First report of three novel Bartonella species isolated in rodents and shrews from nine provinces of Thailand

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Abstract

Background and Aim: Bartonella spp. are Gram-negative zoonic bacteria that are transmitted to humans by several types of animal hosts, including rodents. Several studies have been conducted on the prevalence of Bartonella infections in rodents. However, the risk of rodent-associated Bartonella spp. infection in humans remains unclear. This study aimed to estimate the prevalence and genetic heterogeneity of Bartonella spp. in rodents and shrews from nine provinces of Thailand using culture and molecular techniques.

Materials and Methods: A total of 860 blood samples from rodents and shrews across nine provinces of Thailand were collected from January 2013 to June 2016. Bartonella spp. were isolated from all samples using conventional culture techniques and polymerase chain reaction. Phylogenetic tree analysis was used to align the Bartonella sequences obtained from this study.

Results: The prevalence of Bartonella spp. in rodents and shrews was 11.5% (99/860, 95% confidence interval: 9.38–13.64%). The following nine species of Bartonella were detected: Bartonella tribocorum, Bartonella rattimassiliensis, Bartonella queenslandensis, Bartonella elizabethae, Bartonella chanthaburi spp. nov., Bartonella satun spp. nov., Bartonella cooperiensis, Bartonella ranong spp. nov., and Bartonella henselae. The prevalence of Bartonella-positive animals differed significantly among provinces.

Conclusion: To the best of our knowledge, the three novel Bartonella spp. isolated from rodents and shrews across Thailand were detected for the first time in this study. Further studies on the epidemiology of Bartonella infection in rodents and its interaction with human health should be conducted in accordance with the Thai government’s “One Health” approach to humans, animals, and the environment.

Keywords: Bartonella spp., phylogenetic analysis, polymerase chain reaction, rodents.

Introduction

Bartonella spp. are Gram-negative intraerythrocytic bacteria including more than 40 species and subspecies [1]. Several Bartonella spp. have been confirmed as zoonotic pathogens, such as Bartonella elizabethae, Bartonella tribocorum, Bartonella henselae, Bartonella vinsonii. Sub spp. arupensis, and Bartonella tamae, most of which are transmitted by reservoir hosts and blood-sucking arthropods [2].

Rodents are known to be the main reservoir hosts for different Bartonella spp.; however, some species involve other animals as well. B. henselae utilizes cats and Bartonella bovis and Bartonella chamelii utilize cattle as reservoirs [3]. Several Bartonella spp. have been isolated from rodents in several countries, including Thailand [4–9]. These pathogens are associated with various human diseases, such as cat scratch disease (B. henselae), trench fever (Bartonella quintana), Oroya fever (Bartonella bacilliformis), and endocarditis (B. taimae) [10–12]. In particular, past exposure to rats has been reported in three patients from Thailand with fever, myalgia, and headache [13]. Several reports of these infections in rodents in Thailand have been described [6–9, 12–15]. However, the risk of rodent-associated Bartonella spp. infection in humans remains unclear.

The study aimed to estimate the prevalence and genetic heterogeneity of Bartonella spp. in rodents and shrews from nine provinces of Thailand using culture and molecular techniques and phylogenetic analysis.

Materials and Methods

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee of the National Institute of Health (NIH), Thailand.
Study period and location
The study was conducted from January 2013 to June 2016. The blood samples were collected from nine provinces of Thailand: Khon Kaen, Nakhon Phanom, Tak, Chon Buri, Chanthaburi, Ranong, Phuket, Songkhla, and Satun (Figure-1). The samples were processed at the Department of Medical Sciences, NIH Laboratory, Ministry of Public Health, Nonthaburi, Thailand.

Sample collection
We calculated the minimum sample size based on a previous study by Pangjai et al. [6] using the Epitools program (www.epitool.net) with 95% confidence interval (CI) and 2.5% precision. Based on the results, 401 samples should have been collected. Overall, 860 small mammals, comprising 800 rodents and 60 shrews, were captured using traps from nine provinces of Thailand. Animal species were identified by their morphological characteristics before they were euthanized using a Carbon oxide (CO₂) chamber. A total of 0.5–2 mL of blood samples were aseptically collected through cardiopuncture and immediately placed in sterile ethylenediamine tetra-acetic acid tubes. The samples were transported to the Department of Medical Sciences, NIH Laboratory under chilled conditions and stored at −20°C until further processing.

