Prevalence of antibodies to *Bartonella henselae* in patients with suspected cat scratch disease (CSD) in Italy

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Abstract. Cat scratch disease (CSD) is a relatively new diagnosed illness with clinical signs of self-limiting regional lymphadenopathy accompanied by symptoms of fever and malaise, to encephalopathy and neuropathy, occurring after a cat scratch or flea bite. *Bartonella henselae* is now accepted as the etiologic agent of CSD. From January 1994 to September 1998, 412 patients were evaluated for suspect CSD in Italy. Sera were tested for antibodies to *B. henselae* by a commercially available indirect immunofluorescent assay (IFA), based on *B. henselae*-infected Vero-cells as the antigen substrate. Of the 412 patients, 26 (6.3%) were considered positive having titers of immunoglobulin G (IgG) to *B. henselae* of 64 or higher. In these patients CSD was indeed confirmed by either histopathologic examination of lymph nodes biopsy

or fourfold raise in antibody titers. Nevertheless, sera were tested by IFA for *Afipia felis* and one showed a double reactivity to *B. henselae* and *A. felis*. Finally, three sera, negative to *B. henselae* serology, were positive to *A. felis*. Three hundred and eighty-six patients received alternative diagnoses. One hundred and twenty-five serum samples from control subjects were negative by IFA for either *B. henselae* or *A. felis*. Moreover, a cross-reactivity with sera from patients affected by other diseases was not observed. Our study shows that the ascertained cases of CSD are etiologically determined by *B. henselae*, IFA assay is confirmed as a useful tool in the laboratory diagnosis and, over a 5 years period of study, the incidence of CSD in Italy has been low.

Key words: Antibodies, *Bartonella henselae*, Cat scratch disease, Immunofluorescence, Serology

Introduction

Over the last ten years, a number of different viruses [1, 2], bacteria [3] and mycobacteria [4] have been proposed as the etiologic agent of cat scratch disease (CSD). In 1988, English et al. [5] isolated a bacterial agent from clinical specimens of CSD patients which was in 1991 classified as a new bacterium *Afipia felis* by Brenner et al. [6]. Recently, another agent, a rickettsia-like organism, *Rochalimaea henselae*, has been isolated from either the blood of HIV-infected patients with fever and affected by the bacillary angiomatosis [7, 8] or the lymph nodes of two patients with CSD [9]. Antibodies to *R. henselae* have been detected in the 80–85% sera of patients with CSD [10, 11]. Although the relative role of *A. felis* and/or *R. henselae* in the clinical syndrome known as CSD has been controversial for many years, more recent studies based on the analysis of 16S rRNA gene sequences together with polymerase chain reaction (PCR)-based restriction length polymorphism analysis either of a portion of the citrate synthase gene or a fragment of the ribosomal operon from *Rochalimaea* species subtyping, demonstrate that the etiologic agent is closely related to members of the genus

Rochalimaea [7, 12]. Moreover, *B. henselae*, renamed *Bartonella* [13], and *A. felis* are not closely related when compared by either 16S ribosomal RNA gene sequences or genomic DNA hybridization analysis [7, 8]. More recently, the PCR assay has been performed for the detection and identification of the etiologic agent of CSD [14, 15]. Although PCR is a rapid and specific method for the detection of the organisms in clinical samples, it requires appropriate laboratory equipment. The indirect fluorescent-antibody (IFA) assay developed in 1992 by Regnery et al. [10] at the Centers for Disease Control and Prevention remains the only widely accepted diagnostic procedure for the laboratory confirmation of CSD [16]. Investigators performed serologic analysis on samples obtained from patients with suspected CSD and the comparison of seroreactivity with clinical data indicates that an overwhelming number of patients have antibodies to *B. henselae* but are seronegative for *A. felis* [17–20]. Although CSD affects people of all ages, more than 80% of patients are less than 21 years of age, and the seroprevalence reported by several studies varies widely between different countries or between geographic regions within countries [11, 17, 21–27]. After our communication in

1994 [21], the seroprevalence for *B. henselae* in patients suspected of having CSD in Italy has not been reported. With this aim we have investigated the prevalence of *B. henselae* antibodies in patients with suspected CSD in Italy by IFA assay.

Patients and methods

Four hundred and twelve patients, more than 85% older than 5 years and less than 15 years of age, admitted to the Pediatric or Infectious Diseases Departments from different regions of Italy for evaluation of suspected CSD from January 1994 to September 1998, were prospectively examined. Age and gender for each group of patients are shown in Table 1. Suspected cases were those patients with previous contact with a cat, presence of a scratch and/or a primary dermal or eye lesion and regional lymphadenopathy with negative laboratory tests for other causes of lymphadenopathy, including atypical clinical expression of CSD.

