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ARTICLE

Cat-scratch disease in Northern Italy: atypical clinical manifestations in humans and prevalence of *Bartonella* infection in cats

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Abstract In this paper, we report an investigation on cat-scratch disease (CSD) in Northern Italy. Seventy-four cases of CSD were diagnosed at the San Matteo hospital, Pavia, during the period 2005–2010. Of these 74 patients, 18 (24.3 %) reported atypical clinical manifestations such as ocular papillitis, maculopapular eruptions, vertebral infection, pulmonary infiltrates, and granulomatous hepatitis. Contact with cats was documented for 61 patients (82.4 %), while cat-related trauma was reported for 49 patients (66.2 %). We subsequently investigated the presence of *Bartonella* infection in cats belonging to the above patients and in other domestic and stray cats from three provinces of Northern Italy. Among the 27 domestic cats tested, nine of the 11 belonging to the CSD patients and two of the remaining 16 were infected by *B. henselae* (81.8 % vs. 12.5 %). Out of over 1,300 stray cats examined, 23.1 % were seropositive for *B. henselae*; after culturing and genotyping, 17 % were found to be infected by *B. henselae* (15.5 %) or *B. clarridgeiae* (1.5 %).

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Introduction

Cat-scratch disease (CSD) is a zoonosis caused mainly by *Bartonella henselae*, an emerging, or reemerging, human pathogen [1] and, occasionally, by other *Bartonella* species, such as *B. clarridgeiae* [2, 3]. In the United States, CSD is the most common cause of chronic lymphadenopathy in children and adolescents, other than lymphadenitis caused by evident cutaneous infections, and is mostly a self-limiting disease [4]. *B. henselae* is responsible for a specific type of lymphadenitis characterized by microabscesses surrounded by reticulohistiocytic cells. The balance between host and bacterial factors may produce different clinical courses and involvement of different organs [1]. Cats are the reservoir for these bacteria and fleas play an important role in transmission among cats. Animals become infected during the flea blood meal or by ingestion of infected fleas and flea feces. *Bartonella* infection in cats produces a prolonged and often intense intravascular bacteremia, with the localization of bacteria inside and on the surface of erythrocytes and inside endothelial cells. The bacteremia may last up to 24 months and generally causes no significant clinical signs [5]. Prevalence is generally high in both domestic and stray cats, especially in warm and humid climates [6].

We report our experience of 74 cases of CSD in patients observed from October 2005 through December 2010. We also report the laboratory findings from 27 domestic cats, 11 of them belonging to the above patients, and from stray cats of three provinces of Northern Italy, screened from 2001 through 2008.

Materials and methods

This study was approved by the ethical review board of the hospital Fondazione IRCCS Policlinico San Matteo, Pavia (hereafter, San Matteo hospital).

Patients

Data regarding patients with a final diagnosis of CSD, accessing the outpatient facilities of the Infectious Diseases Department of San Matteo hospital from October 2005 through December 2010, were collected. The diagnosis of *Bartonella* infection required meeting at least three of the following criteria: (a) contact with cats regardless of the presence of an inoculation site; (b) negative serology for other causes of lymphadenopathy, with the pus aspirated from involved nodes negative for mycobacteria, aerobic and anaerobic bacteria, and a positive polymerase chain reaction (PCR) assay for CSD bartonellae; (c) positive indirect fluorescent antibody (IFA) assay with a titer ≥1:64; (d) biopsy specimen showing granulomatous inflammation consistent with CSD or a Warthin–Starry silver stain [7]. All patients were clinically monitored until disease resolution. Ultrasound (US) was performed if a patient had persistent, painful cervical or inguinal lymphadenopathy. In some cases, specimens obtained by US-guided needle aspiration of lymph nodes were analyzed with PCR analysis and isolation in culture was attempted.

Cats

Eleven domestic cats belonging to a subsample of the patients with CSD diagnosis, as well as 16 domestic cats sampled during routine investigations, were tested for *Bartonella* infection by serological analysis and blood culture (see below). Serology and blood cultures were also performed on samples obtained from stray cats collected during the period from January 2001 to May 2008 (1,317 were investigated by serology; 1,340 by blood cultures). This survey on stray cats captured from courtyards of urban areas (Pavia, Bologna, and Varese) was part of a national program for the control of stray pets according to the Italian National Law (281/1991).

Blood cultures

Blood cultures were obtained on the above cat samples using blood agar base heart infusion (Biolife, Milan, Italy) supplemented with 5 % defibrinated fresh rabbit blood. Refrigerated or frozen blood samples (about 1.5 ml) were directly plated onto the medium and incubated in 5 % CO_2 at 37 °C for up to 4 weeks. Identification of the isolates was based on morphological and growth characteristics as previously described [8], and then confirmed by PCR targeting the 16S–23S rDNA intergenic region, as described below.

