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Original Article

Molecular detection of *Rickettsia felis* in ticks and fleas from the environment in Hanoi and Phu Tho province, Vietnam

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Abstract
Introduction: Surveillance for *Rickettsia* spp. is necessary given the recent emergence and re-emergence of various rickettsioses in Vietnam. However, data on their circulation in off-host arthropods from natural environments remain limited. This study aimed to investigate the presence and distribution of *Rickettsia* species in ticks and fleas collected from the environment in Hanoi and Phu Tho provinces, northern Vietnam, between September and December 2021.
Methodology: The ticks and fleas were collected using dragging, light traps, and carbon dioxide traps. Arthropods were identified morphologically and screened for Rickettsia species using real-time PCR targeting the 17kDa antigen gene. Positive samples were further analyzed using species-specific real-time PCR assays and multilocus sequence typing (MLST) for confirmation and phylogenetic analysis.
Results: A total of 758 arthropods were collected, including 748 ticks (747 larval ticks grouped into 101 pools and 1 adult tick) and 10 fleas. The minimum field infection rate (MFIR) of *Rickettsia* spp. in ticks was 2.94% (22/748), while the prevalence in fleas was 50% (5/10). *Rickettsia felis* was detected in 9 larval tick pools and 2 individual fleas by species-specific real-time PCR and MLST. No other Rickettsia species were identified.
Conclusions: This study provides the first evidence of *Rickettsia* spp. in off-host ticks and fleas from the natural environment in Vietnam. These findings indicate a potential risk of environmental exposure to *Rickettsia* and emphasize the need for integrated vector surveillance strategies.

Key words: *Rickettsia*; *Rickettsia felis; rhipicephalus; boophilus;* ticks; fleas; Vietnam.

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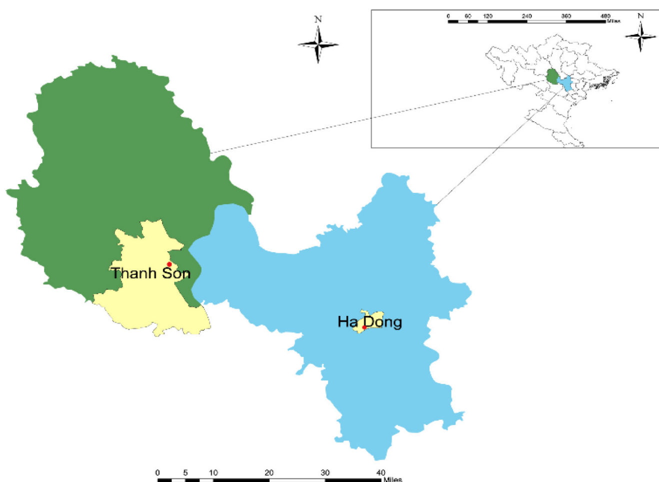
Introduction

The genus *Rickettsia* includes small, obligate intracellular, non-motile, pleomorphic, Gram-negative coccobacillary bacteria. The clinical presentations of infections in humans due to these agents are diverse and include asymptomatic or mild symptoms, multiple organ dysfunction, and death. Rickettsiae are transmitted to humans primarily through the bites of infected arthropods, including ticks, fleas, mites, and lice [1]. Many of these arthropod vectors also serve as reservoirs for the pathogens [1].

Rickettsial infections are re-emerging and widely distributed in all regions in Vietnam [2]. There are currently three *Rickettsia* species reported in Vietnam, including *Rickettsia felis, Rickettsia typhi,* and *Rickettsia conorii: Rickettsia felis* is very common in fleas [3,4] and also found in ticks collected from dogs [5], and has been reported to cause disease in humans [6,7]; antibodies against and DNA of *Rickettsia typhi* and *Rickettsia conorii* have also been reported in humans [2,8,9]. DNA evidence of *Rickettsia* spp. has been reported in humans, small mammals, and arthropods, but the diversity of species present in Vietnam is unknown since serological assays used to identify the presence of a current or previous rickettsial infection only identify rickettsiae at the group level (i.e., typhus group and spotted fever group) [2,10]

Currently, there is no available information on the circulation of *Rickettsia* spp. in arthropod vectors collected from the natural environment, despite the fact

Figure 1. Two study sites are shown in the Map. Sampling locations include red dot sampling points located in two districts of Thanh Son and Ha Dong (yellow).



that these vectors represent a direct source of human infection. The identification of potential tick vectors from the environment is essential for epidemiological investigation, prevention, and control of rickettsial infections [1]. Therefore, we conducted this research to determine the presence and distribution of *Rickettsia* spp. that are transmitted by arthropods identified from the natural environment in Hanoi and Phu Tho. This appears to be the first report of *Rickettsia* spp. in arthropods from the natural environment in Vietnam.