Isolation of Bartonella
Bartonella was isolated according to a previously described method [16] with slight modifications. Briefly, frozen blood samples were thawed at 25°C, and 200 μL of each sample was centrifuged at 1,800xg for 70 min. The sediment was mixed with an equal volume of Medium 199 (Life Technologies, USA) supplemented with sodium pyruvate and fetal bovine serum (Life Technologies, United States). The mixture was then inoculated onto brain heart infusion agar (BHIA, Difco, United States) plates containing 5% defibrinated rabbit blood. The plates were incubated at 35°C under 5% CO₂ for 2–4 weeks. Consequently, Gram-negative coccobacilli grew as small, rough, and grayish colonies and required long culture periods, which were tentatively considered as Bartonella species. The bacteria were subcultured in fresh media and all isolates were maintained in Trypticase Soy Broth with 20% glycerol (v/v) for further characterization.

DNA extraction and polymerase chain reaction (PCR) amplification
The genomic Bartonella DNA was detected using specific PCR primers as described previously by Boonmar et al. [16]. Genomic DNA was extracted from each isolate using InstaGene Matrix (BioRad, Hercules, United States). Primers targeting the β-subunit of RNA polymerase (rpoB) [17] (primer pair sequences, 5’-CGCATTTGCTACTTCTGATG-3’ and 5’-GTAGACTBATTAGAACGCTG-3’) and citrate synthase (gltA) [18] (primer pair sequences, 5’-AATGCAAAAAGAACATGGC-3’ and 5’-GGGGACCAGCTCATGGTGG-3’) were used for PCR. PCR was performed using 20 μL of reaction mixtures containing 20 ng of extracted DNA, 200 μmol/L of each deoxynucleotide triphosphate, 1.5 mmol/L of MgCl₂, 0.5 U of Go-Taq DNA polymerase (Promega, Madison, Wisconsin, United States), and 1 pmoL of each primer. The thermal cycling conditions of PCR included a denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min, with a final step of 72°C for 7 min. Positive and negative controls were included in each experiment. Finally, 10 μL of each PCR product was subjected to electrophoresis on 1.5% agarose gels containing ethidium bromide and visualized on an ultraviolet transilluminator. The expected length of PCR products was 825 bp (rpoB primers) and 379 bp (gltA primers).

Phylogenetic analysis
The Clustal X program [19] was used to align Bartonella sequences obtained from this study. The data will be deposited in the GenBank/EMBL/DDBJ databases. A phylogenetic tree was drawn based on the aligned sequences of gltA and rpoB genes using the neighbor-joining method with Kimura’s two-parameter distance method in MEGA 11 [20]. Bootstrap analysis was conducted using 1,000 resamples. The Brucella melitensis strain 16M sequence was used as an out-group.

Statistical analysis
Pearson’s Chi-square test and Fisher’s exact test were used to comparatively analyze the prevalence of
animal species among provinces using the IBM SPSS Statistics software. The differences observed were considered statistically significant at \( p \leq 0.05 \).

### Results

A total of 860 small mammals were captured from nine provinces of Thailand, including 399 *Rattus* spp., 50 *Bandicota* spp., 351 other spp., and 60 *Suncus murinus* (shrews). Overall, 11.5% of blood samples from rodents (99/860, 95% CI: 9.38–13.64%) were positive for nine *Bartonella* species; the rodents included 86/399 *Rattus* spp. (21.5%), 5/50 *Bandicota* spp. (10%), 3/299 *Mus musculus* (1.0%), and 5/60 *Suncus murinus* (8.3%). The incidence and identities of the nine *Bartonella* spp. were as follows: 27.3% of *B. tribocorum*, 20.2% of *Bartonella rattimassiliensis*, 15.2% of *Bartonella queenslandensis*, 10.1% of *B. elizabethae*, 8.1% of *Bartonella chanthaburi* spp. nov., 6.1% of *Bartonella satun* spp. nov., 6.1% of *Bartonella cooperisplainsensis*, 5.1% of *Bartonella ranong* spp. nov., and 2.0% of *B. henselae* (Table-1).

