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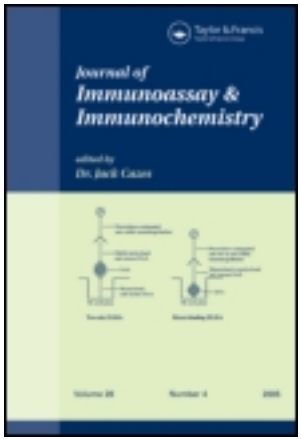

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PREVALENCE OF ANTIBODIES ANTI-*BARTONELLA HENSELAE* IN WESTERN SICILY: CHILDREN, BLOOD DONORS, AND CATS

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PREVALENCE OF ANTIBODIES ANTI-*BARTONELLA HENSELAE* IN WESTERN SICILY: CHILDREN, BLOOD DONORS, AND CATS

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□ *To evaluate seroprevalence of* B. henselae *infection both in Sicilian children and healthy blood donors. Furthermore, circulation of* Bartonella *in the natural reservoir was also studied. Two hundred forty-three children, living in Sicily (Palermo), affected by various diseases, without clinical features suggesting* B. henselae *infection, together with 122 healthy blood donors were serologically investigated for IgG and IgM antibodies by indirect fluorescent antibody test (IFAT). One hundred twenty stray and 62 pet cats were also analyzed only for IgG. Among children 25.1% had IgG antibodies to* B. henselae*; 18.5% showed a titer 1:64, 2.4% 1:128, 2.4% 1:256, 0.8% 1:512, 0.4% 1:1024, and 0.4% 1:5120. Among healthy blood donors 11.4% had IgG class antibodies to* B. henselae*; 9.8% showed a titer 1:64 and 1.6% 1:128. All the human serum samples did not show positive results for* B. henselae *IgM class antibodies. Stray cats (68.3%) and pet cats (35.4%) also had IgG class antibodies to* B. henselae. *We demonstrated high frequency of serologic evidence of past* B. henselae *infection, in young Italian children, affected by various diseases, apparently free of any clinical features suggesting* B. henselae *infection. This observation is supported by high circulation of* Bartonella *in cats.*

Keywords *Bartonella henselae*, cat scratch disease, indirect fluorescent antibody test

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INTRODUCTION

Bartonella (B.) henselae infection is a common zoonosis, with worldwide distribution. Bacterial infection caused by *B. henselae* is the most common cause of a benign regional lymphadenopathy, known as "cat scratch disease" (CSD), in children and young adults.[1–3]

The availability of a sensitive immunofluorescence assay has significantly increased our awareness of human clinical manifestations of *B. henselae* infection, allowing identification, particularly in children, of new clinical features, such as distal ileitis, mimicking Crohn disease, and pseudoinfectious mononucleosis.[4–6] While most cats infected by *B. henselae* never develop detectable clinical signs of disease, the spectrum of *B. henselae*–associated illness, in diseased cats, comprises self-limiting febrile illness, lymphadenopathy, mild to moderate transient anemia, and transient neurologic dysfunction.[7]

Human and cat seroprevalence of antibodies to *B. henselae* may differ, considerably, in different geographic regions, and among individuals living in the same geographic area.[1,8–10] There currently are not clear data about the prevalence of human symptomatic *B. henselae* infection, because it is often underdiagnosed, but there are consistent data on the seroprevalence of *B. henselae* both in humans[11–14] and in feline populations.[15,16]

In this report we carried out a retrospective evaluation of the seroprevalence (IgG and IgM antibodies against *B. henselae*) in 243 children, living in Sicily, affected by various diseases, who were apparently free of any clinical features suggesting *B. henselae* infection. The same analysis was done in 122 healthy blood donors, not gender- and age-matched, from the same geographic area. Furthermore, we have studied the circulation of *Bartonella* in the natural animal reservoir (cats) by detecting IgG *B. henselae* antibodies in 120 stray and 62 pet cats, living in the same geographic area.