DNA extraction and PCR

DNA was extracted from clinical specimens of patients, from cats' blood samples, and from bacterial cultures by using the High Pure Template Preparation Kit (Roche Diagnostics, Monza, Italy), following the manufacturer's instructions. The detection of *Bartonella* spp. was performed by PCR, as described by Jensen et al. [9]. This method is based on the amplification of the 16S–23S rDNA intergenic region, and allows the detection of six *Bartonella* species (*B. bacilliformis*, *B. clarridgeiae*, *B. elizabethae*, *B. henselae*, *B. quintana*, *B. vinsonii* subsp. *berkhoffii*), as well as the differentiation among these species based on the length of the amplification product. *B. henselae* Houston 1 (ATCC 49882) and *B. clarridgeiae* (ATCC 51694) were used as positive controls. *B. henselae* subtyping was performed using two primer pairs targeted on the 16S rRNA gene, specific for the two subtypes (I and II) of *B. henselae* [10].

Serological analysis

For the analysis of cat sera, an indirect immunofluorescence antibody test for *B. henselae* was performed as described by Chomel et al. [11], with *B. henselae* cultivated on HEP-2 cells. For human sera, a commercial IFA assay for *B. henselae* was used (Daltec Instrument Srl, Milan, Italy). Serum samples were considered to be positive when a titer ≥1:64 was obtained.

Results

The medical records of 74 patients (38 females, 36 males; mean age 27.2 years; range 2–74 years) affected by CSD were retrieved. A history of prolonged contact with domestic cats was documented for 61 patients (82.4 %), while cat-related trauma was reported for 49 patients (66.2 %). Only one patient out of 74 presented with a history of immunodepression (she had been diagnosed with breast carcinoma 18 months before the onset of CSD and treated with surgery and chemotherapy). All of the patients had a positive IFA antibody test using the *B. henselae* antigen. US examination was performed on nine patients with tender, enlarging axillary lymphadenopathy. US showed enlarged lymph nodes, usually with central necrosis and surrounding edema. Purulent material from fine-needle aspiration, carried out on the above nine cases, was used in PCR assays. All of these patients were PCR-positive for *B. henselae*, six of which were subtype I, while the remaining three were subtype II.

Attempts to isolate *Bartonella* from pus and tissue samples were unsuccessful. Atypical clinical manifestations were observed in 18 out of 74 (24.3 %) patients (Table 1). Diffuse maculopapular eruptions were observed in five patients with axillary lymphadenopathy; lesions were reddish-brown and persisted for 2–3 weeks. In two cases, *Bartonella* infection involved the liver and the spleen, with US imaging showing fleeting hypoechoic lesions, suggesting the presence of microabscesses; liver enzymes were normal; the predominant lesion on histopathology was a necrotizing granuloma. A 58-year-old man with CSD presented with axillary lymphadenitis and, 2 months later, was diagnosed with a vertebral *Bartonella* infection (L4–L5) on computed tomography (CT), with detection of *B. henselae* subtype I by PCR on intervertebral disk biopsy. Ocular papillitis was observed in a 17-year-old woman with axillary lymphadenitis. Seven patients presented with groin localization of CSD lymphadenopathy. An immunocompetent 12-year-old male with fever, regional adenopathy, multifocal hepatosplenic granulomas, and high serum antibody titers for *B. henselae* developed diffuse bilateral reticulonodular pulmonary infiltrates in the absence of respiratory symptoms. *Bartonella* infection was serologically demonstrated in a 45-year-old patient who presented a 1-month history of fever. Serology was positive in all of the patients with a titer greater than 1:64.

A total of 27 domestic cats were analyzed for *Bartonella* spp. by culture and PCR. Of the 11 cats belonging to CSD patients, nine (81.8 %) returned positive, six of which for *B. henselae* type I and three of which for *B. henselae* type II. For all the nine patients for which successful PCR amplification for *B. henselae* was obtained and for which we could examine the cat, there was a perfect match between the genotypes found in the cat's owner and his/her respective cat. Among the other 16 domestic cats, only two (12.5 %) were infected with *B. henselae* type I. Out of the 1,317 serum and blood samples sera collected from stray cats and tested by IFA, 304 resulted positive (23.1 %). Seroprevalence ranged from 10.8 % (Varese area) to 25 % (Pavia area) (Table 2). Among the 1,344 blood cultures, 229 (17 %) returned positive for *Bartonella* spp., 208 (15.5 %) were identified as *B. henselae*, and 21 (1.5 %) were identified as *B. clarridgeiae*. Genotyping was performed on a subsample (192/208) of the strains isolated for *B. henselae*: 64 (33 %) returned as *B. henselae* type I, 88 (46 %) returned as *B. henselae* type II, and the remaining 40 cases (21 %) were found to be co-infections with both types.

