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Serological evidence of *Bartonella henselae* infection in healthy people in Catalonia, Spain

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SUMMARY

Cat scratch disease (CSD), bacillary angiomatosis, hepatic peliosis and some cases of bacteraemia, endocarditis, and osteomyelitis are directly caused by some species of the genus *Bartonella*. The purpose of this study was to determine the prevalence of IgG antibodies against *Bartonella henselae* in healthy people and to identify the epidemiological factors involved. Serum samples from 218 patients were examined by indirect immunofluorescence assay (IFA). Significance levels for univariate statistical analysis were determined by the Mann–Whitney *U* test, χ^2 test and Fisher's exact test. Of 218 patients, 99 were female and 119 male, with a median age of 34·36 years (range 0–91 years). Nineteen (8·7%) reacted with *B. henselae* antigens. Of all the factors concerning the seroprevalence rate being studied (age, sex, contact with animals, residential area), only age was statistically significant. Our serological data seems to indicate that *B. henselae* is present in Catalonia and could be transmitted to humans.

INTRODUCTION

The number of zoonotic *Bartonella* spp. identified in the last 15 years has increased considerably, since the first HIV-infected patient with unusual vascular proliferative lesions of bacillary angiomatosis (BA) was described in 1983 [1]. Of the 21 species of *Bartonella* currently described, 10 are acknowledged as human pathogen species. *B. bacilliformis*, *B. quintana*, and *B. henselae* are the most frequently described species [2–4], while *B. elizabethae*, *B. vinsonii*, *B. washoensis*, *B. grahamii*, *B. clarridgeiae*, *B. koehlerae* and *B. alsatica* were recently identified as being responsible for some cases of human infection [5–10]. Cat scratch disease (CSD), BA, hepatic peliosis and some cases of bacteraemia, endocarditis, osteomyelitis, uveitis and neurological disorders are directly caused by some species of the genus *Bartonella* [11–13]. To determine the real incidence of *Bartonella* infection, it is necessary to study the seroprevalence in the general population as well as the principal reservoirs and vectors involved in infection transmission. In the present study, we serologically tested 218 samples across Catalonia for evidence of *Bartonella* spp. antibodies and analysed possible corresponding risk factors for infection.

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MATERIAL AND METHODS

Geographical area

The study was undertaken in Vallès Occidental, Catalonia, a predominantly urban area near the coast in the northeast of Spain. A total of 11 municipalities (356 266 inhabitants) participated in the study.

Samples

Serum samples from 218 patients who had attended Sabadell Hospital were collected for the survey. The collection of samples took place during a 5-month period, from September to January. The samples include adults undergoing minor surgery and children cared for non-infectious diseases in the Paediatrics Emergency Service.

Taking into account a previous analysis of the actual population of Vallès Occidental regarding sex, age and residential area, subjects were selected in order to obtain a representative sample. Thus, the study population was stratified by sex, by age (0–14 years, 15–29 years, 30–44 years, 45–64 years, $\geqslant$65 years) and by residential area: rural ($<$5000 inhabitants), semi-rural (5000–50 000), and urban ($>$50 000).

Informed consent was obtained from all adult participants and from parents or legal guardians of minors. Each patient completed a questionnaire in which the following variables were registered: age, gender, place of residence, contact with pets, stray dogs, and occupation. Those inhabitants unable to answer the epidemiological survey were excluded.

The sample of heparinized blood was sedimented and the supernatant was collected and stored at -80 °C. A serological survey was carried out according to the ethical guidelines of the ethics committee of Sabadell Hospital.

Serological technique

Human serum samples were evaluated by indirect immunofluorescence assay (IFA). We used commercial slides (*Bartonella* IFA IgG; Focus Technologies Inc., Herndon, VA, USA) to determine antibodies to *Bartonella* spp. The kit for detecting IgG antibodies utilizing Vero cells infected with either *B. henselae* or *B. quintana* was used according to the manufacturer's instructions. The serum samples were initially diluted 1/64. Any serum samples found to be positive at the initial dilution were further titrated. Positive and negative controls were included in each test. We considered specimens showing no fluorescence at IgG titres of 1/64 as negative and specimens with bright fluorescence at a dilution of $\geqslant$1/64 as positive. The intensity of each specific fluorescence test was subjectively evaluated and independently graded by two of the authors [14].