MATERIALS AND METHODS

Subjects

We analyzed IgG and IgM antibodies to *B. henselae* in serum samples, stored at −20°C, taken from 243 children, living in Sicily, affected by various diseases (bacterial and viral infections, neoplasms, and inflammatory and metabolic diseases), who were, randomly, observed during February 2004–February 2005, as in- and outpatients, at the Children Hospital of Palermo (Italy). One hundred twenty-four were male, and 119 were female. The age range was 2 months to 14 years (mean age 8.3 ± 2.5 years).

Ninety-six patients out of 243 underwent clinical examination for growth control (general health status check-up) and for minor illness, such

as upper respiratory tract infection. Forty-eight were affected by metabolic diseases (i.e., type 1 diabetes mellitus), 33 by nephrologic disease (i.e., acute renal failure and glomerulonephritis), 26 by infectious disease (i.e., lower respiratory tract infection and acute gastroenteritis), 16 by neoplasms (i.e., leukemia/lymphoma), 14 by immunologic disease (i.e., primary immunodeficiency), and, finally, 10 by a pathology requiring surgical intervention (i.e., acute appendicitis).

No patients had clinical features specifically suggesting *B. henselae* infection, including subacute lymphoadenopathy. Informed consent for collection of additional blood samples was given by each patient's parent(s).

We also examined serum samples for IgG and IgM antibodies to *B. henselae*, stored at −20°C, taken from 122 healthy blood donors, not gender- and age-matched, selected from the Blood Bank of our Department. Seventy-five were male, and 47 were female. The age range was 18–56 years (mean age 29.5 ± 8.1 years). No subjects had clinical features specifically suggesting *B. henselae* infection, including subacute lymphoadenopathy.

We also studied serum samples, stored at −20°C, taken from 120 stray cats, living in urban stray-cat colonies, and 62 pet cats, living in private homes, in the same area of our human population, to evaluate the circulation of *Bartonella* in the natural reservoir by detecting IgG *B. henselae* antibodies.

Immunofluorescence Assay

All serum samples were examined by indirect fluorescent antibody test (IFAT) for the presence of IgG and IgM class antibodies to *B. henselae*.

For antigens, we used our in-house prepared slides to increase the test sensitivity.[17] Slides were prepared by using *B. henselae* strain Houston-1 as antigen, obtained from the collection of the National Reference Center of Rickettiosis, Marseille, France. Briefly, bacteria were grown in a VERO cell line for 2 weeks, then infected cells were recovered and lysed by sonication. Cell suspensions were centrifuged at 1,500 rpm (700 × *g*) for 10 min to remove cell debris, and bacteria were purified by using a sucrose gradient [phosphate-buffered saline (PBS) with 25% sucrose (BioMérieux, Marcy-l'Etoile, France)]. The resulting pellet was washed in PBS twice (6,000 × *g* for 10 min), and purified bacteria were pelleted by centrifugation (10,000 × *g* for 10 min), resuspended in sterile distilled water, and stored at −80°C before use. This whole-cell antigenic preparation was plated onto microscopy slides for immunofluorescence (BioMérieux, Marcy-l'Etoile, France) and used for detection of IgG and IgM antibodies by IFAT.

For the IFAT, the slides were stained for 30 min, at 25°C, in thermostat, in humid chamber, with 10 μL of the serum, diluted in PBS 1:64 for IgG and 1:20 for IgM, respectively, for human samples, and 1:64 for IgG for

cat samples. Slides were then washed twice in PBS (pH 7.2, for 5 min), and then briefly rinsed with distilled water. After being air dried, slides were incubated for 30 min, at 37°C, in thermostat, in humid chamber, with a fluorescein-isothiocyanate anti-human IgG or IgM conjugate for human samples, and a fluorescein-isothiocyanate anti-cat IgG conjugate for cat samples [Kirkegaard and Perry Laboratories (KPL)-Europe, Milan, Italy], diluted 1:100 in PBS containing 0.2% Evans blue (Biomerieux, Marcy-l'Etoile, France), for 30 min. The slides were washed as described above, air dried, mounted with tamponed glycerine, and then examined with a fluorescence microscope (Leica Microsystems, Germany) at a ×40 magnification.

