High Prevalence and Low Diversity of *Rickettsia* in *Dermacentor reticulatus* Ticks, Central Europe

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We collected 1,671 *Dermacentor reticulatus* ticks from 17 locations in the Czech Republic, Slovakia, and Hungary. We found 47.9% overall prevalence of *Rickettsia* species in ticks over all locations. Sequence analysis confirmed that all tested samples belonged to *R. raoultii*, the causative agent of tick-borne lymphadenopathy.

The ornate dog tick, *Dermacentor reticulatus*, is a proven vector of pathogens of public health and veterinary importance, including tick-borne encephalitis virus, Omsk hemorrhagic fever virus, rickettsiae, *Babesia* spp., and several others (*1*). *D. reticulatus* ticks are now expanding into new areas of northern and central Europe (*1*), where a higher prevalence of associated diseases can be expected.

Although intensively studied during the past decade, bacteria of the genus *Rickettsia* have been overshadowed by other tickborne pathogens of primary medical importance. Rickettsiae of the typhus group and spotted fever group (SFG) present the greatest health risks. The *D. reticulatus* tick is a vector for SFG rickettsiae. Among *Rickettsia* species, *R. raoultii* and *R. slovaca* are recognized as causative agents of rickettsioses with typical lymphadenopathies, called tick-borne lymphadenopathy or *Dermacentor*-borne necrosis erythema and lymphadenopathy (*2*), which are widespread in Eurasia (*1*). *R. helvetica*, which causes milder symptoms, was also reported from *D. reticulatus* ticks (*1*,*3*).

We analyzed 1,671 *D. reticulatus* ticks (851 female and 820 male) for prevalence, diversity, and distribution of SFG rickettsiae in the Czech Republic, Slovakia, and Hungary. Ticks were collected by flagging for previous studies conducted during 2009–2020 from 7 locations in the Czech Republic, 7 in Slovakia, and 5 in Hungary (Appendix, https://wwwnc.cdc.gov/EID/article/28/4/21-1267-App1.pdf). We selected places with a high abundance of *D. reticulatus* ticks for analyses, to promote high detection probability (Table). We used a duplex quantitative PCR method aiming for *gltA* gene fragments of *Rickettsia* (147 bp). We calculated prevalence (Sterne's exact method if n <1,000, adjusted Wald method if n >1,000) and basic statistical comparisons in Quantitative Parasitology 3.0 (*4*). We also amplified fragments of 2 outer-membrane protein genes, *ompA* (590 bp) and *ompB* (475 bp), by conventional PCR and selected a subset of 5–10 positive samples from each location (144 total) for sequencing (Macrogen, https://www.macrogen.com) and identifying species (Appendix).

We identified all isolates as *R. raoultii*. Our *ompA* gene sequences were 99.83% identical to haplotypes from Italy (GenBank accession no. HM161792.1) and Denmark (accession no. MF166732.1). We used *ompB* gene sequences to create a phylogenetic tree (Figure;

Table. Locations of *Dermacentor reticulatus* tick sampling and observed prevalence of *Rickettsia* spp., Central Europe