Table-2 shows the geographic distribution of the nine *Bartonella* spp. isolated from rodents and shrews. Of all animals carrying these pathogens, 22/414 (5.31%) were captured in the northeastern region of Thailand, 1/41 (2.5%) in the northern region, 5/40 (12.5%) in the central region, 25/148 (16.9%) in the southern region, and 46/217 (21.2%) in the southern region. The prevalence of the nine *Bartonella* spp. in the nine provinces was as follows (in descending order): 35.1% (40/114 animals) in Ranong, 31.0% (14/45) in Nakhon Phanom, 16.9% (25/148) in Chanthaburi, 12.5% (5/40) in Chonburi, 7.7% (3/39) in Phuket, 5.4% (2/37) in Songkhla, 3.7% (1/27) in Satun, 2.5% (1/41) in Tak, and 2.2% (8/369) in Khon Kaen. Among the northeastern provinces, *Bartonella* prevalence in Nakhon Phanom was significantly higher than that in Khon Kaen \( (p < 0.001) \). Further, among the southern provinces, the prevalence in Ranong was significantly higher than that in Satun \( (p < 0.001) \). The phylogenetic tree of the 99 *Bartonella*-positive sequences of gltA and rpoB fragments is shown in Figure-2. Table-3 shows the GenBank accession numbers of the nucleotide sequences obtained from this study, which were deposited in the GenBank.

### Discussion

The prevalence of rodent-associated *Bartonella* spp. has shown high diversity, with more than 20 such species reported worldwide. It is known that more than one *Bartonella* spp. can circulate in rodent communities, and the presence of multiple *Bartonella* genotypes in the same host has been reported \([3, 5, 21]\), leading to emerging bartonellosis, particularly in Southeast Asia \([22, 23]\). The prevalence of these pathogens was reported to be 6% in Indonesia \([24]\), 9.3–42.9% in China \([25]\), 10.1–30.4% in Lao PDR \([26]\), and 13.5–13.8% in Malaysia \([27]\), depending on the diagnostic method, location, environmental conditions, presence...
Table-2: Geographic distribution of nine *Bartonella* species isolated from rodents and shrews in nine provinces, Thailand.

| Province          | Number examined | Number of positive (%) | Number of animals infected with *Bartonella* species |
|-------------------|-----------------|------------------------|-----------------------------------------------------|
|                   | Province        | Number                | *Bartonella coopersplainsensis* | *Bartonella elizabethae* | *Bartonella henselae* | *Bartonella queenslansdensis* | *Bartonella rattimassiliensis* | *Bartonella tribocorum* | *Bartonella chanthaburi spp. nov.* | *Bartonella satun spp. nov.* | *Bartonella ranong spp. nov.* |
| North-Eastern     | Khon Kaen       | 369                   | 8 (2.2)*                            | 0                         | 0                       | 0                       | 3/(Rr = 1, Bi = 2)             | 2/(Bi = 1, Rr = 1)       | 3/(Mm = 3)                        | 0                         | 0                         | 0                         |
|                   | Nakhon Phanom   | 45                    | 14 (31)*                            | 0                         | 0                       | 0                       | 3/(Rr = 3)                    | 11/(Re = 8, Rn = 1, Rr = 2) | 0                         | 0                         | 0                         |
|                   | subtotal         | 414                   | 22 (5.31)                           | 0                         | 0                       | 0                       | 3/(Rr = 1, Bi = 2)            | 5/(Bi = 1, Rr = 4)       | 0                         | 0                         | 0                         |
| Northern Central  | Tak             | 41                    | 1 (2.5)                             | 0                         | 0                       | 0                       | 0                             | 1/(Bi = 1)                    | 0                         | 0                         | 0                         |
|                   | Chon Buri       | 40                    | 5 (12.5)                            | 2/(Bi = 1, Rr = 1)        | 0                       | 0                       | 0                             | 1/(Rr = 1)                    | 0                         | 0                         | 0                         |
| Southern          | Chanthaburi     | 148                   | 25 (16.9)                           | 4/(Rr = 4)                | 0                       | 0                       | 2/(Rr = 2)                    | 8/(Rr = 8)                   | 2/(Rr = 2)                | 5/(Rr = 5)                | 4/(Rr = 4)                | 0                         |
|                   | Ranong          | 114                   | 40 (35.1)**                         | 0                         | 10/(Rn = 8, Rr = 2)     | 2/(Rn = 1, Rr = 1)       | 10/(Rn = 2, Rr = 2)           | 3/(Rn = 2, Rr = 1)          | 10/(Rn = 8, Rr = 2)       | 1/(Rr = 1)                | 0                         | 4/(Sm = 4)                |
|                   | Phuket          | 39                    | 3 (7.7)                             | 0                         | 0                       | 0                       | 2/(Rr = 2)                    | 0                         | 0                         | 1/(Rr = 1)                | 0                         | 0                         |
|                   | Songkhla        | 37                    | 2 (5.4)                             | 0                         | 0                       | 0                       | 0                             | 0                         | 1/(Rt = 1)                | 0                         | 0                         | 1/(Sm = 1)                |
|                   | Satun           | 27                    | 1 (3.7)**                           | 0                         | 0                       | 0                       | 0                             | 0                         | 1/(Rt = 1)                | 0                         | 0                         | 1/(Rt = 1)                |
|                   | Subtotal        | 217                   | 46 (21.12)                          | 0                         | 10/(Rn = 8, Rr = 2)     | 2/(Rn = 1, Rr = 1)       | 10/(Rn = 2, Rr = 3)           | 10/(Rn = 8, Rr = 2)        | 2/(Rr = 1, Rn = 1, Rr = 2) | 2/(Rr = 1, Rn = 1, Rr = 2) | 5(Sm = 5)                |
|                   | Total           | 860                   | 99 (11.5)                           | 6 (6.1)                   | 10 (10.1)                | 2 (2.0)                 | 15 (15.2)                     | 20 (20.2)                    | 27 (27.3)                | 8 (8.1)                    | 6 (6.1)                   | 5 (5.1)                   |

