https://doi.org/10.18502/jad.v20i2.22397

Original Article

The GroEL Gene Molecular Marker in the Detection of Pathogens *Rickettsia conorii* subspecies *indica* at the Species Level in the Ixodidae Ticks Collected from Domestic Animals in Different Agro-Climatic Regions in Tamil Nadu, India

Soundaramourthy Padmaja, *Ayyanar Elango

Division of Vector Biology and Control, ICMR-Vector Control Research Centre, Department of Health Research, Ministry of Health and Family Welfare, GOI, Medical Complex, Indira Nagar, India

***Corresponding author:** Dr Ayyanar Elango, E-mail: elangoar@yahoo.co.in

(Received 25 Jan 2026; accepted 16 May 2026)

Abstract

Background: Indian tick typhus (ITT) is a type of spotted fever caused by intracellular *Rickettsial* pathogens, specifically *Rickettsia conorii* subspecies *indica*, which exhibits unique clinical symptoms in humans compared to other spotted fevers. Human cases have been reported from several states based on hospital-based studies. However, research on the detection of these pathogens in ticks is limited.

Methods: A tick survey was conducted in different agro-climatic regions of Tamil Nadu. A total of 3,016 ticks belonging to 12 species in 532 pools were tested for the presence of the groEL gene of *Rickettsia conorii indica* using indigenously designed primers.

Results: Of the 532 pools tested, 44 pools (8.27 %) were positive for the groEL gene, resulting in an overall Minimum Infection Rate (MIR) of 1.50 (95% CI=1.11–1.98). The bacteria were identified in all agro-climatic regions except in the Cauvery delta region. The MIR of the screened tick species were 4.11 (95% CI=1.73–8.43) in *Rhipicephalus microplus*, 1.34 (95% CI=0.90–1.92) in *Haemaphysalis bispinosa*, and 2.85 (95% CI=1.56–4.83) in *Haemaphysalis intermedia*. The MIR of the ticks collected from cattle was 0.34 in cattle (95% CI=0.15–0.67), 5.50 (95% CI=3.97–7.44) in goats, and 1.37 (95% CI=0.36–3.71) in sheep.

Conclusion: The present study reports that the groEL gene as preliminary marker for the detection of the ITT-causing pathogen in field-collected ticks. The positivity of the groEL gene in the salivary glands needs further experimental confirmation. The primer needs further evaluation by comparing with MLST (Multilocus sequence typing) sequencing.

Keywords: *Haemaphysalis bispinosa*; Spotted fever group; Agro-climatic region; *Rhipicephalus sanguineus*; groEL

Introduction

Rickettsial diseases are emerging globally and are caused by various bacteria belonging to six genera within the order Rickettsiales. These bacteria are transmitted to humans through the bite of ticks, mites, fleas, or lice. There are three major types of *Rickettsia* species classified as the spotted fever group (SFG), the typhus group (TG), and the scrub typhus group (SG) based on serological characteristics (1). Globally, the Spotted fever group includes 26 species of *Rickettsia*, of which 16 are known to transmit to humans and cause zoonotic disease through Ixodidae ticks (2). Mediterranean spotted fever is caused by *Rickettsia conorii*, with *Rhipicephalus sanguineus* serving as the vector for transmitting this bacterium to humans (3). The *Rickettsia conorii* group contains five subspecies: *R. conorii conorii*, *R. conorii caspia*, *R. conorii israelensis*, *R. conorii indica*, and *R. conorii raoulti* as determined by Multilocus Sequence Typing (4, 40). *Rickettsia conorii* subspecies *indica* is a potential causative agent of Indian Tick Typhus (ITT) in India (5). This *Rickettsia* species was isolated in 1950 from *Rh. sanguineus* collected in India. The clinical manifestations of the disease differ from those

http://jad.tums.ac.ir
Published Online: June 30, 2026

Copyright © 2026 The Authors. Published by Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0/). Non-commercial uses of the work are permitted, provided the original work is properly cited.

https://doi.org/10.18502/jad.v20i2.22397

of Mediterranean Spotted Fever, presenting with a rash that includes purpura and eschar at the site of the bite (6,7). However, there have been no reported isolations of *R. conorii indica* from patients in India. The complete genome sequence of *R. conorii indica* was carried out in 2012 (5).

