systems.

323 This study accurately identified a species of *Rickettsia*. In livestock systems, it is essential to implement
324 targeted tick surveillance due to the presence of ticks in southeastern Iran. The use of sensitive molecular
325 diagnostics across various host species revealed a moderate yet regionally consistent prevalence of
326 infection. These findings will enhance epidemiological maps in Anbarabad, Kerman Province, emphasizing
327 the critical importance of accurately identifying tick-borne pathogens for public health objectives. To
328 effectively address emerging zoonotic threats in high-risk ecological environments, the implementation of
329 sophisticated diagnostic tools and strategies centered on host genes will be essential.

330 **Acknowledgments**

331 The authors would like to express their gratitude to the Kerman University research deputy.

332 **Authors' contribution**

333 Conceptualization: [S.S., A.H., M.K.], ...; Methodology: [All Authors], ...; Formal analysis and
334 investigation: [All Authors], ...; Writing - original draft preparation: [All Authors]; Writing -
335 review and editing: [All Authors], ...; Funding acquisition: [M.K.], ...; Supervision: [M.K., A.M].

336 All authors checked and approved the final version of the manuscript for publication in the present
337 journal

338 **Ethics**

339 This study has been approved by the Ethics Committee of Kerman University of Medical Sciences.

340 The Ethic approval code is IR.KMU.AEC.1404.063

341 **Conflict of Interest**

342 The authors declare that they have no conflict of interest.

343 **Grant Support**

344 The authors confirm that they did not receive any financial assistance for the research, authorship,
345 and/or publication of this article

346 **Data availability**

347 The datasets generated during and/or analyzed during the current study are available from the
348 corresponding author on reasonable request.

349

350

351

352 **References**

- 353 1. Sadr S, Hajjafari A, Sazmand A, Santucci C, Masala G, Soroushianfar M, Nazemian S, Rahdar A,
354 Pandey S, Guettari M, Borji H. “Nanobiosensors for revolutionizing parasitic infections diagnosis: a critical
355 review to improve global health with an update on future challenges prospect.” *Europ J Med Res* vol. 30,1
356 484. 16 Jun. 2025. Doi: [10.1186/s40001-025-02685-2](https://doi.org/10.1186/s40001-025-02685-2)
- 357 2. Shaheen MN. The concept of one health applied to the problem of zoonotic diseases. *Rev Med Virol.*
358 2022;32(4):e2326. Doi: [10.1002/rmv.2326](https://doi.org/10.1002/rmv.2326)
- 359 3. Logiudice J, Alberti M, Ciccarone A, Rossi B, Tiecco G, De Francesco MA, et al. Introduction of vector-
360 borne infections in Europe: emerging and re-emerging viral pathogens with potential impact on One Health.
361 *Pathogens.* 2025;14(1):63. Doi: [10.3390/pathogens14010063](https://doi.org/10.3390/pathogens14010063)
- 362 4. Castelli M, Nardi T, Gammuto L, Bellinzona G, Sabaneyeva E, Potekhin A, et al. Host association and
363 intracellularity evolved multiple times independently in the Rickettsiales. *Nat Commun.* 2024;15(1):1093.
364 Doi: [10.1038/s41467-024-45351-7](https://doi.org/10.1038/s41467-024-45351-7)
- 365 5. Dixit R, Manikandan S, Gopalan N, Mohanty BS, Behera SK. Temporal trends in diagnostic evolutions
366 for rickettsial diseases including scrub typhus: a bibliometric study. *Pathog Glob Health.* 2025;119(3-
367 4):75–86. Doi: [10.1080/20477724.2025.2475278](https://doi.org/10.1080/20477724.2025.2475278)
- 368 6. Pustijanac E, Buršić M, Millotti G, Paliaga P, Iveša N, Cvek M. Tick-borne bacterial diseases in Europe:
369 threats to public health. *Eur J Clin Microbiol Infect Dis.* 2024;43(7):1261–95. Doi: [10.1007/s10096-024-
370 04836-5](https://doi.org/10.1007/s10096-024-04836-5)
- 371 7. Addo SO, Amoah S, Unicorn NM, Kyeremateng ET, Desewu G, Obuam PK, et al. Molecular detection
372 of tick-borne pathogens in Kumasi: with a first report of zoonotic pathogens in abattoir workers. *Biomed*
373 *Res Int.* 2024;2024:4848451. Doi: [10.1155/2024/4848451](https://doi.org/10.1155/2024/4848451)
- 374 8. Dantas-Torres F, de Sousa-Paula LC, Otranto D. The *Rhipicephalus sanguineus* group: updated list of
375 species, geographical distribution, and vector competence. *Parasites Vectors.* 2024;17:540. Doi:
376 [10.1186/s13071-024-06572-3](https://doi.org/10.1186/s13071-024-06572-3)
- 377 9. Ghodrati S, Lesiczka PM, Zurek L, Szekely F, Modrý D. *Rhipicephalus sanguineus* from Hungarian
378 dogs: tick identification and detection of tick-borne pathogens. *Vet Parasitol Reg Stud Reports.*
379 2024;50:101007. Doi: [10.1016/j.vprsr.2024.101007](https://doi.org/10.1016/j.vprsr.2024.101007)
- 380 10. Hart TM, Sonnert ND, Tang X, Chaurasia R, Allen PE, Hunt JR, et al. An atlas of human vector-borne
381 microbe interactions reveals pathogenicity mechanisms. *Cell.* 2024;187:4113–27.e13. Doi:
382 [10.1016/j.cell.2024.05.023](https://doi.org/10.1016/j.cell.2024.05.023)
- 383 11. Carbone G, De Bona A, Septelici D, Cipri A, Nobile A, Esposito S. Beyond mosquitoes: a review of
384 pediatric vector-borne diseases excluding malaria and arboviral infections. *Pathogens.* 2025;14:553. Doi:
385 [10.3390/pathogens14060553](https://doi.org/10.3390/pathogens14060553)
- 386 12. Levine ZC, Sene A, Mkandawire W, Deme AB, Ndiaye T, Sy M, et al. Investigating the etiologies of
387 non-malarial febrile illness in Senegal using metagenomic sequencing. *Nat Commun.* 2024;15:747. Doi:
388 [10.1038/s41467-024-44800-7](https://doi.org/10.1038/s41467-024-44800-7)

