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Detection of *Rickettsia* species in rodents collected in the Northern provinces of Vietnam, 2020–2022

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Abstract. Rickettsiae, which are obligate intracellular bacterial parasites, have a life cycle commonly associated with arthropod vectors and animal hosts. Rodents are recognized as their natural reservoir hosts and play a role in transmitting the bacteria to humans through arthropod vectors. The true extent of rickettsial infections in Vietnam remains underestimated due to a limited understanding of the disease's epidemiology, resulting in a significant public health burden. **The aim** of this investigation was to assess the prevalence of *Rickettsia* in wild-living rodents in Dien Bien, Son La, and Phu Tho provinces of Northern Vietnam between 2020 and 2022. **Materials and methods.** A total of 396 wild-living rodents were collected from the Northern provinces of Vietnam during 2020–2022. Real-time polymerase chain reaction (PCR) was employed to detect the percentage of *Rickettsia*-positive specimens. Subsequently, the *ompB* and *17kDa* genes were extracted, amplified, and sequenced from the *Rickettsia*-positive rodents. **Results and discussion.** Among the 396 rodents examined, the majority were *Rattus norvegicus* (56.1%), followed by *R. tanezumi* (37.4%), *R. nitidus* (2.5%), and *R. germaini* (1.8%). *R. korodentsensis* and *R. argentiverter* constituted the remaining rodents, accounting for 2.2% of the total. The real-time PCR analysis revealed that 27 blood samples out of the 396 rodent samples collected, tested positive for *Rickettsia* (6.81%). *R. germaini* was shown to have the highest positive rate 1/8 (12.5%), followed by *R. nitidus* 1/10 (10.0%), *R. tanezumi* 14/149 (9.5%), and *R. norvegicus* 11/222 (4.9%). No other tick-borne pathogens were detected in any of the provinces. Sequencing of the positive samples as regards the *ompB* and *17kDa* genes revealed the closest relation to *R. felis*. These findings highlight the potentially high risk of *R. felis* infection in humans and animals within the studied areas.

Key words: rickettsial diseases, epidemiology, *Rickettsia felis*, Northern mountainous provinces of Vietnam.

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Обнаружение видов *Rickettsia* у грызунов, добытых в северных провинциях Вьетнама в 2020–2022 гг.

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Риккетсии, являющиеся облигатными внутриклеточными бактериальными паразитами, имеют жизненный цикл, как правило, связанный с членистоногими переносчиками и животными-хозяевами. Грызуны считаются их естественными резервуарами-хозяевами и играют определенную роль в передаче бактерий человеку через членистоногих-переносчиков. Истинные масштабы риккетсиозных инфекций во Вьетнаме остаются недооцененными из-за ограниченного понимания эпидемиологии заболевания, что приводит к значительным нагрузкам на общественное здравоохранение. **Цель** исследования состояла в оценке распространенности риккетсий у диких грызунов в провинциях Дьенбьен, Шонла и Футхо Северного Вьетнама в период с 2020 по 2022 г. **Материалы и методы.** Всего в 2020–2022 гг. в северных провинциях Вьетнама добыто 396 диких грызунов. Полимеразная цепная реакция в реальном времени (ПЦР) использовалась для определения доли образцов, содержащих риккетсии. В дальнейшем гены *ompB* и *17kDa* были извлечены, амплифицированы и

секвенированы. **Результаты и обсуждение.** Среди 396 исследованных грызунов большинство относились к виду *Rattus norvegicus* (56,1 %), на втором месте вид *R. tanezumi* (37,4 %), затем *R. nitidus* (2,5 %) и *R. germaini* (1,8 %). Остальные грызуны относились к видам *R. korodentsensis* и *R. argentiverter*, что составляло 2,2 % от общего числа. ПЦР-анализ в реальном времени показал, что 27 образцов крови от 396 добытых грызунов дали положительный результат на риккетсии (6,81 %). Обнаружено, что *R. germaini* имеет самый высокий показатель положительных результатов – 1/8 (12,5 %), за ним следуют *R. nitidus* – 1/10 (10,0 %), *R. tanezumi* – 14/149 (9,5 %) и *R. norvegicus* – 11/222 (4,9 %). Других возбудителей, переносимых клещами, ни в одной из провинций не выявлено. Секвенирование положительных образцов по генам *ompB* и *17kDa* выявило наиболее близкое родство с *R. felis*. Эти данные подчеркивают потенциально высокий риск инфицирования *R. felis* для людей и животных, находящихся на исследуемых территориях.