Before the development of the Enzyme–Linked Immunosorbent Assay (ELISA), the Weil-Felix test was used to detect antibodies for *Rickettsia*, which reacts with the Proteus vulgaris OX2 and OX19 antigen for diagnosing spotted fever (8). The test was primarily used for initial screening of suspected acute febrile illnesses, although it exhibited lower sensitivity and specificity. Following the introduction of ELISA for the diagnosis of various diseases, the detection of spotted fever cases increased significantly. The first human case was reported in a French traveller who visited India, and no cases had been detected using either molecular or strain-specific antibody tests in India (9). Subsequently, serological tests identified *R. conorii* in human cases reported from various states, including Karnataka (10,11), Uttar Pradesh (12), Puducherry (13, 14), Andhra Pradesh (15, 16), Tamil Nadu (17, 18), Odisha (19) and Chandigarh (20), using IgM/IgG ELISA. Molecular detection of *R. conorii* in human cases has been reported from Tamil Nadu (21) and Gorakhpur (22) using Polymerase Chain Reaction (PCR).

There are currently no species-specific molecular markers available for *Rickettsia* species. Multilocus sequence typing (MLST) utilizing molecular markers such as 16S rRNA, gltA, ompA, ompB and sca4 has been employed to differentiate between closely related *Rickettsia* species (4, 23–25). At present, the only available technique for confirmation of *R. conorii indica* is the minimum sequence pairwise sequence similarity of 100%, 100%, 99.9%, 99.9% and 100% for 16S rDNA, glt A, omp A, omp B and sca 4 genes, respectively, by MLST (37). Studies showed that the *Rickettsia* Indian Tick typhus strain showed nucleotide similarity of 100%, 100% and 99.4 with the *R. conorii* strain Malish 7 molecular markers *atpA*, *dnaK* and 17-kDa protein, respectively. Further, the *Rickettsia* Indian Tick typhus strain showed nucleotide similarity of 100%, 100% and 99.9% with the *R. conorii indica* reference strain *ompA* and *ompB*, respectively (26). However, it is difficult to amplify all the marker genes from a single sample and to sequence all five markers for surveillance of the pathogen in the tick samples. Therefore, it is essential to develop a single molecular marker for the identification of *R. conorii indica*. The GroEL genes, which belong to the chaperone family that encodes the 60-kDa heat shock protein, have been used as a molecular marker for the differentiation of *Rickettsia* spp (27). These genes are conserved in both prokaryotes and eukaryotes and serve as housekeeping genes essential for cell survival. The present study aims to develop a single molecular marker for the detection of *R. conorii indica* using the groEL gene. The present study aims to develop and test a species-specific primer from the complete genome of *R. conorii indica*.

Materials and Methods

Tick survey on domestic animals

A tick survey was conducted in various agroclimatic regions of India from 2021 to 2023, with species diversity reported elsewhere (28). A total of 8068 ticks were collected from domestic animals. The ticks were identified at the species level, and the ticks were pooled from 1 to 25 ticks based on the village, host, and sex. From these, the pools containing a minimum of 1 to a maximum of 9 ticks were selected for the present study (29, 30). A total of 532 pools consisting of 3016 (36%) ticks belonging to 12 species were selected randomly. DNA extraction was carried out according to the standard protocol described in previous studies (31), and the extracted DNA was stored at -70 °C until processing. One tick that tested positive for groEL was dissected to obtain the

https://doi.org/10.18502/jad.v20i2.22397

salivary glands and ovaries for pathogen screening. DNA was extracted from both the salivary and ovarian glands.

GroEL primer design with Primer3

The complete genome of *R. conorii indica* is available under the whole-genome shotgun project as NCBI Reference Sequence: NZ_ AJHC01000007.1. The groEL gene is located within the sequence from 955 to 2601. The sequence was downloaded, and primers were designed using the Primer3 online tool. A primer sequence with an expected amplicon size of 401bp was selected for screening the DNA samples.