Methodology

Ethics statement

This study was approved by the Biomedical Research Ethics Committee of Hanoi Medical University, Certificate No. 129/GCN-HDDDNCYSH-DHYHN dated June 19, 2020.

Study design

The collection sites for the study included agricultural and natural areas in mountainous and plain terrains. Northern Vietnam has a seasonally hot and humid tropical climate, which makes it a typical endemic area for arthropod vectors of *Rickettsia* spp. The arthropods used in this study were collected from the natural environment and areas of agricultural activities (i.e., fields, barns, forest edges…). The arthropod specimens were then laboratory-identified, pooled, and their DNA was extracted for the identification of the Rickettsia species with molecular biology methods.

Place and time

September 2021 in Phu Luong commune, Ha Dong district, Hanoi Capital (GPS: 20°56'N, 105°46'E) and December 2021 in Tat Thang commune, Thanh Son district, Phu Tho province (GPS: 22°07'N, 105°14'E) (Figure 1).

Collection and identification of arthropod vectors

Collection

Ticks were collected using the dragging method [11] and carbon dioxide (CO_2) traps [12]. In the dragging method, a white cloth was pulled across the station using a cloth roller, with sampling stops every 10 meters to inspect the cloth and remove attached ticks. CO_2 traps were checked at 30-minute intervals during deployment. Fleas were captured using overnight light traps [13], which were set at dusk and inspected the following morning for specimens, and were additionally sampled using the same CO_2 trap protocol. At each trap station, a different ID was assigned. The sample collectors recorded the following information: GPS location, date, habitat, and names of pre-classified ectoparasites. All ectoparasites collected in the same ID were transferred with forceps to a tube containing 96% ethanol and transported to the laboratory in a CryoShipper for later identification.

Identification

Species, stage, and sex were identified using a morphological identification key developed by the National Institute of Malariology, Parasitology and Entomology (NIMPE) (Vietnam) [14-17].

Pooling samples

Each group of ten individual nymph/larval ticks with the same ID, species, and stages would be pooled together (DNA was extracted from eight individuals, and the others were stored at −80°C). In cases where the number of nymphs/larval ticks was under 10, all the individuals would be pooled together (DNA was extracted from three-quarters of the individuals, and the others were stored at −80°C. Adult fleas and ticks were kept separately, which were cut from the abdomen (Figure 2).

DNA extraction

Each individual or pooled sample was washed three times with sterile distilled water, followed by breakage of the chitin layer using an Eppendorf pestle. Samples were then incubated with Proteinase K at 56ºC for 16 hours, and DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All DNA samples after extraction were checked for concentration and purity

using the NanoDrop measurement method and stored at -80ºC for further analysis.

Real-time PCR and phylogenetic analysis.

All samples were screened using a real-time PCR reaction (i.e., Rick17b) targeting the 17kDa outer membrane antigen (17kDa) gene to detect *Rickettsia* spp. [2]. The 17kDa-positive samples were subsequently assessed by real-time PCR to detect *R. typhi* and *R. felis* as previously described [18]. A Ct cut-off of 38 was used to report positive results.

Nested PCR (nPCR) was performed on positive samples to develop amplicons for subsequent gene fragment sequencing. Multi-locus sequence typing (MLST) using fragments of five rickettsial genes, 17kDa, *gltA*, *ompB*, *ompA,* and *sca4,* was performed as previously described and used in previous studies [19]. The positive control used was DNA from *Rickettsia conorii*. Sterile distilled water was used as the negative control in each PCR reaction. To prevent cross-contamination, DNA extraction, PCR mixture preparation, amplification, and agarose gel electrophoresis were performed in separate places, and filtered pipette tips were used.

PCR products were purified and sequenced at https://base-asia.com/. Sequences were aligned using BioEdit software, and BLAST with the Basic Local Alignment Search Tool was performed on the National Center for Biotechnology Information website (http://ncbi.nlm.nih.gov/).