Serum was considered to be positive for *B. henselae* when the IgG titer was ≥1:64 both for human and for cat samples.[15,16] Human serum was considered positive for IgM to *B. henselae* when the titer was ≥1:20. In all assays, a negative and a positive control was used, at known titer, and positive serum samples were titered to the maximal positive dilution.[15,16]

Statistical Analysis

Significance was tested using Student's *t*-test by variance analysis (Student–Newmann–Keuls test). Correlations between groups were performed using the χ^2 test. The p value of <0.05 was considered as significant.

RESULTS

As shown in Table 1, of the 243 children evaluated, 61 (25.1%) had IgG class antibodies to *B. henselae*. Forty-five (18.5%) showed a titer 1:64, 6 (2.4%) a titer 1:128, 6 (2.4%) a titer 1:256, 2 (0.8%) a titer 1:512, 1 (0.4%) a titer 1024, and 1 (0.4%) a titer 1:5120. Of the 122 healthy blood donors, 14 (11.4%) had IgG class antibodies to *B. henselae*; 12 (9.8%) showed a titer 1:64 and 2 (1.6%) a titer 1:128. None of the human serum samples, both children and adults, showed positive results for IgM class antibodies to *B. henselae*.

TABLE 1 Anti-*B. henselae* IgG Antibodies in Human Serum Samples from Sicily, Administrative Department (Province) of Palermo, by Immunofluorescence Assay (Positive Result: ≥1:64)

	Sera		IgG Class Antibodies to *B. henselae* Titers (Reciprocal)						
Samples	Examined	Positive	64	128	256	512	1024	2560	5120
Children	243	61* (25.1)	45	6	6	2	1	–	1
Blood donors	122	14* (11.4)	12	2	–	–	–	–	–

The percentage of positive sera, inside the examined groups, is shown in parentheses.
*None of the human serum samples showed positive results of testing for IgM to *B. henselae*.

TABLE 2 Anti-*B. henselae* IgG Antibodies in Cat Serum Samples from Sicily, Administrative Department (Province) of Palermo, by Immunofluorescence Assay (Positive Result: ≥1:64)

	Sera		IgG class antibodies to *B. henselae* titers (reciprocal)						
Samples	Examined	Positive	64	128	256	512	1024	2560	5120
Stray cats	120	82 (68.3)	25	17	26	8	6	–	–
Pet cats	62	22 (35.4)	4	9	4	3	–	1	

The percentage of positive sera, inside the examined groups, is shown in parentheses.

Stray cats had higher seroprevalence than pet cats, and their antibody levels were significantly higher (Table 2). In particular, of the 120 stray cats evaluated, 82 (68.3%) had IgG class antibodies to *B. henselae*. Twenty-five (20.8%) showed a titer 1:64, 17 (20.7%) a titer 1:128, 26 (31.7%) a titer 1:256, 8 (6.6%) a titer 1:512, and 6 (5.0%) a titer 1:1024. Of the 62 pet cats evaluated, 22 (35.4%) had IgG class antibodies to *B. henselae*. Four (6.4%) showed a titer 1:64, 9 (14.5%) a titer 1:128, 4 (6.4%) a titer 1:256, 3 (4.8%) a titer 1:512, 1 (1.6%) a titer 1:2560, and 1 (1.6%) a titer 1:5120.

Finally, we found significant differences between children versus healthy blood donors ($\chi^2 = 9.2$, $p < 0.01$), and pet cats versus stray cats ($\chi^2 = 17.8$ $p < 0.001$).