DNA extraction

The DNA was extracted from a tick specimen using a modified manual method described in previous studies (31). Briefly, the tick specimens were removed from alcohol and washed three times with sterilized water. The tick specimens were then transferred to a 1.5 mL tube containing lysis buffer and 2 mm glass beads. The tubes were shaken at 2000 rpm for four hours at room temperature. After shaking, the ticks were removed, and the lysate was transferred to silica suspension pre-equilibrated with 200µl of binding buffer. Following this, 500µl of absolute ethanol was added and vortexed for one minute. The tubes were centrifuged at 11,000 rpm for one minute, and the supernatant was decanted. Subsequently, 500µl of protein wash buffer was added and vortexed for one minute. The supernatant was removed by decanting, and the wash buffer was added again. The tubes were vortexed for one minute and centrifuged at 11,000 rpm for one minute. The supernatant was decanted, and 500µl of absolute alcohol was added. The tubes were vortexed for one minute and centrifuged for one minute at 11,000 rpm. The alcohol was decanted, and the tubes were incubated at 52 °C for five minutes to remove any remaining alcohol. Finally, the DNA was eluted by adding 60 µl of ribonuclease-free water.

Conventional PCR

The conventional PCR reaction was standardized for detecting *R. conorii indica* using the groEL gene with indigenously designed primers. Briefly, the 25µl PCR reaction mixture consists of 12.5µl Ampliqon Master mix (Amplicon A/S, Odense M, Denmark), 1µl of each forward (5'TACAAGAGCGGCAGTTGAGG3') and reverse (5'TTGGCATTGGCTCTGCCTTA3') primer, 10.5µl of RNase-free water, and 1µl of DNA as the template for PCR. The PCR was conducted on an Eppendorf thermocycler with 35 cycles, each consisting of an initial denaturation at 95 °C for 5 minutes, followed by annealing at 57 °C for 1 minute, and extension at 72 °C for 1 minute. The final extension was performed at 72 °C for 10 minutes. A positive control for *R. conorii indica* groEL was obtained from a previous tick DNA source, which has been confirmed by sequencing. The PCR product was analyzed using 1.5% agarose gel, and the results were documented using a gel documentation system. The positive amplicon was excised with a gel cutter and purified using the Macherey-Nagel™ NucleoSpin™ gel kit. Cycle sequencing was performed with the forward and reverse primers separately using the Big–Dye terminator kit, and the reaction was further purified with the NucleoSpin Gel and PCR Clean-up Mini Kit. Sanger sequencing was conducted using the Agilent 2100 Bioanalyzer. The consensus sequence was obtained using BioEdit software. The cleaned consensus sequence was subjected to a BLAST search against the NCBI database. The confirmed sequences were aligned with reference sequences of the *R. conorii indica* groEL gene (NZ_AJHC01000007.1). The sequences were submitted to NCBI through Bankit.

Minimum infection rate (MIR) calculation

The minimum infection rate of pooled ticks was calculated using an Excel Add-in (32). The calculation was based on the ratio of the number of positive pools to the total number of specimens tested (33, 34).

https://doi.org/10.18502/jad.v20i2.22397

Number of positive pools
Minimum Infection Rate (MIR) =------- x 100
Total specimens tested

Results

A total of 532 pools containing 3,016 ticks belonging to 12 tick species collected from domestic animals across seven agro-climatic regions of Tamil Nadu were screened with conventional PCR with indigenously designed primers for the *R. conorii indica* groEL gene. Of these, 44 pools were found to be positive with an expected amplicon size of 401 bp (Fig. 1), resulting in a MIR of 1.50 (95% CI=1.11–1.98). Among these 44 pools, the groEL gene was detected in *Rh. microplus*, *Ha. bispinosa* and *Ha. intermedia*. The highest MIR was observed in *Rh. microplus* (4.11, 95% CI=1.73–8.43) (Table 1). Of these 44 positive pools, four amplicons were sequenced and confirmed the presence of *R. conorii indica*. Among these 44 positive pools positive for groEL, one pool tested positive in each of the salivary gland and ovary dissected from *Ha. bispinosa*. The consensus sequence was submitted to NCBI, where it showed 100 % similarity with the groEL gene. The groEL gene was identified in ticks collected from cattle, goats, and sheep. The highest MIR was found in goats (5.50; 95% CI= 3.97–7.44) (Table 2). The groEL gene was positive in all agro-climatic regions except for the Cauvery delta. The highest MIR was reported from the High rainfall region (3.89; 95 % CI=2.68–5.46) (Table 3). Among the different life cycle stages of ticks, including male, female, nymph and larva, the male exhibited the highest MIR (1.77; 95% CI=1.18–2.55) (Table 4).

