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"*Candidatus* Bartonella thailandensis": a new genotype of *Bartonella* identified from rodents

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Abstract

Bartonella species, intracellular parasite of erythrocytes and endothelial cells, are zoonotic pathogens of wild and domestic animals including rodents. Many species of rodents are commensally infected with a few *Bartonella* species in Asia. However, there are only few reports on detection of *Bartonella* in Thailand. Our objective was to detect the presence of *Bartonella* species in rodents from Thailand. Among 247 rodents captured in five provinces from Thailand we identified *Bartonella* species using molecular methods targeting 3 genes i.e citrate synthase (*gltA*), *ß*-subunit of the RNA polymerase (*rpoB*) and cell division protein gene (*ftsZ*) and the 16-23S rRNA intergenic spacer (ITS). Overall, we found 21 rodents being infected with a *Bartonella* species including 7 *B. coopersplainsensis*, 4 *B. phoceensis*, 6 *B. queenslandensis*, 1 *B. rochalimae*, 1 *Bartonella* sp. RN24BJ and 2 genotypes of a new *Bartonella* that we propose to give the provisional status "*Candidatus* Bartonella thailandensis". To the best of our knowledge, these *Bartonella* species have been detected for the first time in Thailand.

Keywords: *Candidatus* Bartonella thailandensis, rodent

1. Introduction

Bartonella species are intracellular parasites of erythrocytes and endothelial cells of several animals including rodents, ruminants, cats and dogs. *Bartonella* species are considered emerging pathogens in humans. A wide range of mammalian reservoir hosts including rodent and arthropod vectors have been involved in the natural cycle of various *Bartonella* spp. The persistent bacteremia is more readily documented in the primary reservoir species and may occur less frequently or to a much lower level in accidental hosts. In the natural host, clinical manifestation of the infection may be minimal or unrecognized (Chomel et al., 2003). Some *Bartonella* species such as *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis* and *B. washoensis* have been isolated from humans and linked to a rodent reservoir (Ellis et al., 1999; Kosoy et al., 2003). The close relation between rodents and humans throughout the world makes the study of rodent-borne *Bartonella* essential to determine the extent to which rodents may serve as a source of human infections (Castle et al., 2004).

Bartonella species associated with animals and fleas have been recovered in Asia (Ying et al., 2002; Parola et al., 2003; Castle et al., 2004), Australia (Fournier et al., 2007; Gundi et al. in press) and Europe (Gundi et al., 2004). Different species of mice (*Apodemus* spp., *Mus* spp.), rodents (*Bandicota* spp., *Eothenomys* spp., *Rattus* spp.), shrews (*Crocidura* spp., *Sorex* spp) and voles (*Microtus* spp., *Clethrionomys* spp.) also act as a primary vertebrate reservoir of *Bartonella* species that were reported in Asia (Table 1).There are only a few reports on detection of *Bartonella* in Thailand. Castle et al. (2004) reported a prevalence of 9% in *Bandicota indica* and *Rattus* spp. in Chiangrai, Thailand and Parola et al. (2003) identified *Bartonella* BNfRs in flea (*Nosopsyllus fasciatus*) collected from *Rattus surifer* in Kanchanaburi province, on the Thai-Myanmar border. The newly described *B. tamiae* pathogen was isolated from human patients in Thailand (Kosoy et al., 2008) while *B. henselae* was recently isolated from biopsy skin lesions of bacillary angiomatosis (Paitoonpong et al.,

2008). The objective of our study was to detect the presence of *Bartonella* species from rodents captured in Thailand by using molecular tools to identify the species.

2. Materials and methods

2.1. Study area

During May and July 2005, rodents were captured in five districts of Thailand near Myanmar (Sangkhlaburi district, Kanchanaburi province; Pobphra district, Tak province), near Laos PDR (Ponepisai district, Nongkhai province) and near Cambodia (Pongnamron district, Chanthaburi province; Sangkha district, Surin province). The localities of study sites were rice paddies, forests and plantations where populations were mainly made up of farmers. Everyday, 200 wire mesh traps were put following rodent signs, rodent burrow, feces footprint or rodent runway from 6 p.m. to 6 a.m. The traps were set up for 5 days in each province. This study was conducted on the animal welfare by the authority of National Institute of Health, Thailand.

2.2. Samples preparation

Each animal was identified before being euthanasied. Blood samples were collected, separated by centrifugation and total genomic DNA were extracted using NucleoSpin Blood Kit (Macherey-Nagel GmbH, Germany) and kept at -20°C. Samples were handled under sterile conditions to avoid the risk of cross-contamination.