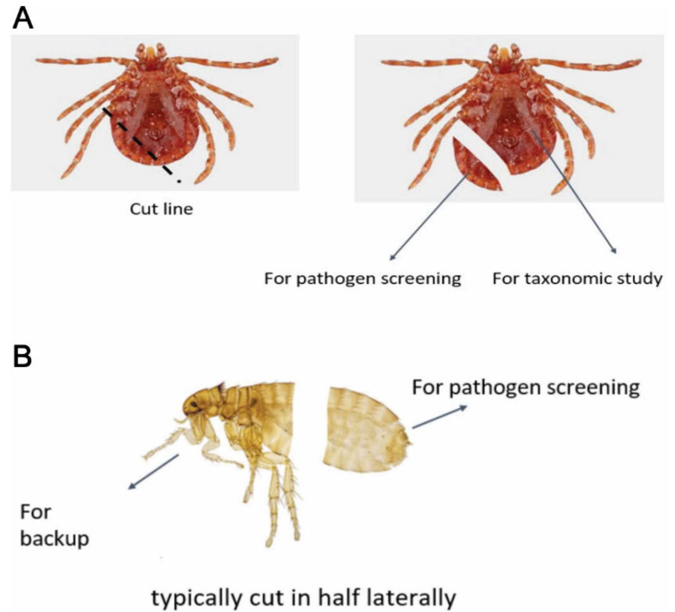
Phylogenetic trees were constructed using the MEGA version 4.0 software and the maximum-likelihood method to infer evolutionary relatedness.

Results

Sample collection

A total of 747 larval ticks were collected using the dragging method. In Ha Dong district, Hanoi Capital, 545 larval ticks were collected over 18 stations (GPS: 20°56'N, 105°46'E) in September 2021. In Thanh Son district, Phu Tho province, Vietnam, 202 larval ticks were collected over 58 stations (GPS: 22°07'N, 105°14'E) in December 2021. Additionally, 10 flea

Figure 2. (A) Method for processing adult tick samples: a small portion is used for molecular analysis, while the remaining portion is utilized for morphological identification. **(B)** Method for processing adult flea samples: the posterior half is used for molecular analysis, and the anterior half is stored at −80°C as a backup sample.



samples were collected from 40 light traps in Phu Tho (n = 5) and 24 carbon dioxide traps in Hanoi (n = 5). The only adult tick was obtained from a light trap in Phu Tho.

Tick and flea identification

Based on microscopic examination and morphological keys, we determined that 100% of the larval tick samples obtained belonged to the genus *Rhipicephalus* and the subgenus *Boophilus*. Larvae were characterized by six legs, the presence of five setae anterior to the spiracular plate, absence of festoons, a blunt palpal apex, and rounded lateral margins of the basis capituli [16,17]. The only adult tick obtained was from a light trap in Phu Tho and was identified as *Rhipicephalus sanguineus* [14].

Ten fleas were collected and determined to be *Ctenocephalides felis orientis* (6 males, 3 females) and *Ctenocephalides felis felis* (1 male) based on

Table 1. Number and identification of ticks and fleas collected in Hanoi and Phu Tho, Vietnam.

Arthropods	Number of Arthropods		
	Total	Hanoi	Phu Tho
Larval ticks (# of ticks/# of pools), - *Rhipicephalus (Boophilus)* spp.	747/101 [a]	545/69 [a]	202/32 [a]
Adult tick, - *Rhipicephalus sanguineus*	1 [b]	0	1 [b]
Ticks (larvae + adult)	**748/102**	**545/69**	**203/33**
Fleas (individuals)	**10** [b]	**5** [b]	**5** [b]
- *Ctenocephalides f. orientis* (6M, 3F)	**9**	**5**	**4**
- *Ctenocephalides f. felis* (1M)	**1**	**0**	**1**

[a] Larvae tick were placed in pools; [b] One adult tick and 10 fleas were assessed individually. F: Female; M: male.

morphological identification keys, as presented in Table 1 [15].

Rickettsia Identification

Results of the genus-specific 17 kDa real-time PCR assay showed that the overall minimum field infection rate for *Rickettsia* spp. was 27/758 (3.56). The minimum field infection rate for the larval tick pools and adults is shown in Table 2. The single adult tick collected from a light trap and identified morphologically as *R. sanguineus* was *Rickettsia* negative (Table 1). Of the 10 individual flea samples, 5 (50%) were *Rickettsia* spp. positive by the Rick17b genus-specific real-time PCR assay targeting the 17 kDa antigen gene. The only adult tick sample was negative for the 17kDa gene.