NCBI accession number

The expected band was sequenced and submitted to NCBI. The sequences deposited in GenBank- one each from the male, female, salivary gland and ovary- were accession numbers: PV534567.1, PV066075.1, PV247038.1, and PV247037.1, respectively.

Phylogenetic tree

A phylogenetic tree was constructed using the Neighbor-Joining method, with 1,000 bootstrap replications performed in MEGA 7 software for *R. conorii indica* (Fig. 2).

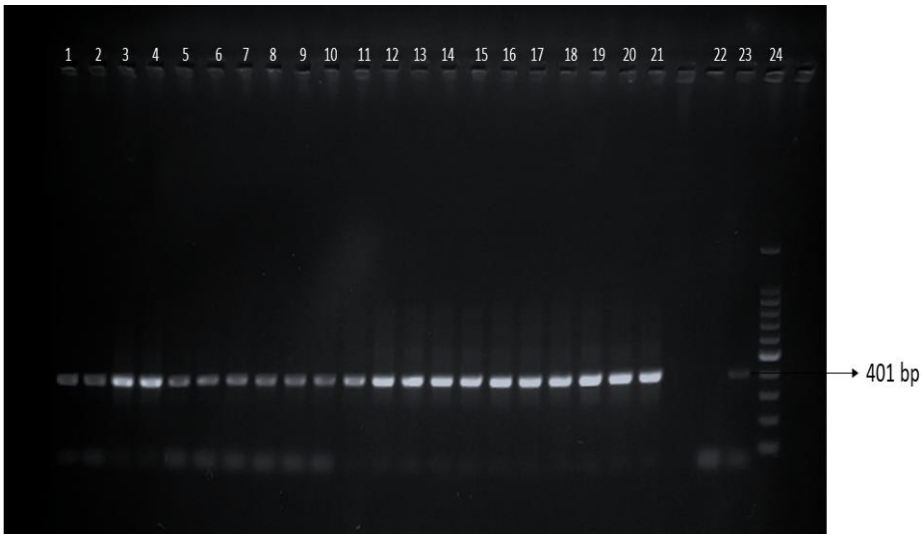


Fig. 1. PCR amplification of *Rickettsia conorii* subspecies *indica* groEL gene in the tick species. Legend lane 1–8: *Haemaphysalis bispinosa*, lane 9–16: *Rhipicephalus microplus*, lane 17–21: *Ha. intermedia*, lane 22: negative control, lane 23: positive control, lane 24: DNA size marker 100bp

http://jad.tums.ac.ir
Published Online: June 30, 2026

https://doi.org/10.18502/jad.v20i2.22397

Table 1. *Rickettsia conorii* subspecies *indica* infection rate in different tick species collected from domestic animals in Tamil Nadu, India, 2021–2023

Species	No. of Ticks	No. of Pools tested	No. of +ve Pools (%)	MIR	95% Confidence Interval (CI)	
					Lower Limit	Upper Limit
Amblyomma integrum	20	5	0 (0)	0.00	0.00	12.26
Rhipicephalus annulatus	67	8	0 (0)	0.00	0.00	4.57
Rhipicephalus microplus	163	21	6 (28.5)	4.11	1.73	8.43
Haemaphysalis bispinosa	1954	357	26 (7.2)	1.34	0.90	1.92
Haemaphysalis intermedia	465	56	12 (21.4)	2.85	1.56	4.83
Haemaphysalis spinigera	8	1	0 (0)	0.00	0.00	17.89
Hyalomma anatolicum	66	11	0 (0)	0.00	0.00	4.75
Hyalomma hussaini	34	6	0 (0)	0.00	0.00	7.90
Hyalomma marginatum	4	2	0 (0)	0.00	0.00	39.18
Rhipicephalus haemaphysaloides	195	49	0 (0)	0.00	0.00	1.85
Rhipicephalus sanguineus	16	8	0 (0)	0.00	0.00	14.62
Rhipicephalus simus	24	8	0 (0)	0.00	0.00	11.54
	3016	532	44 (8.2)	1.50	1.11	1.98



Fig. 2. The Phylogenetic tree shows the relationship between the sequence of the groEL molecular marker of *Rickettsia conorii* subspecies *indica* and other tick-borne pathogens. A phylogenetic tree was constructed using MEGA software