Ключевые слова: риккетсиозные инфекции, эпидемиология, *Rickettsia felis*, северные горные провинции Вьетнама.

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Rickettsial infections have been reported in various regions of Vietnam [1]. These infections, caused by Gram-negative obligate intracellular bacteria of the genus Rickettsia, are primarily transmitted through arthropods associated with rodents [2]. The genus Rickettsia comprises approximately 20 well-characterized species that are pathogenic to humans [3]. Presently, Rickettsia is categorized into four distinct groups: the spotted fever group (SFGR), which includes species such as *R. conorii*, *R. rickettsii*, and *R. japonica*; the typhus group (TG), including *R. typhi* and *R. prowazekii*; the ancestral group (AG) represented by species such as *R. bellii* and *R. canadensis*; and the transitional group (TRG) containing *R. felis* and *R. akari* [2]. Clinical manifestations commonly associated with rickettsioses include such symptoms as fever, headache, rash, and occasional eschars at the tick bite sites [4]. PCR, DNA sequencing, and genetic analysis are widely employed techniques for Rickettsia detection. Commonly targeted genes for detection and diagnosis include citrate synthase (*gltA*), the 17kDa lipoprotein precursor antigen gene (*17kDa*), and outer membrane proteins A and B (*ompA* and *ompB*) [5].

Vietnam is considered a high-risk region for rickettsial agents circulating in rodents and ectoparasites. Numerous studies have elucidated the crucial role of arthropod vectors in transmitting these bacteria between animals, occasionally spilling over the infection to humans [6]. Among hosts, rodents are recognized as pivotal in the transmission of Rickettsia from vectors to humans [3]. A study conducted to investigate the presence of rickettsial pathogens in rodents in Ha Giang province, northern Vietnam, found that 133 individuals (24.8%) were positive for Rickettsia. Specifically, 5.3% were positive for *Rickettsia typhi*, and 19.5% were infected with the spotted fever group Rickettsia [7]. Despite these findings, the extent of *Rickettsia* spp. occurrence in small mammals and their precise role in disease transmission remain unclear. Hence, this survey was undertaken to explore the presence of *Rickettsia* spp. in wild-living small rodents in the Northern provinces of Vietnam.

Materials and methods

Sample collection. Trap cages measuring 24×14×14 cm were utilized for the study. Approximately 100 traps were deployed at each site. The trapping period spanned 2–3 days at each location, with morning trap inspections. Captured animals were anesthetized for blood collection. Whole blood was gathered in EDTA anticoagulant tubes and stored at –80 °C before transportation to the laboratory at the Institute of Biomedicine, Vietnam-Russia Tropical Center. Spleen tissues were also collected from the same specimens and stored in 1x PBS buffer (pH 7.4) before being frozen for tissue crushing.

A total of 396 wild-living rodents were collected from the Northern mountainous provinces of Vietnam between 2020 and 2022. Species identification of the rodents was conducted using morphological keys [8]. In total, 370 blood samples and 396 tissue samples (spleen, lung, liver, and brain) were collected and stored at –80 °C for further analysis.

DNA extraction. Tissue samples were homogenized to create a uniform solution using a TissueLyser LT (Qiagen, Germany). Subsequently, DNA was extracted

from 200 µl of rodent suspension (or blood) utilizing the "AmpliSens® RIBO-sorb" (Amplisens, Russia) in accordance with the manufacturer's instructions. The elution of DNA was carried out in 50 µl TE buffer and then the product stored at -20°C until PCR amplification.

