Small Indian mongooses and masked palm civets serve as new reservoirs of *Bartonella henselae* and potential sources of infection for humans

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Abstract

The prevalence and genetic properties of *Bartonella* species were investigated in small Indian mongooses and masked palm civets in Japan. *Bartonella henselae*, the causative agent of cat-scratch disease (CSD) was isolated from 15.9% (10/63) of the mongooses and 2.0% (1/50) of the masked palm civets, respectively. The bacteraemic level ranged from 3.0×10^1 to 8.9×10^3 CFU/mL in mongooses and was 7.0×10^3 CFU/mL in the masked palm civet. Multispacer typing (MST) analysis based on nine intergenic spacers resulted in the detection of five MST genotypes (MSTs 8, 14, 37, 58 and 59) for the isolates, which grouped in lineage 1 with MST genotypes of isolates from all CSD patients and most of the cats in Japan. It was also found that MST14 from the mongoose strains was the predominant genotype of cat and human strains. This is the first report on the isolation of *B. henselae* from small Indian mongooses and masked palm civets. The data obtained in the present study suggest that these animals serve as new reservoirs for *B. henselae*, and may play a role as potential sources of human infection.

Keywords: *Bartonella henselae*, cat-scratch disease, masked palm civet, mongoose, multispacer typing
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Introduction

Bartonella bacteria are small, fastidious, gram-negative, vector-transmitted pathogens. Since the early 1990s, more than 20 species including three subspecies of *Bartonella* have been identified and at least 13 species are known to be zoonotic agents [1,2]. Cat-scratch disease (CSD) is one of the most common zoonoses caused by *Bartonella henselae* and the cat (*Felis catus*) is recognized as the main reservoir for *B. henselae*. The prevalence of the organism in cats varies from 0% in Norway to 68% in the Philippines, and varies according to the housing status of cats (pet or stray) and the geographical location [3]. Except for domestic cats, *B. henselae* was isolated from wild African lions and cheetahs [4]. Antibody to *B. henselae* was also detected in free-ranging and captive wild felids such as bobcats, leopards, jaguars, pumas and tigers [5,6]. These data suggest that wild Felidae are reservoir hosts of *B. henselae* in nature.

Both the small Indian mongoose (*Herpestes auropunctatus*) and the masked palm civet (*Paguma larvata*) belong to the suborder Feliformia of the order Carnivora along with the felids. Since small Indian mongooses were introduced in 1910 from Bangladesh to Okinawa Prefecture, Japan, they have readily adapted to the new environment and have been having serious effects on the unique ecosystem and causing extensive damage to agricultural crops and the poultry industry in the

area [7]. Masked palm civets are widely distributed from Northern India to Southeast Asia and China, and the introduced individuals have also expanded their habitat and caused serious damage to agricultural products and intrusion into human dwellings in Japan [8].

Hence, the increased populations of small Indian mongooses and masked palm civets have resulted in many opportunities for these species to appear in the peridomestic environment and come into contact with either residents or animal control workers. Although these animals present serious risks as sources of zoonoses such as leptospirosis, rabies, severe acute respiratory syndrome, salmonellosis, yersiniosis and campylobacteriosis [9–12], no epidemiological studies on *Bartonella* infection in mongooses and masked palm civets have been conducted.

Several genotyping methods have been developed and applied for the characterization of *Bartonella* isolates. It is reported that multispacer typing (MST) using nine variable intergenic spacers is the most discriminatory genotyping method for *B. henselae* isolates and is used to investigate the relationships between human and cat isolates [13,14].

The aim of the present study was to investigate the prevalence of *Bartonella* species in small Indian mongooses and masked palm civets in Japan. Furthermore, we evaluated the possibility that these animals serve as a source of CSD for humans by MST of the isolates.

Material and Methods

Sample collection

During the period from 2009 to 2012, blood samples were collected from 63 small Indian mongooses in Okinawa Prefecture and 50 masked palm civets in Chiba ($n = 26$) and Kanagawa ($n = 24$) Prefectures, Japan. Blood samples from the mongooses and masked palm civets were collected by cardiopuncture after euthanasia following the guidelines for invasive alien species prepared by the Japanese Veterinary Medical Association, and then transferred into EDTA-containing collection tubes. Blood samples from the mongooses were immediately stored at − 70°C, whereas those from the masked palm civets were stored at − 20°C for 2–12 months after collection. The samples were sent to the Laboratory of Veterinary Public Health, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University for examination of *Bartonella*.

Isolation and identification of *Bartonella* bacteria

Frozen blood samples were thawed at room temperature and submitted for the isolation of *Bartonella* species following previously reported procedures [15]. Bacterial colonies were tentatively identified as *Bartonella* species based on colony morphology and the long culture period (>1 week), and subsequently the CFU/mL of blood were calculated by additional quantitative culture. For further characterization, five colonies were picked from each sample and subcultured on fresh blood agar plates using the same conditions as the primary culture.

The genomic DNA of each isolate was extracted using InstaGene Matrix (Bio-Rad, Hercules, CA, USA). Identification of *Bartonella* was performed using *Bartonella*-specific PCR for six housekeeping genes including the 16S ribosomal RNA gene (16S rRNA), the cell division protein gene (*ftsZ*), the citrate synthase gene (*gltA*), the heat-shock protein gene (*groEL*), the riboflavin synthase alpha chain gene (*ribC*) and the RNA polymerase beta subunit-encoding gene (*rpoB*). The primers and PCR conditions used for the PCR amplification of 16S rRNA [16], *ftsZ* [17], *gltA* [18], *groEL* [19], *ribC* [20] and *rpoB* [18] have been previously published.

For DNA sequencing of 16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC* and *rpoB*, the PCR products were purified using a Spin Column PCR product purification kit (Bio Basic Inc., Markham, Ontario, Canada), and then sequenced directly by using dye terminator chemistry and a Genetic Analyzer model 3130 (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer's instructions. The sequence alignments were assembled and edited using the AUTOASSEMBLER program in GENETYX-WIN software, version 9 (Genetyx Corp., Tokyo, Japan), and compared with those of other known *Bartonella* species deposited in the GenBank/EMBL/DDBJ database by using the BLAST program.

Multispacer typing and phylogenetic tree based on nine intergenic spacers

Internal fragments of approximately 300–500 bp of nine intergenic spacers (S1–S9) were amplified by PCR as described previously [13]. Positive and negative controls were prepared using DNA from *B. henselae* Houston-1^T and nuclease-free distilled water, respectively. The PCR products of S1–S9 were purified and sequenced directly. Vector sequencing was applied only when obtaining extra bands for S1. The band with the expected size was purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), subcloned using the plasmid pGEM-T Easy vector system (Promega), and sequenced using the same protocol as described for direct sequencing [15]. MST genotypes were determined for ten strains from the mongooses and one strain from the masked palm civet. Ten strains from cats were also subjected to MST analysis. Out of ten cat strains, seven are derived from Okinawa Prefecture where the mongooses were