Location	Country	Year collected	Coordinates	No. positive/total no. collected	Prevalence, % (95% CI)
Hodonín	Czech Republic	2020	48°51′22″N 17°05′18″E	64/90	71.1 (60.6–79.6)
Lanžhot	Czech Republic	2011	48°41′18″N 16°59′22″E	49/90	54.4 (43.9–64.5)
Lednice	Czech Republic	2009	48°49′08″N 16°48′23″E	20/90	22.2 (14.5–32.1)
Lednice	Czech Republic	2020	48°49′08″N 16°48′23″E	14/75	18.7 (11.2–29.2)
Mikulčice	Czech Republic	2009	48°47′57″N 17°05′35″E	66/90	73.3 (63.4–81.8)
Moravská Nová Ves	Czech Republic	2009	48°46′54″N 17°04′36″E	53/90	58.9 (48.3–69.0)
Moravská Nová Ves	Czech Republic	2020	48°46′23″N 17°02′59″E	44/90	48.9 (38.3–59.5)
Číčov	Slovakia	2011	47°46′28″N 17°46′05″E	40/90	44.4 (34.4–55.0)
Ďulov Dvor	Slovakia	2011	47°47′24″N 18°10′14″E	6/90	6.70 (3.0–13.8)
Jurský Chlm	Slovakia	2011	47°48′09″N 18°31′01″E	31/90	34.4 (24.9–45.0)
Klížska Nemá	Slovakia	2011	47°44′51″N 17°49′42″E	20/90	22.2 (14.5–32.1)
Klúčovec	Slovakia	2011	47°47′49″N 17°43′29″E	57/90	63.3 (52.8–72.9)
Lándor	Slovakia	2011	47°47′31″N 18°08′03″E	67/90	74.4 (64.5–82.7)
Studienka	Slovakia	2011	48°31′18″N 17°08′02″E	62/90	68.9 (58.4–77.9)
Dunaremete	Hungary	2011	47°53′33″N 17°30′52″E	39/90	43.3 (33.3–53.9)
Hévíz	Hungary	2013	46°47′14″N 17°11′54″E	53/90	58.9 (48.3–69.0)
Kisbodak	Hungary	2011	47°53′53″N 17°30′31″E	45/90	50.0 (39.4–60.6)
Kondorfa	Hungary	2006	46°53′42″N 16°23′57″E	29/77	37.7 (27.2–49.3)
Szendehely	Hungary	2017	47°50′60″N 19°06′26″E	41/79	51.9 (40.5–62.7)

