Comparative Immunology, Microbiology and Infectious Diseases 35 (2012) 71–76

Contents lists available at SciVerse ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Presented at the 6th International Meeting on Rickettsia and Rickettsial Diseases at Heraklion, Crete, Greece on June 5–7, 2011

A multi-gene analysis of diversity of bartonella detected in fleas from algeria

Idir Bitam[a,b,*], Jean Marc Rolain[b,1], Violaine Nicolas[d], Yi-Lun Tsai[c], Philippe Parola[b,1], Vijay A.K.B. Gundi[b,1], Bruno B. Chomel[c], Didier Raoult[b,1]

[a] *Service d'Ecologie des Systèmes Vectoriels, Institut Pasteur d'Algérie, Algiers, Algeria*
[b] *URMITE, CNRS-IRD UMR 6236, Faculté de Médecine et de Pharmacie, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France*
[c] *Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA*
[d] *UMR CNRS 7205 Origine Structure et Evolution de la Biodiversité, Département Systématique et Evolution, Muséum Nationale d'Histoire Naturelle, CP 51, 45 rue Buffon, 75005 Paris, France*

ARTICLE INFO

Article history:
Received 18 July 2011
Received in revised form 5 November 2011
Accepted 10 November 2011

Keywords:
Bartonella
Flea
Rodents
Algeria

ABSTRACT

We report the molecular detection of several *Bartonella* species in 44 (21.5%) of 204 fleas from Algeria collected from 26 rodents and 7 hedgehogs. *Bartonella elizabethae* and *B. clarridgeiae* were detected in the fleas collected on hedgehogs. *Bartonella tribocorum* and *B. elizabethae* were detected in fleas collected from rats and mice, and sequences similar to an unnamed *Bartonella* sp. detected in rodents from China were detected in rats as well as a genotype of *Bartonella* closely related to *Bartonella rochalimae* detected in fleas collected on brown rats (*Rattus norvegicus*).

1. Introduction

The genus *Bartonella* contains aerobic, fastidious, gram–negative bacilli belonging to the alpha-2 subgroup of the class *Proteobacteria*. Recently, the number of *Bartonella* species isolated has increased markedly. These bacteria are considered to be emerging pathogens involved in an increasing number of recognized diseases [1]. Currently, 22 *Bartonella* species are recognized, all being associated with mammalian hosts. To date, 11 species have been implicated in human diseases [1].

Fleas are found on mammals worldwide and are vectors of several major bacterial zoonoses, including plague caused by *Yersinia pestis*, murine typhus caused by *Rickettsia typhi*, flea-borne spotted fever caused by *R. felis* [2], and Cat-Scratch Disease (CSD) caused by *Bartonella henselae*. In recent years, several rodent flea species have been detected to carry *Bartonella* species including new *Bartonella* genotypes, for which the medical importance is not yet known. For instance, *Bartonella* spp. were detected in 38 *Oropsylla hirsuta* and in three *Oropsylla tuberculatus cynomuris* prairie dog fleas in the United States [3] and from *Ctenocephalides felis* collected from domestic cats and in a *Nosopsyllus fasciatus* flea collected from a red spiny rat (*Rattus surifer*) in Thailand [4]. We have recently detected the presence of *Bartonella* species in rodents and hedgehogs trapped in Algeria using molecular methods. We detected in hedgehogs two *Bartonella* species which are known to be human pathogens in hedgehogs: *Bartonella elizabethae* and *Bartonella clarridgeiae*. The *Bartonella* species detected in Algerian rodents was close to a *Bartonella* species isolated from rodents in China [14]. Several methods of phylogenetic reconstruction based on distinct models of sequence evolution exist. It is well known that each method have several advantages and disadvantages and that the

* Corresponding author at: Service d'Ecologie des Systèmes Vectoriels, Institut Pasteur d'Alger, Hamma, 1, Rue du Docteur Laveran, Hamma, Alger 16000, Algeria. Tel.: +213 21 673365/+33 4 91 32 43 75; fax: +213 21 673365/+33 4 91 830390.
E-mail address: idirbitam@yahoo.fr (I. Bitam).
[1] Tel.: +33 4 91 32 43 75; fax: +33 4 91 830390.

0147-9571/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cimid.2011.11.002

bioinformatics tool used for phylogenetic analysis may have some influence on the topologies of the resulting trees [41]. The most common traditional approaches to reconstructing phylogenies are the Neighbour Joining (NJ) and the maximum parsimony (MP) methods. Recently, Bayesian (BA) approaches to phylogenetic inference have provided a method for simultaneously estimating trees and obtaining measurements of uncertainty for every branch. In this study, we have compared BA, NJ, and MP methods for phylogenetic analysis of *Bartonella* species using *rpoB*, *gltA* and *ftsZ* genes [41].

The aim of this study was to detect *Bartonella* species by means of polymerase chain reaction (PCR) amplification and sequencing in fleas collected on rodents and hedgehogs and to see the evidence for human zoonotic infections with flea-borne bacteria in the districts of Oran and Mascara, Algeria.