2.3. Samples analysis

The DNA samples were screened for the presence of *Bartonella* spp. by the use of a real-time quantitative PCR targeting the 23S rRNA gene with Taqman probe. The primers and probe using in this study were: Barto23SF, 5'-GAGTAAGTGGTAGCGGAGCG-3'; Barto23SR, 5'-GCCGACTCACCCTGCTCAG-3' and Barto23Sprobe, 6-FAM-GAGAGCTCCTGGAGGTATCGGAAG-TAMRA; size of the PCR product was 172 bp. Positive samples at screening were further studied by PCR amplification and DNA

sequencing of 3 genes including citrate synthase (*gltA*) (Birtles and Raoult. 1996), *ß*-subunit of the RNA polymerase (*rpoB*) (Renesto et al., 2001) and cell division protein gene (*ftsZ*) (Zeaiter et al., 2002) and 16S-23S rRNA intergenic spacer (ITS). Sequencing of PCR products were carried out using the BigDye Terminator Cycle sequencing Ready Reaction (ABI PRISM, Applied Biosystems) and sequences obtained were compared to those available in GenBank. Phylogenetic relationships among the studied Bartonellae were inferred from sequence alignment from concatenated gene sequences using the maximum parsimony and neighbor-joining methods within the MEGA version 4.1 software package.

3. Results

3.1. Rodent species and prevalence of Bartonella infection

A total of 247 rodent samples representing nine species within three different genera were collected and identified during the study including 2 species of bandicoot rats (*Bandicota* spp.), mouse (*Mus* spp.) and 6 species of rats (*Rattus* spp.) (Table 1).

Overall, the prevalence of *Bartonella* was 12.7% (17 of 134) in the provinces on the border of Cambodia, 3.7% (2 of 54) in the provinces on the border of Myanmar and 3.4% (2 of 59) in the province on the border of Laos PDR as determined using our *Bartonella* genus RT-PCR. *Bartonella* DNA was detected in 6 rodent species: *B. savilei*, *M. cervicolor*, *R. berdmorei*, *R. exulans*, *R. rattus* and *R. surifer* (Table 1).

3.2. Bartonella identification

Using standard PCR and sequencing of all four loci: *gltA*, *rpoB*, *ftsZ* and ITS, we identified the presence of 7 *B. coopersplainsensis*, 4 *B. phoceensis*, 6 *B. queenslandensis*, 1 *B. rochalimae*, 1 *Bartonella* sp. RN24BJ previously identified from *R. norvegicus* and 2 genotypes of a new *Bartonella* in *R. surifer*. The percentage similarity of DNA sequences from known *Bartonella* species ranged from 96 to 97% on *gltA*, 96 to 97% on *ftsZ* and 96 to 98% on *rpoB*. Among known validated species, the two new species shared 90.1 to 90.2%

126 homology with *B. phoceensis* for partial *rpoB*, 90.3 to 90.9% homology with *B. washoensis*
127 for *ftsZ* and 92.0 to 92.6% homology with *B. grahamii* for *gltA* gene. The GenBank accession
128 numbers for this new species are as follows: FJ411481 (*ftsZ*), FJ411482 (*gltA*), FJ411483
129 (*rpoB*), FJ411484 (ITS) and FJ411485 (ITS).

130 According to the current knowledge, newly encountered *Bartonella* strains should be
131 considered a new species if a 327-bp *gltA* fragment and a 825-bp *rpoB* fragment share
132 <96.0% and <95.4% sequences similarity with those of the validated species as in the current
133 case. We propose for this new *Bartonella* species the provisional name "*Candidatus* B.
134 thailandensis" since it was first detected in rodents from Thailand. Figure 2 shows the
135 phylogenetic position of this new species among members of the genus *Bartonella* based on
136 comparisons of concatenated sequences of 4 genes.

137 **4. Discussion**

138 We present in this study the molecular detection of *Bartonella* species detected in
139 rodents from Thailand. Although blood culture is more sensitive and warranted in order to
140 obtain isolates, this was not possible in this study and should be done in the future. We have
141 used the current molecular criteria previously published for detection, identification and
142 discrimination of bacteria of the genus *Bartonella* (La Scola et al., 2003). These are only few
143 reports on detection of *Bartonellae* in animals from Thailand (Parola et al., 2003; Castle et al.,
144 2004). The overall percentages of infected rodents in our study (8.5%) was similar to that of
145 Castle et al. (2004) who found a prevalence of 9% in rodents (*B. indica*, *R. rattus* and *R.*
146 *losea*) trapped from Chiangrai province in northern part of Thailand (Castle et al., 2004).
147 However, the *Bartonella* species detected in the present study were clearly different from
148 Castle et al. (2004) since these species have never been reported in Thailand before. In this
149 previous report, phylogenetic analysis of *Bartonella* species isolated in rodents indicates that
150 isolates from *B. indica* represent a new *Bartonella* species and those from *Rattus* spp. were