Real-time PCR detection. A genus-specific real-time PCR targeting the Rickettsia genus was conducted using primers and probes as outlined in Table 1. For the Rickettsia qPCR assays, 8 µl of the template was mixed with 0.5 µM of both forward and reverse primers, 0.25 µM of the probe (Pan-Rickettsia real-time PCR, Table 1), and 10 µl of the pre-mixed GoTaq® qPCR and RT-qPCR Systems (Promega, USA). The reaction mixture was brought to a volume of 20 µl with the addition of water. Amplification for the qPCR assay was carried out using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, USA) taking into account the following temperature and cycle parameters: an initial denaturation for 3 minutes at 95 °C followed by 45 cycles of denaturation (95 °C for 10 s) and annealing and elongation (60 °C for 30 s, FAM/Green). The reaction exhibited logarithmic amplification, and all controls were performed as references [5].

Direct amplification through nested PCR (nPCR) was performed to identify target genes, utilizing partial *ompA*, *ompB*, *17kDa*, and *gltA* genes specific to Rickettsia species. The PCR was carried out in a final reaction volume of 20 µl, comprising 3 µl of DNA, 2.5 pmol of each primer, and the premix reagent (2X PCR Master mix Solution, i-StarTaq, iNtRON, Korean). The thermal cycling conditions are outlined in Table 1.

Electrophoresis and purification of PCR products. The PCR products were visualized through 1.5% agarose gel electrophoresis to determine their sizes (Table 1). Subsequently, these PCR products underwent purification using the Jenjet PCR purification kit (Thermo, USA). The purified PCR products were then sent for sequencing using Sanger method.

Sequencing and phylogenetic analysis. Sequencing of Rickettsia-positive nPCR amplicons was performed by Macrogen Inc. (Daejeon, Korea). The obtained sequences were uniformly edited using Bioedit sequence alignment editor software. To identify referenced species, NCBI BLAST (National Center for Biotechnology Information) – BLASTN, a nucleotide BLAST, was utilized. Sequence alignment and column alignment were carried out using ClustalX2 software. The phylogenetic tree was constructed using MEGA-X software, employing the neighbor-joining method, bootstrap analysis (1,000 reiterations) was also performed.

Table 1

Sequences of probes and primers used for detection of rickettsial DNA by real-time PCR and nested PCR

Target	Assay	Primer name	Nucleotide sequence (5'–3')	Product size (bp)	PCR profile (°C/s)				Reference
					Denaturation	Annealing	Extension	Cycles	
All *Rickettsia* spp.	Pan-Rickettsia real-time PCR	PanR8_F	AGCTTGCTTTTGGATCATTTGG		95/10 min	60/30 *FAM	–	45	[9]
		PanR8_R	TTCCTTGCCTTTTCATACATCTAGT						
		PanR8_P	Fam-CCTGCTTCTATTTGTCTTGCAGTA ACACGCCA-BHQ1						
All SFGR except *Rickettsia helvetica*	*ompA* nested	Rr190k. 71p	TGGCGAATATTTCTCCAAAA	650	95/30	42/35	60/120	35	[10]
		Rr190k. 720n	TGCATTTGTATTACCTATTGT						
		Rr190k. 71p	TGGCGAATATTTCTCCAAAA	532	95/30	48/60	65/120	35	
		Rr190k. 602n	AGTGCAGCATTCGCTCCCCCT						
All SFGR	*ompB* nested	Rc.rompB. 4362p	GTCAGCGTTACTTCTTCGATGC	475	95/15	54/15	72/30	35	[11]
		Rc.rompB. 4836n	CCGTACTCCATCTTAGCATCAG						
		Rc.rompB. 4496p	CCAATGGCAGGACTTAGCTACT	267	95/15	56/15	72/30	35	
		Rc.rompB. 4762n	AGGCTGGCTGATACACGGAGTAA						
All SFGR	*17kDa* nested	R17122	CAGAGTGCTATGAACAAACAAGG	…	95/15	52/15	66/30	35	[12]
		R17500	CTTGCCATTGCCCATCAGGTTG						
		Tz 15	TTC TCA ATT CGG TAA GGG C	246	95/15	52/15	66/30	35	
		Tz 16	ATA TTG ACC AGT GCT ATT TC						
Rickettsia prowazekii	*gltA* nested	RpCS.877p	GGGGGCCTGCTCACGGCGG	381	95/15	54/15	72/30	35	[10]
		RpCS.1258n	ATTGCAAAAAGTACAGTGAACA						
		RpCS.896p	GGCTAATGAAGCAGTGATAA	337	95/15	54/15	72/30	35	
		RpCS.1233n	GCGACGGTATACCCATAGC						