All 27 *Rickettsia*-positive DNA samples were further assessed by species-specific real-time PCR for *R. felis* and *R. typhi* and by multilocus sequence typing (MLST) using *Rickettsia*-specific gene fragments of 17kDa, *gltA, ompB, sca4,* and *ompA*. Amplicons were not obtained for *sca4* and *ompA* genes for any of the samples analyzed.

Among the 27 Rickettsia-positive samples, 11 were identified as being infected with *R. felis* (4 by real-time PCR only, 3 by both real-time PCR and sequencing, and 4 by sequencing only). These included 9 tick pools and 2 individual fleas (Table 2). The remaining 16 Rick17b real-time PCR-positive pooled samples did not yield positive results in the species-specific real-time PCR assays or amplicons suitable for sequencing.

Phylogenetic Analysis

Twenty-two larval ticks and five adult flea samples tested positive for the 17kDa *Rickettsia*-specific real-time PCR assay. Of the 27 samples positive for *Rickettsia* spp., seven produced amplicons for at least one of the three genes (17kDa, *ompB, gltA*) that were subsequently successfully sequenced. The seven arthropods with successful sequences were entered into GenBank using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Figure 3. (A) Research team members collecting samples **(B)**, **(C)** flea and tick larvae.

The 17kDa and *gltA* fragment sequences of TTH.BC.021 and TTH.BC.024 were 100% identical to each other and exhibited a 99% (475/477) match to the homologous 17kDa sequence of *Rickettsia felis* in *Ctenocephalides felis felis*, Brazil (Accession numbers MH194356) and a complete match to the homologous gltA gene sequence of *Rickettsia felis* in *Liposcelis bostrychophila* (booklouse), USA (Accession number GQ385243). These sequences belonged to the same cluster of sequences on each phylogenetic tree analyzed (Figure 3).

The partial DNA sequence in the *ompB* gene was successfully amplified in six samples. Four pools (PLU.VE.007, PLU.VE.012.4, PLU.VE.016.2, and TTH.VE.019.2) had 100% identical sequences and differed by only one nucleotide when BLASTed against sample PLU.VE.011.1 and by three nucleotides when compared with TTH.BC.021. The *ompB* fragment

Table 2. *Rickettsia* identification and prevalence based on species-specific Real-time PCR assays and 17kDa, *gltA,* and *ompB* gene sequencing.

Rickettsia assay and location	Minimum field infection rate/ Prevalence			
	Ticks		Fleas	
	Number pool positive/ Number examined ticks	MFIR[a] (%)	Number positive/ Number fleas	Prevalence positive (%)
Total samples positive for *Rickettsia spp.* (real-time PCR)	22/748	2.94	5/10	50
in Hanoi	17/545	3.12	2/5	40
in Phu Tho	5/203	2.46	3/5	60
Total samples positive for *Rickettsia felis*[b]	9/748	1.20	2/5	40
In Hanoi	8/545	1.47	0/2	0
In Phu Tho	1/203	0.49	2/3	66.7
Samples positive for *Rickettsia typhi* (real-time PCR)	0	0	0	0

[a] MFIR (minimum field infection rate/100 ticks) = no. of positive pools/no. of examined ticks in pools × 100 [17]; [b] *R. felis* positive by Real-time PCR only, both Real-time PCR + sequencing, or sequencing only.

sequences obtained in this study were 99-100% identical to previously submitted sequences (Accession numbers MT358275, ON053303, GU182892, MN267050).

Discussion

All collected larval stages belonged to *Boophilus* spp. commonly known as cattle ticks or blue ticks, which is consistent with other field studies using flagging techniques for questing ticks [1]. Notably, the number of larval ticks collected in Hanoi was nearly three times greater than that in Phu Tho. Epidemiologically, the larval stage is critical because, in cases of transovarial transmission of the etiological agents, it is the larvae that transmit vector-borne diseases [1]. In addition, one tick sample was collected at the adult stage using a light trap and was identified as *Rhipicephalus sanguineus,* commonly known as the brown dog tick.