● Reference gene; ◆ The present study sequences; ▲ Sequences from other studies

http://jad.tums.ac.ir
Published Online: June 30, 2026

https://doi.org/10.18502/jad.v20i2.22397

Table 2. *Rickettsia conorii* subspecies *indica* infection rate in different domestic animals in Tamil Nadu, India, 2021–2023

Host	No. of Ticks	No. of Pools tested	No. of +ve Pools (%)	MIR	95% Confidence Interval (CI)	
					Lower Limit	Upper Limit
Buffalo	28	6	0 (0)	0.00	0.00	9.38
Cow	2073	347	7 (2.0)	0.34	0.15	0.67
Dog	25	3	0 (0)	0.00	0.00	9.39
Goat	663	138	34 (24.6)	5.50	3.97	7.44
Sheep	227	38	3 (7.8)	1.37	0.36	3.71
	3016	532	44 (8.2)	1.50	1.11	1.98

Table 3. *Rickettsia conorii* subspecies *indica* infection rate in different agro-climatic regions of Tamil Nadu, India, 2021–2023

Agro-climatic region	No. of Ticks	No. of Pools tested	No. of +ve Pools (%)	MIR	95% Confidence Interval (CI)	
					Lower Limit	Upper Limit
Cauvery Delta	447	77	0 (0)	0.00	0.00	0.83
High rainfall	709	138	27 (19.5)	3.89	2.68	5.46
Hilly region	185	34	2 (5.8)	1.11	0.20	3.62
North Eastern	517	86	2 (2.3)	0.39	0.07	1.28
North Western	552	87	3 (3.4)	0.55	0.15	1.49
Southern Zone	410	74	5 (6.7)	1.27	0.47	2.80
Western Zone	196	36	5 (13.8)	2.74	1.03	6.02
	3016	532	44 (8.2)	1.50	1.11	1.98

Table 4. *Rickettsia conorii* subspecies *indica* infection rate in different life cycle stages of ticks collected from domestic animals in Tamil Nadu, India, 2021–2023

Sex	No. of Ticks	No. of Pools tested	No. of +ve Pools (%)	MIR	95% Confidence Interval (CI)	
					Lower Limit	Upper Limit
Female	1297	230	17 (7.3)	1.33	0.81	2.07
Larvae	39	9	0 (0)	0.00	0.00	7.24
Male	1462	256	25 (9.7)	1.77	1.18	2.55
Nymph	218	37	2 (5.4)	0.94	0.17	3.08
	3016	532	44 (8.2)	1.50	1.11	1.98

Discussion

Indian Tick Typhus cases have been reported in India since 1925 (35) based on clinical and serological methods. The cases, which have been reported sporadically in different regions of India, include those from the Spotted fever group caused by *Rickettsia* species. These species are associated with arthropods, and some are transmitted to humans by hard tick genera such as *Amblyomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes* and *Rhipicephalus*. Transovarial transmission and co-feeding among hard ticks are the main factors in maintaining *Rickettsia* species in nature (36, 37).

Among the spotted fever group of *Rickettsia,* studies on *R. conorii indica* in ticks are quite limited. This pathogen was first isolated from the *Rh. sanguineus* tick species in 1950 and was later isolated from the French travel-

https://doi.org/10.18502/jad.v20i2.22397

lers who visited India (9). However, it has never been isolated from human cases in India. This lack of isolation is due to the absence of a fact-specific molecular marker for directly identifying *Rickettsia* at the species level. Serological methods such as ELISA have indicated the prevalence of *R. conorii* in humans in several studies (38), but it has never been isolated from a human sample. Additionally, information regarding vectors other than *Rh. sanguineus* that transmit *R. conorii indica* is lacking. Employing recent molecular techniques to study this bacterium may enhance detection rates.