Samples of 6 ml of peripheral whole blood were obtained, whenever possible on the day of admission and collected in tubes containing SST[®] gel and clot activator (Vacutainer; Becton Dickinson Vacutainer Systems Eur, Meylan Cedex, France). Sera, immediately separated by centrifugation at $2.300 \times g$ for 10 min, were frozen at -70°C and sent in refrigerate packages to our laboratory, where the serological test was performed.

Immunofluorescent serology

Sera from both patients and control subjects were tested in a two-fold dilution series starting at 1:8 by a *B. henselae*-based IFA test (*Bartonella henselae* IgG Antibody Test, FULLER Laboratories; Fullerton CA, USA; distributed in Italy by 'ARNIKA, Milano, Italy). This assay, using *B. henselae*-infected Vero-cells as the antigen substrate, reveals, in a semi-quantitative mean, immunoglobulin G (IgG) class antibody concentration in the sera of patients suspected of having CSD. Serum testing for IgG antibodies to *B. henselae* was carried out as recommended by the manufacturer, whereas the interpretation of the results was modified on the basis of our experience. Despite instructions (positive if titer 128), titers ≥ 64 were defined as positive for IgG class.

Table 1. Characteristics of CSD patients in each group and IFA results

Age/y (range)	Gender (M/F)	Serology (IFA titer)
5–15	7/11	64
5–15	3/3	128
5	0/1	256
7	1/0	512

Moreover, in order to evaluate the putative sero-conversion, sera reactive at the dilution of 1:64 (as the cut-off screening dilution in sterile phosphate buffer solution) were taken to higher dilutions, upto 1:1024. The negative control serum and dilutions of the positive control serum have been assayed with each daily run. *A. felis* IFA assay was performed as previously described [21]. Based on our experience, positive values for IgG-specific *A. felis* IFA had previously determined to be greater than or equal to 16.

Specificity

Sera of patients with serologically confirmed infections by *Coxiella burnetii*, *Borrelia burgdorferi*, *Brucella*, *Legionella Pneumophila*, *Chlamydia pneumoniae*, *Toxoplasma gondii*, *Cytomegalovirus*, Epstein–Barr virus, *Leptospira*, from our laboratory collection, were assessed for cross-reactive antibodies to *B. henselae*.

Results

Interpretation of results

A positive reaction appears as bright staining (at least 1+) of characteristic cytoplasmic patches within a percentage of the Vero-cells. The size, appearance, and density of the fields have been compared with those of the positive and negative control reactions. IgG titers ≥ 64 were considered supporter for the diagnosis of CSD. When an acute phase titer could not indicate infection clearly, the demonstration of a four-fold increase in the serum samples taken during the convalescent phase was considered diagnostic of infection. Positive sera at this dilution have been re-run to determine their end-point titer. Samples not reactive at the screening dilution of 1:32 were considered negative and, to exclude the illness, further serum specimen had been drawn about 2 weeks after the first, taken immediately after the onset of signs or symptoms.

Laboratory testing

Sera were tested by IFA assay for antibodies to *B. henselae*. Sera from patients with various other diseases were assessed for cross-reactive antibodies to *B. henselae*. Twenty-six (6.3%) of the 412 patients with suspected CSD were seropositive for *B. henselae*, with titers of 64 or more (some titers were very high) (Figure 1). Among the 26 positive sera for *B. henselae*, 18 had a titer of 64, 6 of 128, 1 of 256 and, finally, 1 of 512 (Table 1, Figure 1).

All the 26 CSD patients were under 15 years of age. In addition, serum samples were tested by IFA assay for IgG class antibodies to *A. felis* [21]. Only 3, negative for anti-*B. henselae*, had antibodies to *A. felis* with a titer ≥ 16 ; in one case the titer was 128. Finally, in one case there was a double reactivity with a titer of 128 for *B. henselae* and 64 for *A. felis*. Nev-

Figure 1. Distribution of *B. henselae* specific antibodies among the 26 patients affected by cat scratch disease.

ertheless, human anti-*B. henselae* sera did not cross-react with *A. felis* antigen in the IFA assay. Among the sera tested, 386 (93.7%) were seronegative for *B. henselae* (i.e. titer < 64). However, 18 patients had a low titer of 32 to *B. henselae* in the admission, which did not change after 15 days when a second serum sample was taken. These patients were not considered to have CSD (Tables 1 and 2, Figure 1). In all positive patients CSD diagnosis was confirmed by either the four-fold raise in antibody titers or lymph nodes biopsy (pattern of caseating granulomas, microabscesses and follicular hyperplasia). Finally, 125 serum samples from healthy subjects were tested by IFA assay for *B. henselae* and *A. felis*. Both the tests resulted negative.