closely related to *B. elizabethae* and *B. grahamii* (Castle et al., 2004). However, we have detected in the present study for the first time other *Bartonella* species previously detected in other studies including *B. phoceensis* (previously isolated from *R. norvegicus* in the city of Marseille, France) (Gundi et al., 2004), *B. rochalimae* (previously isolated in a woman with fever, bacteremia and splenomegaly in Peru) (Eremeeva et al., 2007), *B. coopersplainsensis* and *B. queenslandensis* (previously isolated in Australian rats, Gundi et al., in press) and *Bartonella* sp. RN24BJ (previously detected in *R. norvegicus* in China, Li et al, unpublished data). In this study, we have found *B. phoceensis* from 4 rodents (2 samples *R. rattus* of which 1 from Kanchanaburi and 1 from Nongkhai provinces; 2 samples *R. surifer* from Surin provinces) as previously reported in Asia (Ying et al., 2002; Inoue et al., 2008; Lin et al., 2008). However we have not detected in our study either the newly described *B. tamiae* pathogen isolated from human patients from Khon Kaen province, Thailand (Kosoy et al., 2008) or *B. henselae* isolated from a case of bacillary angiomatosis (Paitoonpong et al., 2008). On the other hands, Parola et al. (2003) identified a new genotype of *Bartonella* spp. (*Bartonella* BNfRs) from a *Nosopsyllus fasciatus* specimen collected on a rat (*R. surifer*) from the Thai-Myanmar border that was not detected in our study. Conversely, phylogenetic analysis in of concatenated sequences this study indicates that two rodents (*R. surifer*6 and *R. surifer*12) were likely infected with a new genotype of *Bartonella* that we propose to give the provisional status "*Candidatus* B. thailandensis" according to Murray and Stackerbrandt (1995) guidelines. According to the current molecular criteria from La Scola et al. (2003), our sequences obtained from these 2 rodents fulfilled the criteria for a new *Bartonella* species (La Scola et al., 2003).

The epidemiological importance of rodent-borne *Bartonella* spp. as a cause of disease in animals and humans is emerging, as suggested for a novel rodent *Bartonella*-associated febrile illness in the rural southwestern USA, based on serological evidence (Boulouis et al.,

2005; Iralu et al., 2006). At present, only a limited number of human infections caused by rodent-borne *Bartonella* species have been reported such as *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis*, *B. washoensis*. Further studies are required to determine if rodents could be another natural reservoir of *Bartonella* species i.e *B. rochalimae* identified from *R. norvegicus* in Taiwan (Lin et al., 2008). Further investigations are warranted in order to isolate these new *Bartonella* species and to determine if they can cause any clinical manifestations related to unknown diseases in Thailand. However, previous experience has shown that Bartonella species firstly detected or isolated in animals or arthropods could be later involved in humans. This was the case for example with *B. alsatica* firstly isolated from wild rabbits (Heller et al., 1999) and later identified as human pathogens in a heart valve of a patient with endocarditis (Raoult et al., 2006) and in a patient with cat scratch disease (Angelakis et al., 2008).