Results and discussion

Collection of ticks and rodents. A total of 396 rodents were trapped in three provinces: Dien Bien, Son La, and Phu Tho, between 2020 and 2022 (Fig. 1). The rodent species distribution was as follows: 56.1% *R. norvegicus*, 37.4% *R. tanezumi*, 2.5% *R. nitidus*, and 1.8% *R. germaini*. The remaining rodents, accounting for 2.2%, were identified as *R. korodentsensis* and *R. argentiverter*. During the ectoparasite census, four different species of Trombiculidae mites were discovered: *Gahrliepia (Walchia) lupella*, *G. (W.) chinensis*, *G. (W.) micropelta*, and *Leptotrombidium deliense*. Additionally, a flea species, *Xenopsylla cheopis*, and two gamaside species, *Laelaps nuttali* and *L. sedlaceki*, were also identified.

The real-time PCR showed that, among the 396 rodent samples collected, 27 blood samples were positive for *Rickettsia* spp., accounting for 6.8%, with the involvement of *R. tanezumi* (14/148, 9.5%), *R. norvegicus* (11/222, 4.9%), *R. germaini* (1/8, 12.5%), and *R. nitidus* (1/10, 10.0%) rodents (Table 2).

Sequencing and phylogenetic analysis. All positive samples were tested using nested PCR targeting rickettsial genus-specific genes (*17kDa*, *ompA*, *ompB*, and *gltA* genes) (Table 2). The resulting high-fidelity PCR products were Sanger sequenced and aligned with known sequences identified in the GenBank database, showing a high degree of similarity using ClustalX. Finally, we identified 10 positive specimens with sequences: 8 sequences from the *ompB* gene and 2 sequences from the *17kDa* gene.

The amplicon sequences (8 samples) of the partial *ompB* gene obtained from *R. tanezumi*, *R. norvegicus*, *R. germaini*, and *R. nitidus* demonstrated 99–100% similarity to the sequences of the homologous genes of *R. felis* through BLAST analysis. All the amplicons obtained in the above PCRs had identical sequences to those of the *R. felis* type strain URRWXCal2 (CP000053). Additionally, those amplicons had identical sequences with other strains of *R. felis*, including those with GenBank sequences ON053303 (from a tick, Biomedical Research Center Slovak Academy of Sciences, Slovakia) and GU182892 (from two cases with subacute meningitis, Sweden) (Fig. 2).

Fig. 1. Geographical locations of the rodent collection sites within the framework of this study

Similarly, two sequences of the *17kDa* gene from *R. tanezumi* demonstrated 99–100% similarity to the *17kDa* gene sequences of *R. felis* previously reported. Case 149 and 174 had identical sequences with GenBank sequences KX446946 (from *Xenopsylla cheopis*, Brazil) and MH194356 (from *Rhipicephalus microplus*, Brazil), respectively (Fig. 3).

Phylogenetic trees were generated based on *ompB* and *17kDa* gene sequences using MEGAX software, employing the maximum-likelihood algorithm with 1000 replicates for bootstrap testing (Fig. 2 and Fig. 3). The phylogenetic analysis showed that the causative agent of spotted fever is most closely related to *R. felis.*

In this study, we present evidence regarding the molecular detection of *R. felis* in *R. tanezumi* and *R. norvegicus*. *R. tanezumi* (Temminck, 1844), known as the Asian house rat, is a prevalent commensal rat species in East and Southeast Asia. It is commonly found in both indoor and outdoor environments from South China to Southeastern Asia. Due to its broad habitat range and seasonal migrations, *R. tanezumi* can host various pathogens, including zoonotic agents of public health concern [13].