*Prevalence in Nakhon Phanom was significantly higher than in Khon Kaen (p < 0.001), **Prevalence in Ranong was significantly higher than in Satun (p < 0.001)*
of vectors, and animal host species and their habitats. The prevalence of 11.5% reported in this study is similar to that reported in a study conducted in Malaysia [27]. In line with the previous studies, Bartonella spp. in our study was also frequently isolated from Rattus rattus [7, 8]; however, other studies showed contrasting results [24–26]. The previous studies from Thailand have identified the presence of B. elizabethae, B. henselae, Bartonella clarridgeae, and B. tamiae, which were known to cause infections in humans [6–9, 12–13]. Of these, we did not detect B. tamiae and B. clarridgeae in this study but we found the other two species in addition to B. tribocorum, B. rattiamassiliensis, B. coopersplainensis, B. queenslandensis, and three novel Bartonella spp.

B. henselae is a well-known pathogen in wild and domestic cats and causes cat-scratch disease [28, 29]. It has been isolated from rodents in Thailand in a previous study [7, 8] and is associated with febrile Thai patients [13, 30]. This pathogen was detected in approximately 2% of the rodents from the Ranong Province. Notably, the three novel Bartonella spp. were also found in this province and were isolated from shrews.

B. elizabethae is widely distributed in Asian countries [7, 8, 25]. It is known to be associated with
Table-3: GenBank accession numbers for nucleotide sequences.