In Vietnam, six *Rhipicephalus* species have been reported, including *R. sanguineus* (belonging to the genus *Rhipicephalus*) and *R. microplus* (belonging to the subgenus *Rhipicephalus* (*Boophilus*)), which are particularly important due to their close association with humans [20]. *R. sanguineus* and *R. microplus* exhibit characteristics such as high adaptation to living within human dwellings and being the most frequent ticks infesting dogs and cattle worldwide, which, in consequence, augments human exposure and the risk of bites and transmission of pathogens [20].

Ten flea samples were identified as *Ctenocephalides felis orientis* and *C. felis felis*, two common species in Vietnam [3,4]. Assessing the abundance of host-seeking fleas in human habitats is important as it relates to domestic and peri-domestic settings [21]. The arthropods in this report were primarily collected from residential and agricultural areas (Table 3). Most rural areas in Vietnam, including Hanoi and Phu Tho, are characterized by small-scale livestock farming and artisanal agriculture [22]. This practice requires extensive farmer–livestock contact as well as prolonged periods of work in agricultural areas, which facilitates the transmission of bacterial species through tick or flea bites.

This is the first report of Rickettsia spp. in off-host, questing ticks and fleas in Vietnam. The minimum field infection rate (MFIR) in ticks was 2.94%, while the prevalence in individually tested adult fleas was 50%. The MFIR observed in ticks is lower than that reported in Italy (6.06%) [23] and Kyzylorda, Kazakhstan (12.6–22.7%)[24], but comparable to reports from Almaty, Kazakhstan (0.4–15.1%) [24]; Korea (3.1%) [25] and Japan (2.7%) [26].

The 50% prevalence in adult fleas aligns with findings from Uganda, where *R. felis* DNA was detected in 56% of off-host fleas [21]. There are no reports regarding *Rickettsia* spp. in Vietnam and Southeast Asia in off-host fleas collected by CO2 or

Table 3. Information of *Rickettsia* spp. - Positive samples.

No.	Sample ID	Location	Collected method	Arthropod		Global Positioning System	Habitat	17kDa Real-time PCR	*R. felis*-specific	
									Real-time PCR	Sequencing
1	PLU.BC.006.F	Hanoi	carbon dioxide traps	Female flea	*C. orientis*	20°56' / 105°46'	Pasture	Ct = 35	Neg	Neg
2	PLU.BC.006.M	Hanoi	carbon dioxide traps	Male flea	*C. orientis*	20°56' / 105°46'	Pasture	Ct = 35	Neg	Neg
3	TTH.BC.010	Phu Tho	light traps	Male flea	*C. felis*	21°9' / 105°14'	House	Ct = 28	Neg	Neg
4	TTH.BC.021	Phu Tho	light traps	Male flea	*C. orientis*	21°9' / 105°14'	House	Ct = 22	Ct = 23	*ompB*; 17kDa; *gltA*
5	TTH.BC.024	Phu Tho	light traps	Male flea	*C. orientis*	21°9' / 105°14'	Buffalo stables	Ct = 23	Ct = 25	17kDa; *gltA*
6	PLU.VE.008	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°46'	Pasture	Ct = 33	Ct = 37	Neg
7	PLU.VE.012.1	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Rice field	Ct = 36	Neg	Neg
8	PLU.VE.012.2	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 38	Neg	Neg
9	PLU.VE.012.3	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 37	Neg	Neg
10	PLU.VE.012.4	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 37	Neg	*ompB*
11	PLU.VE.012.5	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 35	Neg	Neg
12	PLU.VE.015.1	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 32	Ct = 35	Neg
13	PLU.VE.015.2	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 38	Neg	Neg
14	PLU.VE.016.1	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 38	Ct = 38	Neg
15	PLU.VE.016.2	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 37	Neg	*ompB*
16	PLU.VE.016.3	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 37	Neg	Neg
17	PLU.VE.007	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°46'	Pasture	Ct = 38	Neg	*ompB*
18	PLU.VE.0011.1	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°46'	Pasture	Ct = 32	Ct = 35	*ompB*
19	PLU.VE.0011.2	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°46'	Pasture	Ct = 38	Neg	Neg
20	PLU.VE.0011.3	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°46'	Pasture	Ct = 37	Neg	Neg
21	PLU.VE.018	Hanoi	dragging	Larvae tick	*Rhipicephalus*	20°56' / 105°47'	Pasture	Ct = 35	Neg	Neg
22	TTH.VE.019.1	Phu Tho	dragging	Larvae tick	*Rhipicephalus*	21°7' / 105°16'	Pasture	Ct = 35	Neg	Neg
23	TTH.VE.019.2	Phu Tho	dragging	Larvae tick	*Rhipicephalus*	21°7' / 105°16'	Pasture	Ct = 38	Neg	ompB
24	TTH.VE.002	Phu Tho	dragging	Larvae tick	*Rhipicephalus*	21°7' / 105°15'	Pasture	Ct = 36	Neg	Neg
25	TTH.VE.055	Phu Tho	dragging	Larvae tick	*Rhipicephalus*	21°9' / 105°14'	Forest edges	Ct = 38	Neg	Neg
26	TTH.VE.024	Phu Tho	dragging	Larvae tick	*Rhipicephalus*	21°9' / 105°14'	Rice field	Ct = 37	Ct = 38	Neg
27	TTH.VE.025	Phu Tho	dragging	Larvae tick	*Rhipicephalus*	21°9' / 105°14'	Rice field	Ct = 37	Neg	Neg