We documented the prevalence of *R. felis* in Dien Bien, Son La, and Phu Tho provinces. The first human infection case with *R. fe*lis was reported in Thailand in 2003 [3]. The presence of *R. felis* in this study showed

Table 2

Rickettsial infection in rodents collected from three Northern provinces of Dien Bien, Son La, and Phu Tho in Vietnam

Species	Number of rodents	Detect *Rickettssia* spp.		
		Real-time PCR (Number, % positive)	*ompB* gene PCR (No., % positive)	*17kDa* gene PCR (No., % positive)
R. tanezumi	148	14 (9.5%)	4 (2.7%)	2 (1.4%)
R. norvegicus	222	11 (4.9%)	4 (1.8%)	0 (0%)
R. germaini	8	1 (12.5%)	0 (0%)	0 (0%)
R. nitidus	10	1 (10.0%)	0 (0%)	0 (0%)
R. korodentsensis	4	0 (0%)	0 (0%)	0 (0%)
R. argentiverter	4	0 (0%)	0 (0%)	0 (0%)
Total	**396**	**27 (6.8%)**	**8 (2.0%)**	**2 (0.5%)**

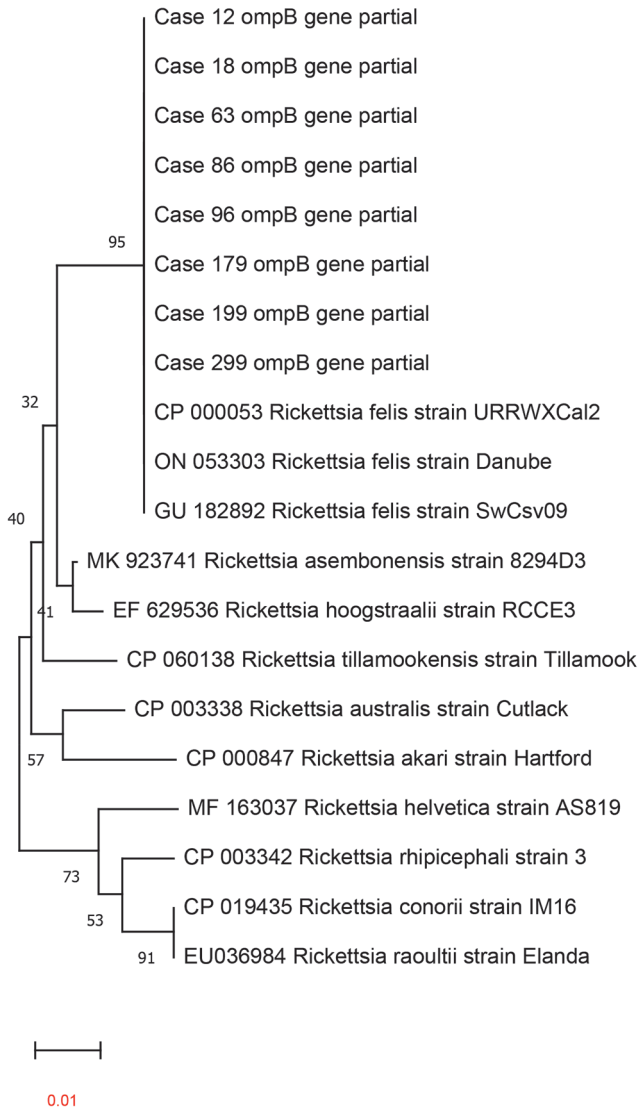

Fig. 2. Phylogenetic analysis of *Rickettsia* spp. obtained through this study. Bootstrap consensus phylogenetic tree constructed based on partial sequences of *ompB* gene by the neighbor-joining method with 1000 bootstrap replicates