Discussion

We report the seroepidemiology of *B. henselae* studied by IFA assay in patients with suspected CSD in Italy over a 5-year period. The results show that 26 (6.3%) of 412 patients with suspected CSD had serum titers to *B. henselae* of 64 or more and, therefore, were considered positive patients. This study demonstrates that the clinical suspect of CSD, based on the presence of regional lymphadenopathy and/or a previous history of contact with a cat, is confirmed by serology for antibody to *B. henselae*. In all positive patients the clinical suspect of CSD was also confirmed by a surgical biopsy. However, there are no generally accepted diagnostic tests because of the lack of univocity on the cause of CSD. Although the CSD

skin test antigen has been individuated for the diagnosis of the disease, this material is unlicensed and difficult to standardize. To diagnose CSD and to exclude more serious illness such as malignant diseases, physicians must now rely on clinical criteria and surgical biopsy. Fastidious growth requirements of bacteria coupled with an intrinsically slow growth rate (colonies requiring 15 to 45 days of incubation to become apparent) restrict the possibilities of rapid diagnosis and identification in the clinical setting. Although the PCR has been proposed as a diagnostic test for CSD, it is not a standardized means and is not yet available for the routine diagnosis of CSD in laboratory of clinical microbiology. The described evaluation of PCR assay positivity among samples from patients suspected of having CSD described is virtually identical to the percentage positivity observed by serologic means since it shows a strong correlation between PCR positivity and antibody titer [14, 15]. The PCR assay offers the advantage of early diagnosis, since it is not dependent on the mounting of a detectable humoral immune response by the patient. However, although the PCR is able to differentiate *B. henselae* from *B. quintana* infections, the test may be affected by various problems of sensibility, inhibitors, or DNA extraction from tissue and other samples. The IFA test, being a more simple and available test, encourages use in clinical microbiology laboratories.

In our hands, the *B. henselae* IFA test – because of its high sensitivity and specificity – confirms the opinion that it may be considered useful in the diagnosis of CSD [10, 17–20, 26, 27]. When in clinically suspected CSD the first titer is low, a titer increase in a serum specimen obtained about 2 weeks after the onset of symptoms should confirm the diagnosis. In addition, from a laboratory point of view, the IFA assay makes the diagnosis of CSD easy and rapid, therefore preventing these patients from unnecessary investigations such as invasive surgical diagnostic procedures.

The observations that most of the enrolled patients (85%) were children (5 up to 5 years) and all the positive patients were under 15 years of age are consistent with other studies in which it is reported that CSD occurs mainly in children and young adults [23, 25–28]. This may be due to the behaviour or

Table 2. Immunoglobulin G (IgG) class antibody titers of patients with cat scratch disease (CSD) caused by *Bartonella henselae* over the years period of the study. Titer ≥ 64 was considered supportive of the CSD diagnosis

	<32	32	64	128	256	512
1994	99	5	1	0	0	0
1995	141	4	5	3	0	0
1996	78	1	3	2	0	1
1997	34	8	4	1	1	0
1998 (until September)	15	0	5	0	0	0
Total	368	18	18	6	1	1

habits of children which lead them to play with cats, creating the possibility of a scratch or a bite.

With regard to the controversy on the etiological role played by *A. felis* and *B. henselae*, it was initially suspected that *A. felis* may be the causative agent of this illness [6]. Although a dual etiological role has been ascertained in a small number of cases of CSD [29–31], our study shows without any controversy that *B. henselae* is implicated in CSD in humans.

Among the emerging infectious diseases, CSD plays an important role and it has to be taken into consideration in the differential diagnosis among the lymphadenitis, above all those occurring in young people. As a clinical sign, the lymphadenitis typically follows the development of a papule occurring one to two weeks after a cat scratch or bite. Usually, in this illness the lymphadenopathy is proximal to the inoculation site and resolves in two to four months [28]. Although the frequency of illness observed in the period of our study in Italy has been low, this might be due to the fact that Italian families with children generally avoid having a housebound cat. Cats have been shown to provide the only known reservoir of *B. henselae* [24]. Several reports on serologic testing of cats showed the seroprevalence of *B. henselae* with a wide difference among regions or countries [11, 17, 21, 24, 25, 32, 33]; also, it is auspicious that in a future work the prevalence in Italy might be determined in order to assess the true incidence of *B. henselae* in cats. In this way, the low frequency in Italian children could be explained.

In conclusion, our results confirm that the *B. henselae* IFA test will be a useful tool in the rapid diagnosis of CSD. The IFA, being a rapid and specific means, affords clinicians an alternative to culture or PCR analysis for laboratory confirmation of CSD diagnosis, thereby permitting them to exclude other diseases and to carry out the antibiotic therapy.

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