ID: Identification; Ct: Cycle threshold; Neg: Negative.

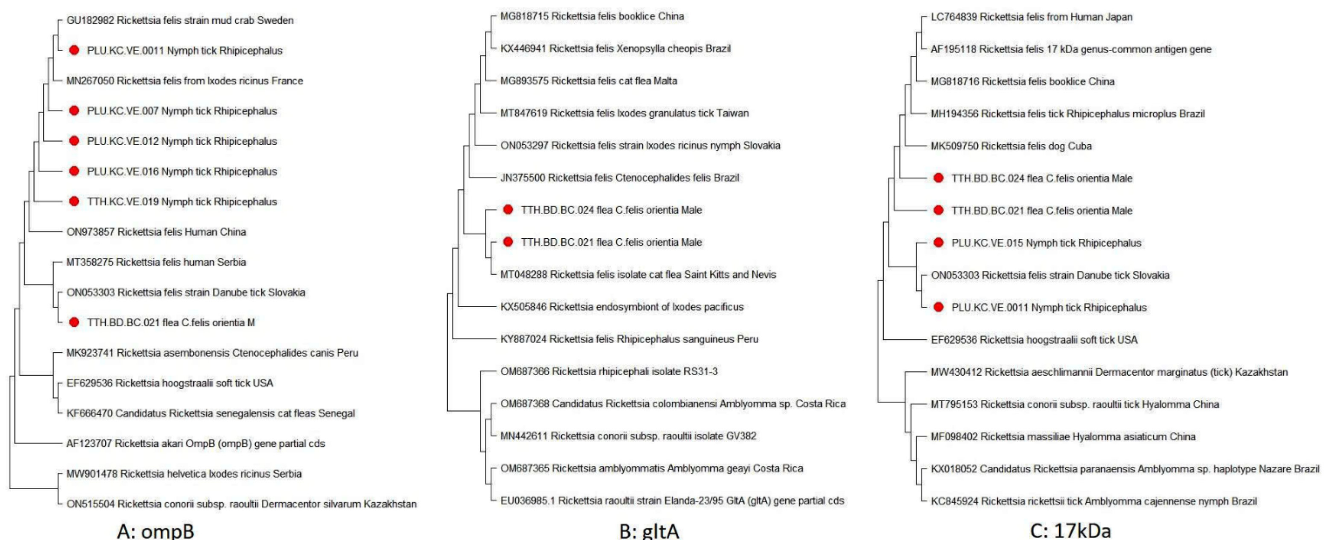
light traps. In Vietnam and Southeast Asia, studies on host-acquired ectoparasites are more common than studies conducted on ectoparasites collected from the environment [27]. In addition, studies collecting questing ticks are more abundant than studies collecting off-host fleas [27].