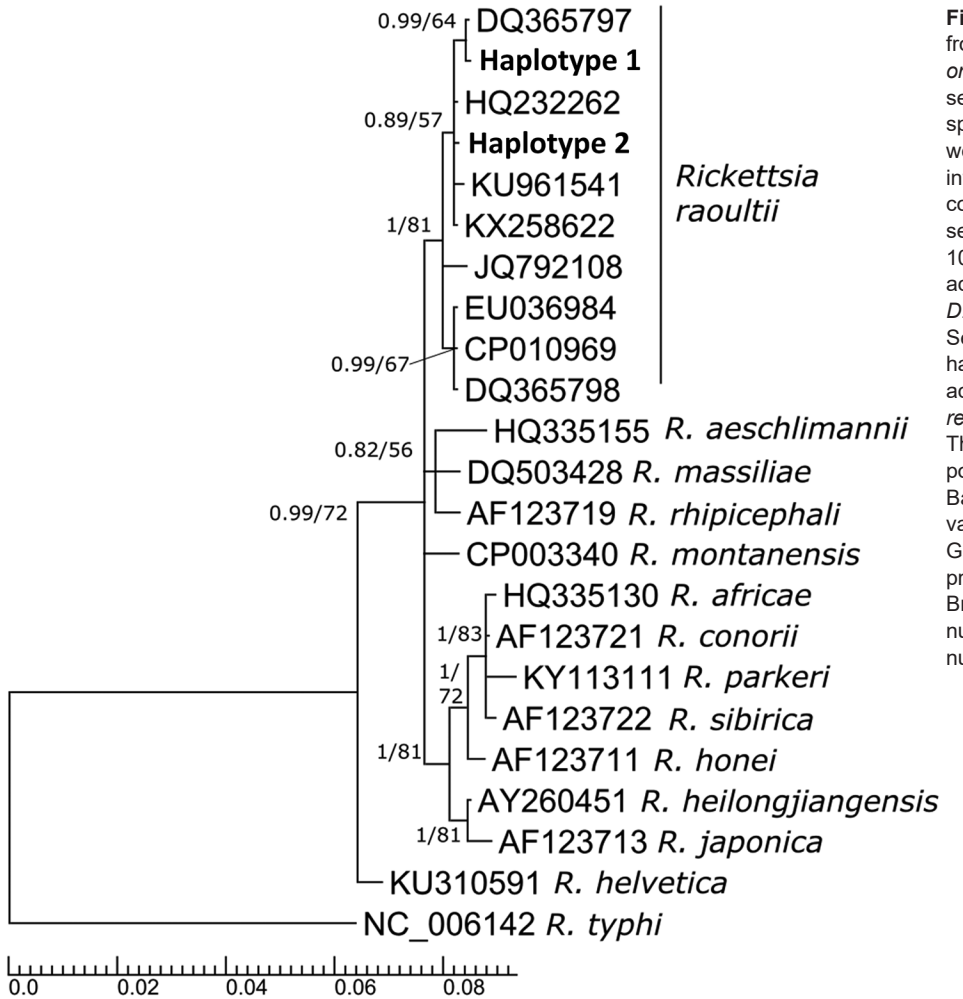

Figure. Phylogenetic tree inferred from outer membrane protein *ompB* region (600 bp) showing 4 separate branches of *Rickettsia* spp. in ticks. The sequences we obtained (bold) were placed into highly supported subclade corresponding with *R. raoultii*. First sequence (haplotype 1) shows 100% identity with GenBank accession no. DQ365797 from *D. reticulatus* ticks from France. Second sequence (haplotype 2) has 100% identity with GenBank accession no. HQ232262 from *D. reticulatus* ticks from Germany. The numbers at the nodes show posterior probabilities under the Bayesian inference/bootstrap values for maximum likelihood. GenBank accession number are provided for reference sequences. Branch lengths indicate expected numbers of substitutions per nucleotide site.

Appendix) in which both sequences were placed into a highly supported subclade formed by sequences of *R. raoultii*. We did not detect either *R. slovaca* or *R. helvetica* at the locations in the study, but the prevalence of these species in *D. reticulatus* ticks is generally low because the main vectors are *D. marginatus* ticks for *R. slovaca* and *Ixodes ricinus* ticks for *R. helvetica* (*2*,*3*).

The mean prevalence of *Rickettsia* in *D. reticulatus* ticks was 47.9% (95% CI 45.5%–50.3%), without significant difference between sexes (p = 0.307 by χ^2 test). Remarkably, we observed the lowest prevalence (6.7%) in Ďulov Dvor, Slovakia, ≈3 km from Lándor, which had the highest prevalence (74.4%) (Table). Differences in the surrounding environments might account for this discrepancy: Ďulov Dvor by an oxbow lake in the middle of arable land and Lándor in a forest along the river Váh. We assumed more abundant interconnected populations of host animals with unrestricted movement live in the forest environment. Data from Lednice, Czech Republic, situated in the middle of farmland, indicated ≈20% prevalence, consistently lower than the ≈60% in nearby areas of floodplain forests along the Morava River near Mikulčice. Comparing findings from the earlier and newer sample collections showed that the proportion of positive ticks remained consistent and variability over time was not significant. Specifically, we compared samples from Lednice (2009 and 2020; p = 0.574 by χ^2 test), Moravská Nová Ves (2009 and 2020; p = 0.178 by χ^2 test), and Mikulčice (2009) and Hodonín (2020), ≈9 km apart (p = 0.739 by χ^2 test).

Distribution of the pathogen in *D. reticulatus* tick populations seems to be very uneven in Central Europe, which is also suggested by other studies (*5*). Our overall prevalence of 47.9% corresponds with similar data showing the prevalence of *R. raoultii* in *D. reticulatus* ticks to be 56.7% in Germany, 57.8% in Hungary, and 50.2% and 45.6% in 2 locations in Slovakia (*5*–*7*). On the other hand, researchers also found much lower prevalences of 10.8% in Slovakia (*8*) 15.6% in the Czech Republic (*3*) and 14.9% in Austria (*9*). Although significant seasonal differences in prevalence were reported (*10*), our data showed that

the high observed prevalence in the study locations remained consistent over a long time period.

