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Serological and molecular detection of *Bartonella* spp. in humans, cats and dogs from northern Sardinia, Italy

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INTRODUCTION

Bartonella spp. are aerobic, gram-negative pleomorphic bacteria, belonging to the α_2 subgroup of the *Proteobacteria*. Many *Bartonella* spp. are pathogens of humans, dogs [1,2], and cats [3]. Numerous domestic and wild animals can serve as chronically infected reservoir hosts for *Bartonella* spp. An increasing number of arthropod vectors have been implicated in the transmission of *Bartonella* spp. [3].

The main source of transmission to humans is cats by means of scratches, whereas infection is less likely to occur by cat bite. Most human cases of infection with *Bartonella henselae*, named cat scratch disease (CSD), present as an acute febrile lymphadenopathy. The presence of cat fleas is essential for the maintenance of the infection within the cat population. The role of dogs as reservoirs of *Bartonella* spp. is less clear, because they seem to be only accidental hosts. Nevertheless, dogs are excellent sentinels for human infections, because a similar disease spectrum develops in dogs [1].

MATERIALS AND METHODS

Samples

- Serum samples and EDTA–blood samples were collected from 97 humans with CSD in 2007.
- Serum samples and EDTA–blood samples were randomly collected from 190 dogs and 55 cats during 2005–2007.

Strains, cell culture, growth conditions, and antigen preparation

B. henselae Houston 1 (ATCC 49882) was cultivated in brucella broth (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with haemin (250 μg/mL) and 8% Fildes solution, and incubated at 37°C in 5% CO_2. Log-phase cultures were pelleted by centrifugation (5000 *g*, 30 min), washed three times in phosphate-buffered saline (PBS, pH 7.2) and inoculated into 25-cm^2 flasks (Corning, NY, USA) of L929 cells (ECACC 85011425) containing Dulbecco's minimal essential medium (Gibco, Introvigen, Grand Island, NY, USA) supplemented with 2 mM L-glutamine (200 mM) and 15% (v/v) fetal bovine serum (Gibco) at 37°C with 5% CO_2. L929 cells infected with *B. henselae* were harvested by centrifugation at 5000 *g* for 15 min and washed twice with PBS. The final pellets were resuspended in PBS and spotted (10 μL) onto each well of 12-well Teflon-coated slides (Immuno-Cell Int., Mechelen, Belgium). Slides were air-dried for 1 h, fixed with cold acetone for 15 min, and used immediately or stored at –20°C until use.

Indirect fluorescent antibody test (iFAT)

Sera were diluted 1 : 40 in PBS with 1% bovine serum albumin (Gibco). An aliquot of 10 μL from each serum was placed on the well of a *B. henselae* L929 slide and incubated in a humidified chamber at 37°C for 30 min. After being washed in PBS, slides were incubated for 30 min at 37°C with fluorescein isothiocyanate-labelled anti-human immunoglobulin (KPL) diluted 1 : 100. The slides were then washed three times in PBS, counterstained with 1% Evans blue, and examined for fluorescence in a Zeiss microscope.

Molecular test

EDTA–blood samples were subjected to DNA extraction (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany). Primers p12B (5′-GAGATGGCTTTTGGAGATTA-3′) and p24E (5′-CCTCCTTCAGTTAGGCTGG-3′), previously described by Relman *et al.* [4], were used to amplify a *Bartonella* 16S rRNA gene fragment by PCR. The 296-bp amplicons were then sequenced. The nucleotide sequences of both strands of the 296-bp amplicon were determined with an automatic DNA sequencer. Alignment of overlapping sequences was performed using the online program ALIGN (http://vega.igh.cnrs.fr/bin/align-guess.cgi). The sequences were analysed using the BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Specific *B. henselae* IgG antibodies were detected in 21 patients (21.6%) with typical symptoms of CSD, in 11 dogs (5.8%), and in two cats (3.6%). *B. henselae* IgM antibodies were detected in three patients (3.1%), in seven dogs (3.7%), and in four cats (7.3%). Three dogs (1.6%) and four humans

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Table 1. Prevalence of IgG and IgM antibodies specific to *Bartonella henselae* in humans, cats and dogs, by iFAT

	Human		Cat		Dog	
Results	**IgG (%)**	**IgM (%)**	**IgG (%)**	**IgM (%)**	**IgG (%)**	**IgM (%)**
IgG or IgM positives	21 (21.6)	3 (3.1)	2 (3.6)	4 (7.3)	11 (5.8)	7 (3.7)
IgG and IgM positives	4 (4.1)		0 (0)		3 (1.6)	
IgG or IgM doubts	0 (0)	0 (0)	1 (1.8)	3 (5.5)	7 (3.7)	17 (8.9)
Total samples tested	97	97	55	55	190	190

(4.1%) were found to be positive for IgM and IgG antibodies (Table 1).

Bartonella spp. DNA was amplified from a blood sample of a woman scratched by a cat with subsequent fever and lymphadenopathy; the patient was also positive for IgM and IgG. *Bartonella* spp. DNA was detected in three cats. The sequence from the human sample and the sequences from two cat samples identified *B. henselae*; the sequence from the third cat was 99% similar to the 16S rRNA gene of several bartonellae (*Bartonella capreoli*, *Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella fuji*, and *Bartonella doshiae*). The cat positive for the bartonella that differed from *B. henselae* was an 11-month-old domestic male cat with submandibular lymph node enlargement and fever, whereas the others, half-breed and stray cats, were without symptoms attributable to *Bartonella* infection.

CONCLUSION

This study represents the first molecular detection of *B. henselae* in cats from Sardinia. We report the positive sequence of a bartonella different from *B. henselae*. This bartonella was isolated in culture, and studies are in progress for its definitive classification.

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