| Nucleotide sequences                  | GenBank accession numbers | gltA   | rpoB   |
|---------------------------------------|--------------------------|--------|--------|
| B. elizabethae_THNRG001               | MF105784                 | MF105860 |
| B. queenslandensis_THNRG003            | MF105785                 | MF105861 |
| B. tribocorum_THNRG006                 | MF105786                 | MF105862 |
| B. tribocorum_THNRG012                 | MF105787                 | MF105863 |
| B. elizabethe_THNRG018                | MF105788                 | MF105864 |
| B. rattimassiliensis_THNRG019          | MF105789                 | MF105865 |
| B. tribocorum_THNRG020                 | MF105790                 | MF105866 |
| B. queenslandensis_THNRG022            | MF105791                 | MF105867 |
| B. henselae_THNRG028                  | MF105792                 | MF105869 |
| B. elizabethe_THNRG029                | MF105793                 | MF105870 |
| B. rattimassiliensis_THNRG032          | MF105794                 | MF105871 |
| B. tribocorum_THNRG033                 | MF105795                 | MF105872 |
| B. queenslandensis_THNRG034            | MF105796                 | MF105873 |
| B. elizabethe_THNRG036                | MF105797                 | MF105874 |
| B. queenslandensis_THNRG037            | MF105798                 | MF105875 |
| B. elizabethe_THNRG043                | MF105799                 | MF105876 |
| B. tribocorum_THNRG044                 | MF105800                 | MF105877 |
| B. elizabethe_THNRG045                | MF105801                 | MF105937 |
| B. queenslandensis_THNRG061            | MF105802                 | MF105878 |
| B. ranong_THNRG068                    | MF105803                 | MF105879 |
| B. ranong_THNRG071                    | MF105804                 | MF105880 |
| B. elizabethe_THNRG073                | MF105805                 | MF105881 |
| B. queenslandensis_THNRG074            | MF105806                 | MF105882 |
| B. tribocorum_THNRG077                | MF105807                 | MF105884 |
| B. tribocorum_THNRG079                | MF105808                 | MF105885 |
| B. tribocorum_THNRG080                | MF105809                 | MF105886 |
| B. queenslandensis_THNRG081            | MF105810                 | MF105887 |
| B. tribocorum_THNRG083                | MF105811                 | MF105888 |
| B. elizabethe_THNRG084                | MF105812                 | MF105889 |
| B. elizabethe_THNRG086                | MF105813                 | MF105890 |
| B. queenslandensis_THNRG091            | MF105814                 | MF105892 |
| B. tribocorum_THNRG094                | MF105815                 | MF105893 |
| B. ranong_THNRG105pOB                 | MF105816                 | MF105894 |
| B. ranong_THNRG106pOB                 | MF105817                 | MF105895 |
| B. rattimassiliensis_THPKTR006         | MF105818                 | MF105896 |
| B. satun_THPKTR014                    | MF105819                 | MF105897 |
| B. rattimassiliensis_THCTIR99          | MF105820                 | MF105898 |
| B. chanthaburi_THCTIR100              | MF105821                 | MF105899 |
| B. coopersplainsensis_THCTIR110       | MF105822                 | MF105900 |
| B. coopersplainsensis_THCTIR119       | MF105823                 | MF105901 |
| B. coopersplainsensis_THCTIR120       | MF105824                 | MF105902 |
| B. coopersplainsensis_THCTIR128       | MF105829                 | MF105906 |
| B. chanthaburi_THCTIR129              | MF105830                 | MF105907 |
| B. chanthaburi_THCTIR130              | MF105832                 | MF105909 |
| B. rattimassiliensis_THCTIR131        | MF105833                 | MF105910 |
| B. rattimassiliensis_THCTIR132        | MF105834                 | MF105911 |
| B. rattimassiliensis_THCTIR135        | MF105835                 | MF105912 |
| B. queenslandensis_THCTIR141          | MF105836                 | MF105913 |
| B. queenslandensis_THKKNRP3-20        | MF105837                 | MF105914 |
| B. queenslandensis_THKKNRP3-21        | MF105838                 | MF105915 |
| B. tribocorum_THKKNRP3-22             | MF105839                 | MF105916 |
| B. tribocorum_THKKNRP3-24             | MF105840                 | MF105917 |
| B. tribocorum_THKKNRP3-25             | MF105841                 | MF105918 |
| B. queenslandensis_THKKNRP3-29        | MF105842                 | MF105919 |
| B. satun_THSTNR8                      | MF105843                 | MF105920 |
| B. chanthaburi_THSKAR26               | MF105844                 | MF105921 |
| B. ranong_THSKAR27                    | MF105845                 | MF105922 |

(Contd.)

deglutination [31] and human neuroretinitis [32]. We found this species in approximately 10% of the rodents in the Ranong province near the Myanmar border, where there are several markets, which are visited by business travelers and workers. The Ranong Province showed the highest prevalence of *Bartonella* infection in animals (35.1%) among all provinces. Thus, the epidemiology of this infection in febrile patients with rodent exposure should be considered.

In this study, *B. tribocorum* was the most prevalent *Bartonella* spp. in rodents (27.3%) followed by *B. rattimassiliensis* (20.2%), both of which had been detected in febrile patients in Thailand in a previous study [13]. Almost all incidences of these species were in *Rattus* rodents, similar to that reported in the previous studies [4, 26, 30, 33].

The other two *Bartonella* spp.; *B. queenslandensis* and *B. coopersplainsensis* were also isolated from *Rattus* rodents. They have been isolated from rodents and fleas in Taiwan in a previous study [34].

We found three novel *Bartonella* spp. in the Chanthaburi, Satun, and Ranong provinces. The public health information concerning *Bartonella* infections in these three provinces remains unknown. Further collaboration between human and animal sectors can help investigate the possibility of new *Bartonella* spp. infections in febrile patients with rodent exposure in these three provinces.

**Conclusion**

To the best of our knowledge, this is the first study that reported the detection of three novel *Bartonella* spp. isolated from rodents and shrews in Thailand. In this study, nine different *Bartonella* spp. available at www.veterinaryworld.org/Vol.15/July-2022/4.pdf.
were detected and most of them were potentially zoonotic, using rodents as reservoir hosts. Further studies on the risk of this infection among humans, rodents, and the environment are needed to advance public health information.

**Authors’ Contributions**

DP, BN, WP, WW, and NC: Collected the samples. DP and WR: Provided technical help during the experiments. PW and MB: Did the statistical analysis. SB: Designed the study and drafted and revised the manuscript. All authors have read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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