Rhipicephalus sanguineus is the primary vector for transmitting *Rickettsia*, particularly *R. conorii*. Other genera involved in transmission include *Haemaphysalis* and *Hyalomma* (11). *Rickettsia conorii* has been identified in *Rh. microplus* and *Rhipicephalus haemaphysaloides* in Maharashtra (39). The *R. conorii* subspecies *raoulti* has been reported from the *Ha. intermedia* tick species in Tamil Nadu, India, using the MLST technique (40). The Spotted Fever Group primers, such as ompA, are more effective at differentiating closely related *Rickettsia* species than the *glt*A and 16S rRNA (27). The GroEL gene marker has been used for the identification of bacterial species, including *Streptococcus* (41), *Bartonella* (42), *Ehrlichia* (43) and *Rickettsia* (27). Further, the GroEL gene has better differentiation of *Rickettsia* and STG (spotted fever group) rickettsiae than the gltA and ompA gene analysis (27). The present study developed a preliminary molecular marker using the groEL gene for the identification of *R. conorii indica*. in the genome extracted from whole samples and tissues of the ovary and salivary gland of ticks. Phylogenetic tree analysis indicates that the groEL gene of *R. conorii* indica is closely related to *R. sibirica* strain (27). However, a further confirmation study is essential to confirm *R. conorii indica* using MLST sequencing. In addition, a PCR-RFLP pattern analysis of the positive samples is necessary for confirmation of *Rickettsia*.

Conclusions

The present study demonstrated that the preliminary marker for the detection of *R. conorii indica* using the groEL marker by PCR in the tick population in India. The groEL gene serves as a molecular marker for distinguishing this species from other *Rickettsia* at the species level. Further evaluation of this preliminary marker on various tick species collected from domestic animals is needed. The main limitation of the present study is that the positive samples were not confirmed with regular MLST typing. Therefore, the primer should be evaluated in the future by comparing the groEL-confirmed samples with MLST sequencing.

Acknowledgements

We really appreciate the great help provided by Mr M Stalin, Sr. Technician 2, Mr N Ramesh, Lab Assistant and Mr B Tilagar, Puducherry, India, for collecting ticks from domestic animals in the field. The project is funded through the internal funds of the ICMR-VCRC, Puducherry, India.

Ethical consideration

Institutional ethics committee approval was obtained from ICMR-VCRC, Puducherry, India, Animal ethics committee (ICMR-VCRC/IAES/2020/1).

Conflict of interest statement

The authors declare that they have no competing interests. This study was supported by the Indian Council of Medical Research - Vector Control Research Centre (ICMR-VCRC), project no. IM2005.