- 389 13. Ghasemi A, Latifian M, Esmaeili S, Naddaf SR, Mostafavi E. Molecular surveillance for *Rickettsia* spp.
390 and *Bartonella* spp. in ticks from Northern Iran. *PLoS One*. 2022;17:e0278579. Doi:
391 [10.1371/journal.pone.0278579](https://doi.org/10.1371/journal.pone.0278579)
- 392 14. Ghavami MB, Alibabaei Z, Jamavar MR, Taghiloo B. Emergent spotted fever group *Rickettsiae*
393 infections among hard ticks in the Islamic Republic of Iran. *East Mediterr Health J*. 2024;30(2):145–55.
394 Doi: [10.26719/emhj.24.030](https://doi.org/10.26719/emhj.24.030)
- 395 15. Ghafar A, Gasser RB, Abbas T, Rehman A, Gauci CG, Jabbar A. Ticks and tick-borne diseases of
396 bovines in a smallholder livestock context: the Pakistani example. *Adv Parasitol*. 2021;114:167–244. Doi:
397 [10.1016/bs.apar.2021.08.009](https://doi.org/10.1016/bs.apar.2021.08.009)
- 398 16. Orkun Ö, Karaer Z, Çakmak A, Nalbantoğlu S. Spotted fever group *Rickettsiae* in ticks in Turkey. *Ticks*
399 *Tick Borne Dis*. 2014;5(2):213–8. Doi: [10.1016/j.ttbdis.2012.11.018](https://doi.org/10.1016/j.ttbdis.2012.11.018)
- 400 17. Zhang EY, Kalmath P, Abernathy HA, Giandomenico DA, Nolan MS, Reiskind MH, et al. *Rickettsia*
401 *africae* infections in sub-Saharan Africa: a systematic literature review of epidemiological studies and
402 summary of case reports. *Trop Med Int Health*. 2024;29(7):541–83. Doi: [10.1111/tmi.14002](https://doi.org/10.1111/tmi.14002)
- 403 18. Tawana M, Onyiche TE, Ramatla T, Mtshali S, Thekisoe O. Epidemiology of ticks and tick-borne
404 pathogens in domestic ruminants across the Southern African Development Community (SADC) region
405 from 1980 until 2021: a systematic review and meta-analysis. *Pathogens*. 2022;11(8):929. Doi:
406 [10.3390/pathogens11080929](https://doi.org/10.3390/pathogens11080929)
- 407 19. Cossu CA, Cassini R, Bhoora RV, Menandro ML, Oosthuizen MC, Collins NE, et al. Occurrence and
408 molecular prevalence of *Anaplasmataceae*, *Rickettsiaceae* and *Coxiellaceae* in African wildlife: a
409 systematic review and meta-analysis. *Prev Vet Med*. 2024;230:106257. Doi:
410 [10.1016/j.prevetmed.2024.106257](https://doi.org/10.1016/j.prevetmed.2024.106257)
- 411 20. Qorbani A, Khalili M, Nourollahifard S, Mostafavi E, Farrokhnia M, Esmaeili S. Diversity of *Rickettsia*
412 species in collected ticks from southeast Iran. *BMC Vet Res*. 2024;20(1):279. Doi: [10.1186/s12917-024-](https://doi.org/10.1186/s12917-024-04142-4)
413 [04142-4](https://doi.org/10.1186/s12917-024-04142-4)
- 414 21. Mostafavi SM, Khalili M, Akhtardanesh B, Nourollahifard SR, Esmaeili S. *Rickettsia* spp. in
415 *Rhipicephalus sanguineus* sensu lato ticks collected from stray dogs in Kerman city, Iran. *Ticks Tick Borne*
416 *Dis*. 2022;13(5):101985. Doi: [10.1016/j.ttbdis.2022.101985](https://doi.org/10.1016/j.ttbdis.2022.101985)
- 417 22. Farhadpour F, Telmadarraiy Z, Chinikar S, Akbarzadeh K, Moemenbellah-Fard MD, Faghihi F, et al.
418 Molecular detection of Crimean–Congo haemorrhagic fever virus in ticks collected from infested livestock
419 populations in a new endemic area, south of Iran. *Trop Med Int Health*. 2016;21(3):340–7. Doi:
420 [10.1111/tmi.12667](https://doi.org/10.1111/tmi.12667)
- 421 23. Champour M, Chinikar S, Mohammadi G, Razmi G, Shah-Hosseini N, Khakifirouz S, et al. Molecular
422 epidemiology of Crimean–Congo haemorrhagic fever virus detected from ticks of one-humped camel
423 (*Camelus dromedarius*) populations in northeastern Iran. *J Parasitol Dis*. 2016;40(1):110–5. Doi:
424 [10.1007/s12639-014-0458-y](https://doi.org/10.1007/s12639-014-0458-y)