Our data suggest an overall high prevalence of *R. raoultii* and its possible long-term stability in *D. reticulatus* tick populations in the studied region, highlighting the enduring high risk of acquiring this rickettsial infection. Besides veterinary consequences (*1*), this risk should be considered by medical personnel and public health authorities because the incidence of tick-borne lymphadenopathy might increase with the reported (*1*) expansion of the vector into new areas and its growing abundance in Central Europe.

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Dr. Balážová is a junior scientist at Veterinary University in Brno, Czech Republic. Her research is aimed at vector-borne zoonotic diseases and the development of new molecular methods for pathogen detection.

References

1. Földvári G, Široký P, Szekeres S, Majoros G, Sprong H. *Dermacentor reticulatus*: a vector on the rise. Parasit Vectors. 2016;9:314. https://doi.org/10.1186/s13071-016-1599-x
2. Parola P, Rovery C, Rolain JM, Brouqui P, Davoust B, Raoult D. *Rickettsia slovaca* and *R. raoultii* in tick-borne rickettsioses. Emerg Infect Dis. 2009;15:1105–8. https://doi.org/10.3201/eid1507.081449
3. Rudolf I, Venclíková K, Blažejová H, Betášová L, Mendel J, Hubálek Z, et al. First report of *Rickettsia raoultii* and *Rickettsia helvetica* in *Dermacentor reticulatus* ticks from the Czech Republic. Ticks Tick Borne Dis. 2016;7:1222–4. https://doi.org/10.1016/j.ttbdis.2016.07.011
4. Rózsa L, Reiczigel J, Majoros G. Quantifying parasites in samples of hosts. J Parasitol. 2000;86:228–32. https://doi.org/10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2
5. Wölfel R, Essbauer S, Dobler G. Diagnostics of tick-borne rickettsioses in Germany: a modern concept for a neglected disease. Int J Med Microbiol. 2008;298:368–74. https://doi.org/10.1016/j.ijmm.2007.11.009
6. Szekeres S, Docters van Leeuwen A, Rigó K, Jablonszky M, Majoros G, Sprong H, et al. Prevalence and diversity of human pathogenic rickettsiae in urban versus rural habitats, Hungary. Exp Appl Acarol. 2016;68:223–6. https://doi.org/10.1007/s10493-015-9989-x
7. Špitalská E, Sparagano O, Stanko M, Schwarzová K, Špitalský Z, Škultéty Ľ, et al. Diversity of *Coxiella*-like and *Francisella*-like endosymbionts, and *Rickettsia* spp., *Coxiella burnetii* as pathogens in the tick populations of Slovakia, Central Europe. Ticks Tick Borne Dis. 2018;9:1207–11. https://doi.org/10.1016/j.ttbdis.2018.05.002
8. Švehlová A, Berthová L, Sallay B, Boldiš V, Sparagano OAE, Spitalská E. Sympatric occurrence of *Ixodes ricinus, Dermacentor reticulatus* and *Haemaphysalis concinna* ticks and *Rickettsia* and *Babesia* species in Slovakia. Ticks Tick Borne Dis. 2014;5:600–5. https://doi.org/10.1016/j.ttbdis.2014.04.010
9. Duscher GG, Hodžić A, Weiler M, Vaux AGC, Rudolf I, Sixl W, et al. First report of *Rickettsia raoultii* in field collected *Dermacentor reticulatus* ticks from Austria. Ticks Tick Borne Dis. 2016;7:720–2. https://doi.org/10.1016/j.ttbdis.2016.02.022
10. Hornok S, Meli ML, Gönczi E, Hofmann-Lehmann R. Seasonally biased or single-habitat sampling is not informative on the real prevalence of *Dermacentor reticulatus*-borne rickettsiae—a pilot study. Acta Vet Hung. 2017;65:81–8. https://doi.org/10.1556/004.2017.008

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Spread of SARS-CoV-2 Variants on Réunion Island, France, 2021

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In January 2021, after detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, genomic surveillance was established on Réunion Island to track the introduction and spread of SARS-CoV-2 lineages and variants of concern. This system identified 22 SARS-CoV-2 lineages, 71% of which were attributed to the Beta variant