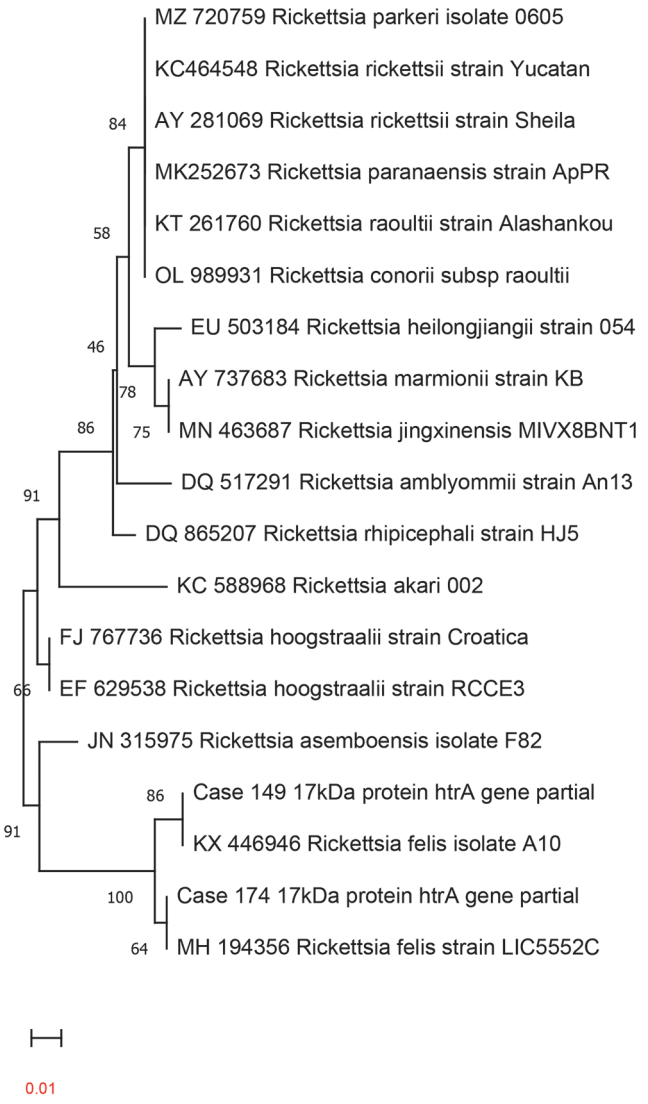

Fig. 3. Phylogenetic analysis of *Rickettsia* spp. obtained through this study. Bootstrap consensus phylogenetic tree constructed based on partial sequences of the *17kD* gene by the neighbor-joining method with 1000 bootstrap replicates

100% similarity with the studied sequence [14]. The geographic distribution of this disease might be linked to multiple vectors and the extensive dissemination of pathogens. Most reported patients had been in contact with dogs and cats, with documented instances of flea transmission from rodents being rare [5]. Despite this, there have been no reports of direct *R. felis* detection from rodents in this region. *R. felis* has been documented in dogs, displaying 100% similarity with the studied sequence [14]. While rickettsial diseases have been reported in Vietnam, information regarding host infection remains limited. This study represents the initial report of *R. felis* detection in rodents in northern Vietnam. Alongside the results of this study, it is outlined that city-dwelling rodents could potentially serve as a reservoir in rickettsial epidemiology within the study areas.

However, this study has its limitations. Primarily, *R. felis* was confirmed through PCR, and the sequencing result was based only on the *ompb* and *17kDa* genes. Additionally, although we collected ectoparasites from captured small mammal hosts, we did not conduct rickettsial detection in these ectoparasites. Hence, further investigations on Rickettsia in parasites are needed to elucidate disease transmission routes and gather additional epidemiological information in the study areas. Moreover, research on the influence of weather and habitat conditions on host and parasite diversity and development requires attention. While all positive samples were confirmed to be infected with *Rickettsia* spp. by real-time PCR, the nested PCR method did not detect nucleic acid fragments of pathogens in some rickettsial-infected rodents due to low bacterial load. Further studies are necessary to ascertain the role of fleas and ticks in the epidemiology of these Rickettsia in rodents and to construct an epidemiological map for *Rickettsia* spp. in Vietnam.

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