DISCUSSION

The relatively low prevalence of CSD and other clinical manifestations related to *B. henselae* infection contrasts with the high frequency with which we found serologic evidence of past infection in young children. In fact, 25.1% of unselected Italian children, affected by various diseases, who were apparently free of any clinical features suggesting *B. henselae* infection, had positive results of serologic testing, which were consistent with past infection. The seroprevalence of *B. henselae* infection in a group of healthy blood donors of the same geographical area was 11.4%.

B. henselae infection is common in stray and domestic cats (*Felis catus*).[1] The distribution of feline infections by *B. henselae* and the cat flea (*Ctenocephalides felis*) appear to be concordant, and experimental evidence has demonstrated that cat fleas can transmit *B. henselae* between cats. However, the potential role of the cat flea as a vector of CSD from cats to humans has not been experimentally evaluated.[2] Anyway, transmission of *B. henselae* to humans is obligatorily associated with feline reservoirs.[18]

Prevalence of infection varies considerably among cat populations (strays or pets), with an increasing grading from low, in cold climates (0% in Norway), to high, in warm and humid climates (17.02% in Brazil, 18.6% in Turkey, 68% in the Philippines).[19–21] However, inside a given country, the prevalence may also vary among cat populations.[9,10,22,23]

Seroepidemiological studies of *B. henselae* infection in the general human population, especially in children, are limited in the literature. A recent Chilean study, examining 181 children and adolescents and 107 technical and professional workers involved in the care of cats, demonstrated a seroprevalence of *B. henselae* of 13.3% in children and 10.3% in occupational risk subjects.[24] Another study, conducted in Central and Northern Jordan, examined the sera from 482 children, and demonstrated a seroprevalence of *B. henselae* of 11%, especially in those having a cat in the household and/or having a history of cat scratches or bites.[25] An Italian retrospective serologic study, evaluating the presence of IgG and IgM antibodies to *B. henselae* in 508 children and adolescents, living in central Italy, who were apparently free of any features suggesting *B. henselae* infection, demonstrated an IgG seroprevalence of 61.6% and an IgM seroprevalence of 3.9%.[12]

To our knowledge, our study is the first to evaluate a large group of individuals—children, affected by various diseases, living in a defined geographic area—and to relate their seroprevalence data with those from healthy blood donors.

The high seroprevalence in children we observed could be related to the high prevalence of this zoonosis in Sicily, as attested by a seroprevalence of 68.3% among stray cats and of 35.4% among pet cats from the same geographic area. The different values of seroprevalences in stray cats and pet cats may be due to the lower environmental exposure to *Bartonella* and the better health state and medical surveillance of pet cats, with respect to stray cats. However, the limitation of our survey is that our cats were randomly chosen and perhaps never had a direct contact to the individuals investigated in this study. On the other hand, the value of our study is both in the analysis of human exposition to *B. henselae* and in the evaluation of bacteria circulating in the natural reservoir.

Therefore, our data support the hypothesis that, as is true for many other childhood infections, *B. henselae* infection is generally asymptomatic in young children and, in most cases, resolves spontaneously. Only a few subjects develop symptoms of classic CSD and systemic visceral manifestations, as a consequence of transient or stable alterations in the immunologic state,[26] but, more likely, CSD, as many other many infections, is not diagnosed or is under-reported.

The observation of different IgG antibody titers in children (25.1%) and in healthy blood donors (11.4%) (see Table 1) could be explained by the fact that they decline rapidly after the exposition to *B. henselae*.[27]

The comparison of seroprevalences of antibodies against *B. henselae* and related organisms in man and reservoir host populations is of general interest, because CSD, bacillary angiomatosis, and *Bartonella*-induced endocarditis are considered "emerging diseases."[21,23] Because cat populations

are also reservoirs for *B. clarridgeiae* and *B. koehlerae*, in addition to *B. henselae*, it would be important to include these species in further evaluation of the seroepidemiology of *Bartonella* species in the cat and the human populations of our region.

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