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Appendix

Materials and methods

We isolated DNA from samples from the Czech Republic and Slovakia using alkaline hydrolysis in 1.25% NH_4OH (*1*) and samples from Hungary using a NucleoSpin tissue kit (Macherey-Nagel, http://www.mn-net.com) according to manufacturer instructions. For pathogen detection we ran real-time PCR using Probes Master mastermix on Light Cycler (both Roche Diagnostics, https://www.roche.com) and for sequencing we ran conventional PCR using PPP mastermix (Top-Bio, http://www.top-bio.com) and VWR Doppio gradient (VWR, https://www.vwr.com) (*2–4*) (Appendix Table).

Commercial provider Macrogen (https://www.macrogen.com) performed sequencing. We analyzed obtained sequences by BLAST algorithm (*5*) and aligned them using Geneious alignment with 21 relevant sequences downloaded from the GenBank database. We selected representative sequences of spotted fever group *Rickettsia* species based on BLAST analysis and phylogeny published elsewhere (*6*). We cut the final alignment to 600 bp and executed phylogenetic analysis in Geneious Prime software (*7*) with *Rickettsia typhi* (GenBank NC_006142) as an outgroup. We tested phylogenetic relationships by Bayesian inference analysis (*8*) and maximum likelihood analysis (*9*). We performed the Bayesian analysis by Geneious Prime plugin MrBayes version 3.2.6 (https://www.geneious.com) using the GTR (general time-reversible) substitution model for 10^6 generations, with trees and parameters sampled every 200 generations. We summarized the trees after removing 10% burn-in. We carried out the maximum likelihood analysis by the Geneious Prime plugin PhyML 3.3.20180621 using the GTR substitution model. We calculated nodal supports with 1000 bootstrap replicates and visualized the tree using TreeGraph 2.12.0 (*10*).

Appendix Table. Methods used for sample examination and preparation of gene fragments for sequencing*

Method	Target gene	Primers and probes, µmol/L	Sample volume/total volume, µL	Program				Ref
				Hotstart	Cycles	Final extension	Cooling	
Duplex quantitative PCR with probe	*gltA*	ApMSPf_upg 0.4 ApMSPr 0.4 gltA-CS-5 0.6 gltA-CS-6 0.6 5'Cy.5- ApMSPp-3'BHQ3 0.2 5'Hex- gltA-CS-3'BHQ2 0.4	2.0/20	95°C/10 min	45× [95°C for 10 s, 51°C for 30 s, 60°C for 30 s]	None	40°C for 10 min	(*2*)
PCR	*ompA*	Rr190.70p0.5 190.701 0.5	2.5/25	95°C/5 min	35× [95°C for 15 s, 54°C for 15 s, 72°C for 30 s]	72°C/3 min	10°C/ ∞	(*3*)
PCR	*ompB*	ompB.4362p 0.5 ompB.4836n 0.5	2.5/25	95°C/5 min	35× [95°C 15s, 54°C 15 s, 72°C 30 s]	72°C/3 min	10°C/ ∞	(*4*)

References

<jrn>1. Kubelová M, Tkadlec E, Bednář M, Roubalová E, Siroký P. West-to-east differences of *Babesia canis canis* prevalence in *Dermacentor reticulatus* ticks in Slovakia. Vet Parasitol. 2011;180:191–6. PubMed https://doi.org/10.1016/j.vetpar.2011.03.033</jrn>

<jrn>2. Balážová A, Baláž V, Ondruš J, Široký P. Duplex qPCR assay for detection and quantification of *Anaplasma phagocytophilum* and *Rickettsia* spp. Ticks Tick Borne Dis. 2020;11:101462. PubMed https://doi.org/10.1016/j.ttbdis.2020.101462</jrn>

<jrn>3. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol. 1991;173:1576–89. PubMed https://doi.org/10.1128/jb.173.5.1576-1589.1991</jrn>