- 425 24. Qorbani A, Khalili M, Nourollahifard S, Mostafavi E, Farrokhnia M, Esmaeili S. An update on spotted
426 fever group serology in Kerman Province, Iran. *Comp Immunol Microbiol Infect Dis*. 2022;88:101862. Doi:
427 [10.1016/j.cimid.2022.101862](https://doi.org/10.1016/j.cimid.2022.101862)
- 428 25. Camprubí-Ferrer D, Díaz Menendez M, Crespillo-Andújar C, Galparsoro HA, Belhassen-García M,
429 Cuadros González J, et al. Executive summary of the Spanish guidelines for the diagnosis and management
430 of imported febrile illnesses from the Spanish Society of Tropical Medicine and International Health
431 (SEM-TSI), the Imported Pathology Group of the Spanish Society of Infectious Diseases and Clinical
432 Microbiology (GEPI-SEIMC), the Spanish Society of Family and Community Medicine (SEMFYC), the
433 Spanish Society of Primary Care Physicians (SEMERGEN) and the Spanish Society of Emergency
434 Medicine (SEMES). *Enferm Infecc Microbiol Clin (Engl Ed)*. 2024;42(7):380–5. Doi:
435 [10.1016/j.eimce.2024.05.011](https://doi.org/10.1016/j.eimce.2024.05.011)