References

1. Ravishankar V, Narayanan S, Shandil RK

https://doi.org/10.18502/jad.v20i2.22397

(2024) Rickettsial infections: prevalence and diagnosis of scrub typhus in India. Front Trop Dis. 27: 5.
2. Seidi S, Omidi AH, Esmaeili S (2024) Distribution of different *Rickettsia* species in countries of the WHO Eastern Mediterranean (WHO-EMRO) region: An overview. Travel Med Infect Dis. 58: 102695.
3. Latifian M, Khalili M, Farrokhnia M, Mostafavi E, Esmaeili S (2022) *Rickettsia conorii* subsp. *israelensis* infection: A case report from southeast Iran. BMC Infect Dis. 22(1): 320.
4. Zhu Y, Fournier PE, Eremeeva M, Raoult D (2005) Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol. 14: 5–11.
5. Sentausa E, El Karkouri K, Robert C, Raoult D, Fournier PE (2012) Genome sequence of *Rickettsia conorii* subsp. *indica*, the agent of Indian Tick Typhus. J Bacteriol. 194(12): 3288–3289.
6. Jayaseelan E, Rajendran SC, Shariff S, Fishbein D, Keystone JS (1991) Cutaneous eruptions in Indian Tick Typhus. Int J Dermatol. 30(11): 11.
7. Murali N, Pillai S, Cherian T, Raghupathy P, Padmini V, Mathai E (2001) Rickettsial infections in South India - how to spot the spotted fever. Indian Pediatr. 38(12): 1393–1396.
8. Amano K, Hatakeyama H, Okuta M, Suto T, Mahara F (1992) Serological studies of antigenic similarity between Japanese spotted fever *Rickettsiae* and Weil-Felix test antigens. J Clin Microbiol. 30(9): 2441–2446.
9. Parola P, Fenollar F, Badiaga S, Brouqui P, Raoult D (2001) First documentation of *Rickettsia conorii* infection (strain Indian tick typhus) in a Traveler. Emerg Infect Dis. 7(5): 909–910.
10. Kalal B, Puranik P, Nagaraj S, Rego S, Shet A (2016) Scrub typhus and spotted fever among hospitalised children in South India: Clinical profile and serological epidemiology. Indian J Med Microbiol. 34 (3): 293–298.
11. Hulmani M, Alekya P, Kumar VJ (2017) Indian Tick Typhus Presenting as Purpura Fulminans with Review on Rickettsial Infections. Indian J Dermatol. 62(1): 1–6.
12. Tripathi CDP, Singh M, Agarwal J, Kanta C, Atam V (2017) Seroepidemiology of spotted fever rickettsiosis in Uttar Pradesh: a prospective study. J Clin Diagn Res. 11(6): DC04–9.
13. Stephen S, Ambroise S, Gunasekaran D, Hanifah M, Balakrishnan S, Pradeep J, Sarangapani K (2018) Serological evidence of spotted fever group rickettsiosis in and around Puducherry, south India-a three years study. J Vector Borne Dis. 55(2): 144–150.
14. Stephen S, Gunasekaran D, Pradeep J, Ambroise S, Sarangapani K (2019) Serological diagnosis of Indian Tick Typhus in and around Puducherry: Application of Indirect Immunofluorescence Assay. J Clin Diagn Res. 13(5): 6–9.
15. Rama Prasad GS, Mekala S, Gadagani N (2019) A rare case of rickettsial infection presented as febrile delirium in a 4 years old child from a government hospital, Andhra Pradesh, India. J Dr YSR Univ Health Sci. 8(4): 272.
16. Akshatha V, Vijayalakshmi J (2020) Prevalence of rickettsial infections in a tertiary care centre. Indian J Microbiol Res. 7(1): 6–9.
17. Saravanan K, Jha L, Prakash J, Aruliah R, David E (2021) Seroprevalence of Scrub typhus, Spotted fever and Murine typhus in Vellore District, Tamil Nadu. Int J Pharm Biol Sci. 11: 56–60.
18. D'Cruz S, Perumalla SK, Yuvaraj J, Prakash JAJ (2022) Geography and prevalence of rickettsial infections in Northern Tamil Nadu, India: a cross-sectional study. Sci Rep. 12(1): 1.

https://doi.org/10.18502/jad.v20i2.22397

19. Jena SS, Kar M, Soreng I, Acharrya S, Misra SR (2022) Meningo-Encephalitis due to spotted fever group *Rickettsia*--A rare case report with serial follow up MRI. Neurol India. 70(1): 412–414.
20. Chugh S, Kumari P, Goel S, Biswal M, Sehgal IS, Sekar A (2022) *Rickettsia conorii* infection with fatal complication. Autops Case Rep. 12: e2021392.
21. Prakash JAJ, Sohan Lal T, Rosemol V, Verghese VP, Pulimood SA, Reller M, Dumler JS (2012) Molecular detection and analysis of spotted fever group *Rickettsia* in patients with fever and rash at a tertiary care centre in Tamil Nadu, India. Pathog Glob Health. 106(1): 40–45.
22. Biswal M, Zaman K, Suri V, Gopi S, Kumar A, Gopi T, Vig S, Sharma N, Bhalla A (2020) Molecular confirmation and characterization of *Rickettsia conorii* in North India: a report of three cases. Indian J Med Res. 151(1): 59–64.
23. Parola P, Paddock CD, Raoult D (2005) Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 18(4): 719–756.
24. Moreira G, Moreira RSS, Neves FA das, Swiontek V, Barradas PF, Gomes-Gonçalves S, Mesquita JR (2025) First Molecular Detection of *Rickettsia conorii* and *Rickettsia helvetica* in Ticks from Dogs in Luxembourg. Pathogens. 14(2): 204.
25. Socolovschi C, Gaudart J, Bitam I, Huynh TP, Raoult D, Parola P (2012) Why are there so few *Rickettsia conorii* conorii-infected *Rhipicephalus sanguineus* ticks in the wild? PLoS Negl Trop Dis. 6(6): e1697.
26. Torina A, Fernández de Mera IG, Alongi A, Mangold AJ, Blanda V, Scarlata F, Marco VD, Fuente JDL (2012) *Rickettsia conorii* Indian tick typhus strain and *R. slovaca* in humans, Sicily. Emerg Infect Dis. 18(6): 1008–1010.
27. Lee JH, Park HS, Jang WJ, Koh SE, Kim JM, Shim SK, Park MY, Kim YW, Kim BJ, Kook YH, Park KH, Lee SH (2003) Differentiation of rickettsiae by groEL gene analysis. J Clin Microbiol. 41(7): 2952–2960.
28. Elango A, Shriram AN, Raju HK, Kumar A (2024) Study on the Ixodid ticks species diversity (Acari: Ixodidae) on domestic ruminants in different agro-climatic regions of Tamil Nadu. Trop Anim Health Prod. 56(2): 73.
29. Babu NN, Jayaram A, Auti AM, Bhandari Y, Shetty U, Arunkumar G (2023) *Rickettsia africae* and other unclassified *Rickettsia* species of the spotted fever group in ticks of the Western Ghats, India. Exp Appl Acarol. 90(3–4): 429–440.
30. Vinayak V, Balasubramanian R, Siba S, Patil J, Bhadale P, Harsuni H, Sithalakshmi S, Varghese J, Alagarasu K (2026) Spotted fever group *Rickettsia* in Haemaphysalis ticks: genetic diversity and first detection of "Candidatus *Rickettsia* jingxinensis" in India. Ticks Tick Borne Dis. 17(1): 102580.
31. Wilson JJ (2012) DNA barcodes for insects. Methods Mol Biol. 858: 17–46.
32. Biggerstaff BJ (2009) PooledInfRate, Version 4.0: A Microsoft® Office Excel© Add-In to Compute Prevalence Estimates from Pooled Samples. Centers for Disease Control and Prevention: Fort Collins. CO, USA.
33. Cowling DW, Gardner IA, Johnson WO (1999) Comparison of methods for estimation of individual-level prevalence based on pooled samples. Prev Vet Med. 39(3): 211–225.
34. Park JW, Lee SH, Lee GS, Seo JJ, Chung JK (2020) Epidemiological Characteristics of Field Tick-Borne Pathogens in Gwang-ju Metropolitan Area, South Korea, from 2014 to 2018. Osong Public Health Res Perspect. 11(4): 177–184.