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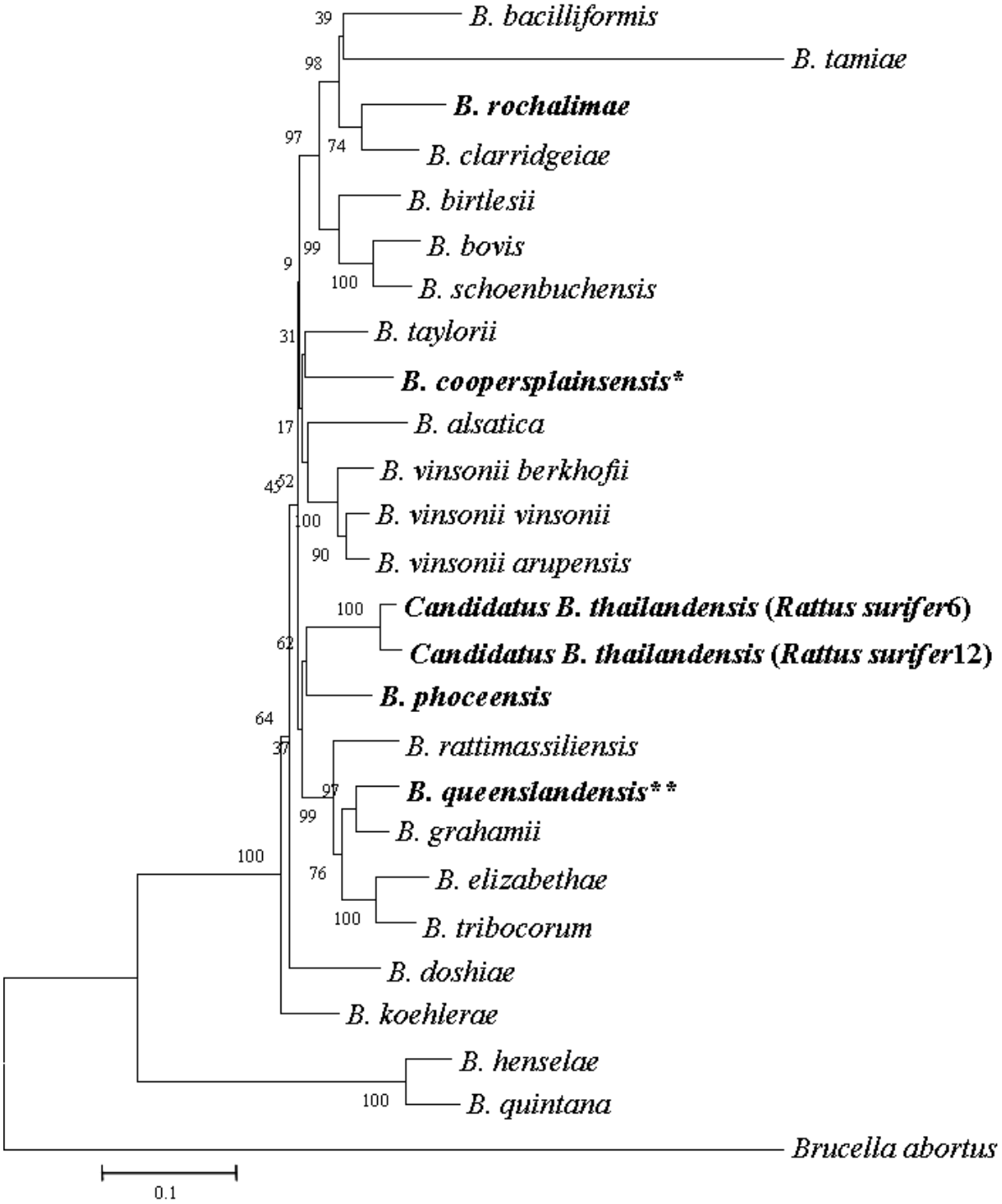
Figure 1. Neighbor-Joining concatenated tree of *rpo*B, *glt*A, ITS and *fts*Z genes on Thai rodent samples with known species of *Bartonell*a.



GenBank accession numbers: *rpoB* (EU111792*, EU111791**); *gltA* (EU111803*, EU111802**); ITS (EU111770*, EU111769**); *ftsZ* (EU111781*, EU111780**)

Table 1. Percentages of *Bartonella* infections in rodents within three neighboring provinces in Thailand, during May to June, 2005.

Rodent species	Province neighboring Cambodia		Province neighboring Myanmar		Province neighboring Laos PDR	Total (Percentage of rodent infections)
	Chanthaburi	Surin	Kanchanaburi	Tak	Nongkhai	
	No. of tested/positive (*Bartonella* infection)	No. of tested/positive (*Bartonella* infection)	No. of tested/positive (*Bartonella* infection)	No. of tested/positive (*Bartonella* infection)	No. of tested/positive (*Bartonella* infection)	
Bandicota indica	6/0	4/0	8/0	1/0	1/0	20/0 (0)
Bandicota savilei	26/2 (*B. queenslandensis* {1}, *B. rochalimae* {1})	3/0	0/0	0/0	4/0	33/2 (6.06)
Mus cervicolor	0/0	0/0	0/0	2/1 (*B. queenslandensis* {1})	0/0	2/1 (50)
Rattus berdmorei	16/5 (*B. coopersplainsensis* {4}, *B. queenslandensis* {1})	4/0	0/0	0/0	0/0	20/5 (25)
Rattus exulans	1/0	1/0	1/0	21/0	45/1 (*Bartonella* sp. RN24BJ {1})	69/1 (1.45)
Rattus fluvescens	7/0	0/0	0/0	1/0	0/0	8/0 (0)
Rattus losea	2/0	5/0	4/0	0/0	2/0	13/0 (0)
Rattus rattus	24/2 (*B. coopersplainsensis* {2})	3/0	12/1 (*B. phoceensis* {1})	3/0	7/1 (*B. phoceensis* {1})	49/4 (8.16)
Rattus surifer	2/0	30/8 (*B. coopersplainsensis* {1}, *B. queenslandensis* {3}, *B. phoceensis* {2}, *Candidatus* B. thailandensis {2})	1/0	0/0	0/0	33/8 (24.24)
Total (Percentage of infection)	84/9 (10.71)	50/8 (16)	26/1 (3.85)	28/1 (3.57)	59/2 (3.39)	