2. Material and methods

2.1. Description of study areas

Fleas were collected in two locations during an entomological survey of flea-borne diseases, including plague, in Algeria [6]. The two sites that represented the original focus of the reported cases of plague were Tafaraoui (35°29′N, 0°32′O) and Douar Ouled Ben Aouali (35°33′N, 0°21′O) in the districts of Oran and Mascara, about 450 km West of the capital, Algiers. These settlement areas are situated at an altitude of 208 m above sea level and have an estimated population of 1400 for Tafraoui and 1375 inhabitants for Douar Ouled Ben Aouali.

2.2. Fleas collection and identification

Fleas were collected from rodents and hedgehogs trapped either from inside houses or from peridomestic areas of these cities. All fleas were identified using standard morphologic taxonomic criteria [5,6] and then stored for one month at ambient temperature in 100% ethanol. Alcohol-preserved fleas were rinsed individually with distilled water for 10 min. DNA was extracted for each flea individually as previously described [6]. The species identification of fleas was confirmed by PCR amplification of a 1951-bp fragment of the 18S rDNA gene, as previously described [6].

2.3. PCR performed and sequencing

The strategy for the detection and identification of *Bartonella* spp. was to initially perform a screening with a real-time PCR assay on a LightCycler machine using primers and Taqman* probes, as previously described [6,7]. For *Bartonella* spp., the precise identification of the species was then performed after PCR amplification and sequencing of several target genes (*ftsZ*, *gltA*, *rpoB*) and the intergenic spacer (ITS), as suggested by La Scola et al. [8]. Sequences were aligned by CLUSTAL W version 1.5 [27] with the default settings. Evolutionary relationships were estimated by constructing phylogenetic trees using Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian Markov-chain Monte Carlo phylogenetic analyses. Two taxa were chosen as outgroups: *Agrobacterium* and *Neorickettsia*. The final alignment comprises 28 sequences of 660 nucleotides for the *rpoB* gene, 183 nucleotides for the *gltA* gene and 313 nucleotides for the *ftsZ* gene. NJ and MP analyses were carried out with PAUP 4b10 program [26] and Bayesian analyses with MrBayes 3.1 [28,29]. The computer program MrModeltest 2.3 [31] was used to evaluate the fit of 24 nested models of nucleotide substitution to the data. The Akaike information criterion revealed that the model which best fits the data was the GTRG + I + G model for the three genes. Bootstrap analysis (500 replicates) was used for NJ (K2P genetic distance) and MP analyses to estimate the robustness of internal nodes. All MP analyses were performed using the tree-bisection-reconnection (TBR) branch swapping option with 10 random addition replicates. Gaps were treated as a fifth state. The program's default priors for parameters of the Bayesian analyses were used for all analyses. We used three heated chains and a single cold chain in all Bayesian analyses, and initiated runs with random trees. We conducted two independent runs with five million generations per run. We sampled trees (and parameters) every 100 generations. Stationarity was assessed by examining the average standard deviation of split frequencies. As the two runs converge onto the stationary distribution, we expect the average standard deviation of split frequencies to approach zero, reflecting the fact that the two tree samples become increasingly similar [30]. Moreover, the Potential Scale Reduction Factor (PSRF) should approach 1.0 as runs converge. For each run, the first 25% of sampled trees were discarded as burn-in. All phylogenetic analyses were run both independently for each gene and for the combined data set.

3. Results

A total of 204 fleas collected from 26 rodents and 7 hedgehogs were tested, including 95 *Xenopsylla cheopis* collected in *Rattus rattus* ($n = 15$) and *Rattus norvegicus* ($n = 6$), 96 *Archeopsylla erinacei* collected in hedgehogs ($n = 7$), and 13 *Leptopsylla segnis* collected in *Mus sretus* ($n = 5$) that were unambiguously identified after 18S rDNA amplification and sequencing (Table 1).

A total of 44 (21.5%) fleas were positive by real-time PCR for *Bartonella* ITS. After PCR amplification and sequencing of the ITS and *ftsZ* gene, 42 of these fleas had sequences identical to *B. elizabethae* ($n = 26$), *B. tribocorum* ($n = 3$), *B. clarridgeiae* ($n = 4$) and an unnamed *Bartonella* species previously detected in rodents from China (Genbank accession number AY461845) with ITS ($n = 9$) (Table 1). Finally, for 2 fleas, the ITS and *ftsZ* sequences had respectively a 98% and 99% homology with a *Bartonella rochalimae*-like isolate (Genbank access numbers DQ683199 and DQ683198), and also a 98% and 99% homology with a *B. rochalimae*-like species isolated from *R. norvegicus* from Taiwan for which the sequences were recently published in GenBank (access numbers EU551157 and EU551155). For this *Bartonella* species, partial *rpoB* and *gltA* gene sequences were also obtained (Table 1) and used to build a phylogenetic tree. Results of independent and combined analyses were congruent whatever the type of phylogenetic analysis