https://doi.org/10.18502/jad.v20i2.22397

35. Megaw JWD (1925) Indian Tick-Typhus. Ind Med Gaz. 60(2): 58–61.
36. Matsumoto K, Ogawa M, Brouqui P, Raoult D, Parola P (2005) Transmission of *Rickettsia massiliae* in the tick, *Rhipicephalus turanicus*. Med Vet Entomol. 19(3): 263–270.
37. Földvári G, Široký P, Szekeres S, Majoros G, Sprong H (2016) *Dermacentor reticulatus*: A vector on the rise. Parasit Vectors. 9(1): 314.
38. Pradeep J, Anitharaj V, Sangeetha B (2024) Human rickettsial infections in India- a review. J Vector Borne Dis. 61(1): 5–22.
39. Padbidri VS, Rodrigues JJ, Shetty PS, Joshi MV, Rao BL, Shukla RN (1984) Tick-borne rickettsioses in Pune District, Maharashtra, India. Int J Zoonoses. 11 (1): 45–52.
40. Nallan K, Ayyavu V, Ayyanar E, Thirupathi B, Gupta B, Devaraju P, Kumar A, Rajaiah P (2023) Molecular evidence of *Rickettsia conorii* subsp. *raoultii* and *Rickettsia felis* in *Haemaphysalis intermedia* Ticks in Sirumalai, Eastern Ghats, Tamil Nadu, South India. Microorganisms. 11 (7): 1713.
41. Goh SH, Driedger D, Gillett S, Low DE, Hemmingsen SM, Amos M, Chan D, Lovgren M, Willey BM, Shaw C, Smith JA (1998) *Streptococcus iniae*, a human and animal pathogen: Specific identification by the chaperonin 60 gene identification method. J Clin Microbiol. 36(7): 2164–2166.
42. Marston EL, Sumner JW, Regnery RL (1999) Evaluation of intraspecies genetic variation within the 60 kDa heat-shock protein gene (groEL) of Bartonella species. Int J Syst Bacteriol. 49(3): 1015–1023.
43. Sumner JW, Nicholson WL, Massung RF (1997) PCR amplification and comparison of nucleotide sequences from the groESL heat shock operon of *Ehrlichia* species. J Clin Microbiol. 35(8): 2087–2092.