Table 1 Atypical clinical manifestations of cat scratch disease (CSD) in 18 patients

No. of patients	Clinical manifestations
5	Maculopapular eruptions
2	Granulomatous hepatitis microabscesses in liver and spleen
1	Vertebral infection (L4–L5)
1	Ocular papillitis
7	Inguinal lymphadenopathy
1	Pulmonary infiltrates and granulomatous hepatitis
1	Fever of unknown origin

Discussion

The diagnosis of CSD is primarily clinical, with laboratory analyses used to confirm initial suspicion [12]. Even though *B. henselae* usually causes a mild infection that is limited to lymph nodes draining from the area where the scratch occurred, atypical manifestations have sometimes been reported (e.g., [13]). In our study, atypical symptoms were observed in 24.3 % of the cases. This percentage is higher than that reported by Carithers in 1985 [13], who observed typical CSD in almost 95 % of the cases. This difference is most likely due to the current availability of advanced techniques for the detection of *B. henselae*, such as serology and PCR.

The isolation of *Bartonella* from humans is generally difficult, especially from nodal tissues. In the presented cases, all attempts to isolate *Bartonella* from pus and tissue cultures failed, the possible explanation being that lymphadenopathy is due to a persistent immune response rather than to the direct presence/persistence of bacteria. PCR is a useful tool for the detection and direct diagnosis of CSD in humans. In our study, PCR analyses performed on pus returned positive for nine patients, six of which hosted *B. henselae* type I and three of which hosted *B. henselae* type II. Although our findings come from a small number of samples, they are in accordance with data from other European countries and Australia, where *B. henselae* type I is more frequently linked to human cases and may be more virulent for humans than type *B. henselae* II [14].

Table 2 Distribution of *Bartonella henselae* isolates from stray cats among three Italian provinces

Locations	Blood culture		Serology	
	No. of examined cats	No. of positive cats (%)	No. of examined cats	No. of positive cats (%)
Pavia	770	180 (23.4)	753	188 (25.0)
Varese	93	8 (8.6)	83	9 (10.8)
Bologna	481	41 (8.5)	481	107 (22.2)
Total	1,344	229 (17.0)	1,317	304 (23.1)

Nine of the 11 (82 %) domestic cats belonging to the patients with CSD returned positive for *B. henselae*, while only two of the 16 (12 %) domestic cats non-related with patients were positive. We emphasize that the strain types isolated from the above nine cats matched those of the respective owners (see "Results"). Although too low for statistical significance, these results confirm the importance of the cat as a reservoir for this zoonotic disease in Northern Italy, in agreement with previous results showing that, among 165 domestic cats sampled in this area, 35 (21 %) were bacteremic and 49 (30 %) were seropositive for *B. henselae* [6, 15].

Among the stray cats sampled in urban areas, 23 % were seropositive and 17 % were bacteremic, confirming our previous finding that *Bartonella* is widespread among stray cats in Northern Italy [6] and indicating the potential source of infection represented by the habit to adopt stray kittens without accurate controls for *Bartonella* and fleas. Seroprevalence is generally higher in older cats than in younger animals, whereas bacteremia is more frequent in younger cats (<1 year old) [11]. This is crucial, given that stray kittens are usually adopted when very young. In these cases, the owner should be informed that the cat must be in good health and flea-free, and that periodic checks for fleas should be carried out. It would, thus, be important to determine not only the serological positivity for *Bartonella*, but also the bacteremia and antibodies titers, in order to obtain a complete evaluation of the health and reservoir status of the cat before adoption. Diagnosis of bacteremia is, indeed, essential in assessing the risk of transmission of *Bartonella* to humans and a non-bacteremic cat with positive serological test should be re-evaluated for possible recurrent bacteremia. Volunteers in stray-cat recoveries and in people temporarily housing stray cats should also be subjected to periodic screenings to assess occult *Bartonella* infections.

CSD may have unusual clinical manifestations and may be challenging for the clinician. CSD, as most human infectious diseases, is a zoonosis. Collaboration between human and animal health professionals, which was conceptualized by Calvin Schwabe as "One Medicine" [16], is crucial in controlling these diseases. Actions taken towards animal populations may, indeed, have greater benefits for public health than just interventions in humans [17, 18].

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Conflict of interest All the organizations involved in this study are non-profit public governmental institutions. No conflict of interest is, thus, declared.

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