Based on real-time PCR and sequencing results, 9 larval tick pools and 2 adult fleas were identified as *R. felis*. Due to its worldwide distribution, *R. felis* infection is considered an emerging threat to human health [28]. *R. felis* is transmitted to humans primarily through infected fleas, mainly the "cat flea" *Ctenocephalides felis.* This pathogen has been widely detected in Vietnam in fleas, ticks parasitizing dogs [3], cats, and rodents [10]. Human cases of rickettsial infection have also been reported among hospital patients in central Vietnam by Le-Viet *et al* [6] and in communities in the Central Highlands by Hoang *et al.* [7]. Human infection results from transmission through an infected flea bite, and patients may present with nonspecific clinical manifestations, such as fever, fatigue, headache, arthralgia, myalgia, eschar, and maculopapular rash [28]. The potential route of transmission of *R. felis* from ticks to humans may be through bites, similar to the transmission route of other tick-borne *Rickettsia* species. The detection of *R. felis* in tick salivary glands, together with molecular evidence, supports this hypothesis [29,30]. The pathogenic mechanism of *R. felis* in ticks remains unclear. Further laboratory studies are required to demonstrate the role of ticks in the maintenance and transmission of *R. felis* to vertebrate hosts, including humans.

The phylogenetic tree based on the partial *gltA*, 17kDa, *ompB* gene sequences confirmed that all the *R. felis* in this report are similar to other *R. felis* reported genotypes detected from Brazil, Slovakia, Cuba, the USA, and France in one cluster (Figure 4). Particularly, the *ompB* sequence fragment from sample TTH.BC.021 obtained from a cat flea in Phu Tho fully matched the sequence of a human *R. felis* infection reported in Serbia [31]; the other sequences were also more than 99% identical with samples from patients in China [32] and Japan (accession number AC LC764839). There was no comparison with the sequences of people–infected *R. felis* in Vietnam, because the reports by Le-Viet *et al.* [6] and Hoang *et al.* [7] did not report the sequences. There needs to be more research analyzing *R. felis* genotypes in humans in Vietnam to clarify the relationship between this bacterial species and disease. Besides, vector ecology studies are needed to understand the factors that drive vector abundance, pathogen host range (i.e., including vector range and reservoirs), host-parasite interactions, hosts, and factors affecting the frequency of contact between humans and disease vectors in Vietnam.

This study has several limitations. Firstly, our research focused solely on several locations in one commune of Hanoi and Phu Tho province, which may not be fully representative of the entire tick population in Vietnam. Secondly, the study collected mainly larvae, while the analysis of larvae has many limitations because the larval-stage ticks are the smallest in size and therefore contain less bacterial nucleic acids. Thirdly, with our pooling strategy, the results might be affected by dilution effects and thus by a reduction in sensitivity. Additionally, despite its extensive use, this

Figure 4. Phylogenetic relationships of the *ompB*, *gltA* and 17kDa genes of *Rickettsia* spp. detected from ticks and fleas in Hanoi and Phu Tho, Vietnam. *Rickettsia* spp. sequences in this figure are marked with red dots.



approach is strongly influenced by the pool size, which is chosen arbitrarily. The underlying assumption of the pooling method is that only one infected individual exists in a positive pool, and for this reason, it estimates the lower bound of the infection rate [33-35], potentially underestimating the actual prevalence of infection. Therefore, our reported prevalence rates may represent a lower bound of the infection rate. Furthermore, although all positive samples were confirmed to be infected with *Rickettsia* spp. by a genus-specific assay, only a limited number of those samples were identified by a species-specific *R. felis* real-time PCR assay. In addition, only seven samples were able to produce gene-specific amplicons by nested PCR, possibly due to low bacterial load, as previously reported, since real-time PCR has higher sensitivity than standard PCR.

Despite these limitations, our study contributed to the initial understanding of the distribution and pathogen characteristics of ticks in the natural environments of Vietnam. Further research is required to determine the role of fleas and ticks in the epidemiology of *Rickettsia* species and to develop an epidemiological map of *Rickettsia* spp. in Vietnam.

Conclusions

In summary, this study identified the presence of *Rickettsia* spp. in questing ticks collected from the environment in Hanoi and Phu Tho province, Vietnam, with an MFIR of 2.94% (n = 22). Additionally, *Rickettsia* spp. were detected in 50% of the individually tested adult fleas (n = 5). Among the Rickettsia-positive samples, 11 were identified as *R. felis*. Phylogenetic relationships revealed a close relationship between *R. felis* that were found in this study with those in other ticks and fleas, and humans. These outcomes demonstrate the need to conduct a continued surveillance program to identify tick-borne rickettsiae and better understand their geographical distributions and potential impact on human and animal health, in addition to risk communication and the development of rickettsial disease prevention strategies.

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Conflict of interest

No conflict of interest is declared.

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