<jrn>4. Choi YJ, Lee SH, Park KH, Koh YS, Lee KH, Baik HS, et al. Evaluation of PCR-based assay for diagnosis of spotted fever group rickettsiosis in human serum samples. Clin Diagn Lab Immunol. 2005;12:759–63. PubMed</jrn>

<jrn>5. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10. PubMed https://doi.org/10.1016/S0022-2836(05)80360-2</jrn>

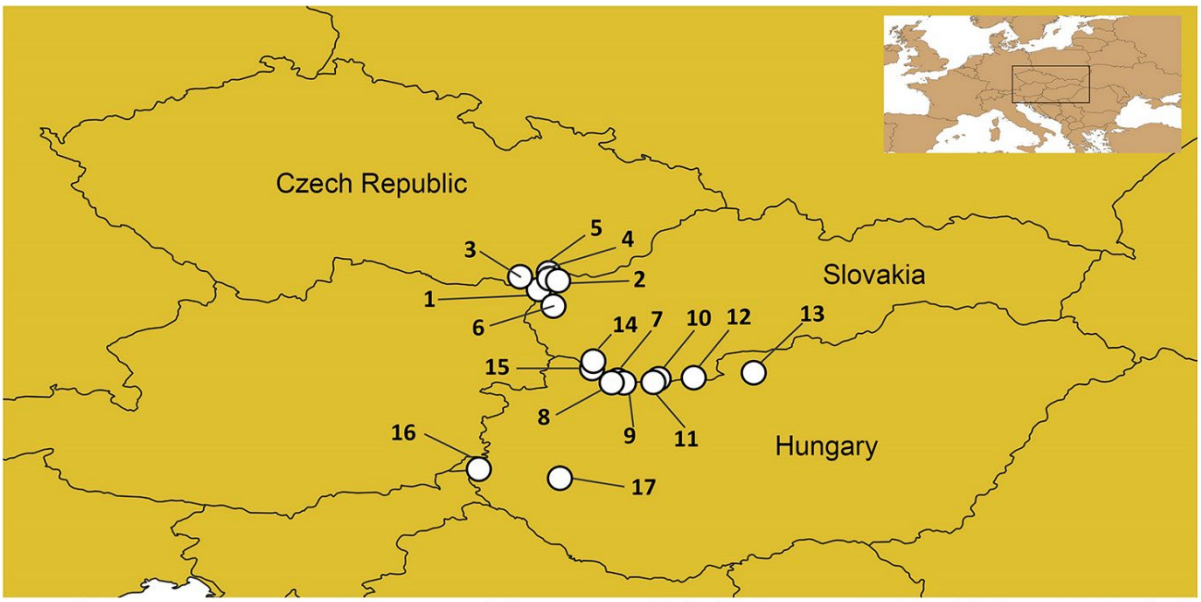
<jrn>6. Sekeyová Z, Danchenko M, Filipčík P, Fournier PE. Rickettsial infections of the central nervous system. PLoS Negl Trop Dis. 2019;13:e0007469. PubMed https://doi.org/10.1371/journal.pntd.0007469</jrn>

<jrn>7. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28:1647–9. PubMed https://doi.org/10.1093/bioinformatics/bts199</jrn>

<jrn>8. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;19:1572–4. PubMed https://doi.org/10.1093/bioinformatics/btg180</jrn>

<jrn>9. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 2003;52:696–704. PubMed https://doi.org/10.1080/10635150390235520</jrn>

<jrn>10. Stöver BC, Müller KF. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics. 2010;11:7. PubMed https://doi.org/10.1186/1471-2105-11-7</jrn>



1. Lanžhot
2. Moravská Nová Ves
3. Lednice
4. Mikulčice
5. Hodonín
6. Studienka
7. Klučovec
8. Kližska Nemá
9. Čičov
10. Dulov Dvor
11. Lándor
12. Jurský Chlm
13. Szendehely
14. Kisbodak
15. Dunaremete
16. Kondorfa
17. Hévíz