Genus Bartonella is known to be a variety of gram-negative, fastidious, hemophagous bacteria in humans, domestic and wild animals. Several Bartonella species are recognized zoonotic and transmitted by blood sucking arthropod vectors. The aim of the present study was to investigate the presence of Bartonella infections in rodents and shrews species in 9 provinces of Thailand. Bartonella strains were isolated from 860 rodents (171 from Rattus norvegicus,125 from R. exulans, 59 from R. norvegicus, 39 from R. rattus, 5 from R. argenteus, 4 from Bandotus savellie, 46 from B. indica, 5 from Bubromys bermoridea, 5 from Maximys rajah, 1 from M. surifer, 10 from Mus caroli 99 from Musculus 23 from Cricetulus albigularius, 3 from Callosciurus notatus, 3 from Menetes bermorei, 2 from Tupaias glis and 60 from Suncus murinus). The prevalence of Bartonella bacteremic animals by province was 2% (8369) of the animals collected in Phuket, 31% (1445) in Nakhon Phanom, 2% (141) in Tak, 12% (540) in Chon Buri, 16% (25149) in Chanthaburi, 35% (40114) in Ranong, 7% (3/39) in Phatthalung, 5% (29773) in Songkhla and 3% (12774) in Satun. Sequence analysis of the citrate synthase and RNA polymerase β subunit genes identified that the 97 isolates from each Bartonella-positive animal were B. tribocorum, B. rattimassiliensis, B. elizabethae, B. queenslandensis, B. cooperensis and B. henselae and this also study found Bartonella and 3 new genotypes of Bartonella species in Thailand for the first time.

Keywords: Bartonella species, Rotent, Shrew, Thailand

Introduction

Genus Bartonella are small, gram-negative aerobic bacilli that are difficult to grow in culture, mainly transmitted by vector. These microorganisms infect erythrocytes of their mammalian hosts, and some species cause a wide spectrum of human illness, such as chronic bacillariemia, fever and endocarditis. Identification of the bacteria is based on results of poly ribonucleic polymerase chain reaction (PCR) assay, followed by sequencing of several housekeeping genes including citrate synthase (gltA), RNA polymerase beta subunit (rpoB), cell division-associated protein (fisZ), heat shock protein (groEL), riboflavin synthase alpha chain (rnc), 16S rRNA and the intergenic spacer region (ITS). Prevalence and Genetic Heterogeneity of Bartonella Strains Isolated from Animal in 9 Provinces, Thailand

Materials and Methods

Sampling: During 2013-2016, 860 blood samples and rodents from 9 provinces in Thailand were collected by cardiopuncture and immediately placed into EDTA tubes into EDTA tubes. These tubes were stored at -80°C and tested at the National Institute of Health, Thailand.

Isolation of bacteria

The frozen blood samples were thawed at room temperature and centrifuged at 1,800 g for 60 min. After centrifugation, the plates were plated onto brain heart infusion agar (BHIA) plates containing 5% CO2 for 48-72 h of incubation. The plates were incubated at 37°C under 5% CO2 for 4-7 days. Two or three of small and rough grey colonies were picked up from each plate, streaked out on fresh plates, and further cultured under the same conditions.

PCR amplification and sequencing of gltA and rpoB genes

Genomic DNA was extracted from each isolate of Bartonella spp. using an Instagene matrix (BioRad, Hercules, CA). The primers used for amplification and sequencing as following 1gltaF (5'-ACTGAGTCTACTGCGTGGACG-3'), 1gltaR (5'-CGCATTGGCTTACTTCGTATG-3'), 2gltaF (5'-GOGGCAACAGGCTGTTGTTG-3'), 2gltaR (5'-TATCATGTTAGAAGCCTG-3'), 1rpoBF (5'-ATGAAACAGAGGATCAGTTG-3'), 1rpoBR (5'-CTGAAAGAAGAAGACGAA-3').

Phylogenetic analysis

The CLUSTAL_X program was used for the alignment of Bartonella sequences. A phylogenetic tree was drawn based on the sequences of gltA and rpoB genes, using the neighbor-joining method with Kimura's two-parameter distance method in MEGA 7. Bootstrap analysis was carried out with 1,000 resamplings.

Results and Conclusion

The previous study, we found the prevalence of Bartonella bacteremic animals by province collected 42.9% in Phang Nga, 26.8 % in Chiang Rai, 20.49% in Sa Kaeo, 16.74% in Nakhon Si Thammarat, 12.08% in Surat Thani, 8.06% in Chaiyaphum, 8.06% in Chumpon, 7.20% in Surat Thani, 3.24% in Loei, 1.31% in Udon Thani, and 1.31% in Songkhla. These results indicate that Bartonella species are widely distributed in small mammals in Thailand and some animal species may serve as important reservoirs of Bartonella species in the country. This is the first report of B. henselae isolated from the rodents in Thailand. B. henselae is an emerging bacterial pathogen, causing cat scratch disease and bacillary angiomatosis. Cats bacteremic with B. henselae constitute a large reservoir from which humans become infected. And also this study found 5 new genotypes of Bartonella species in Thailand for the first time.

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References