Statistical analysis

Statistical analysis was performed using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA) Seroprevalence was determined globally and by residential area. A univariate analysis was performed to determine possible risk factors. Univariate group comparisons were performed using χ^2 and Fisher's exact test. Group differences were determined by odds ratio and 95% confidence intervals. Quantitative variables were compared by Mann–Whitney *U* test. A *P* value of $<0{\cdot}05$ was considered significant.

RESULTS

Of the 218 subjects, 119 were male and 99 female. The mean age was 34·36 years (0–91 years). Subjects were reported from 11 towns, and 145 (66·51%), 59 (27·2%), and 14 (6·5%) subjects lived in urban, semi-rural, and rural areas, respectively; 35 (16%) reported contact with pets, and four (1·9%) with stray dogs. In the group of 161 adults ($\geqslant$18 years), there were 11 (6·8%) students, 27 (16·8%) retired, 37 (23%) housewives, 53 (32·9%) workers, and 26 (16·1%) unemployed. For seven people the occupation was unknown.

Considering titres of $\geqslant$1:64 as positive, the seroprevalence of *B. henselae* in humans was 8·7%, considering samples with antibodies against *B. henselae* only or with *B. henselae* titres twofold higher to *B. quintana* titres. No sample had antibodies against *B. quintana* only. The relationship between the *B. henselae* antibody prevalence and the surveyed items is shown in Table.

Nineteen samples had antibodies against *B. henselae*. Ten of them had an IgG titre of 1:64, four a titre of 1:128, four a titre of 1:256 and one a titre of 1:512.

No difference in seroposivity was observed between males and females. The *B. henselae* seroprevalence was 8·3% in urban areas, 11·9% in semi-rural areas and 0% in rural areas. The mean age ($\pm$S.D.) of seropositive subjects was 42·83$\pm$17·04 years, whereas the

Table *Demographic information from subjects tested for antibodies to* B. henselae

Variables ($n=218$)	No. subjects (%)	n (%) of positive	P value
Sex			0·858
Male	119 (54·6)	10 (52·6)	
Female	99 (45·4)	9 (47·4)	
Age (years)			
0–14	51 (23·4)	1 (5·3)	0·046
15–29	50 (23)	3 (15·8)	0·368
30–44	44 (20·1	8 (42)	0·042
45–64	46 (21·1)	6 (31·6)	0·155
$\geqslant 65$	27 (12·4)	1 (5·26)	0·313
Residence area			
Urban	145 (66·5)	12 (63·1)	0·623
Semi-rural	59 (27)	7 (36·9)	0·185
Rural	14 (6·5)	0 (0)	0·286
Contact with animals			
Pets	35 (16)	6 (31·6)	0·05
Stray dog	4 (1·9)	1 (5·6)	0·296
Occupation (adults, $\geqslant 18$ years, $n=161$)			
Student	11 (6·8)	1 (5·8)	0·015
Retired	27 (16·8)	5 (29·4)	0·172
Housewife	37 (23)	2 (11·8)	0·607
Worker	53 (32·9)	9 (52·9)	0·018
Unemployed	26 (16·1)	0 (0)	0·091
Unknown	7 (4·3)	0 (0)	0·112

mean age of seronegative subjects was $33{\cdot}59 \pm 23{\cdot}56$ years ($P=0{\cdot}053$). Seropositivity was significantly more prevalent in subjects aged 30–44 years ($P=0{\cdot}042$). On the other hand, subjects aged 0–14 years showed a lower seropositive rate ($P=0{\cdot}046$). Of seropositive patients, six (31·6%) had contact with pets ($P=0{\cdot}05$) and one (5·6%) with stray dogs (non-significant). Concerning occupation, students presented lower seroprevalence (5·8%, $P=0{\cdot}015$) and workers had a higher seropositive rate (52·9%, $P=0{\cdot}018$).

DISCUSSION

B. henselae, now regarded as the primary, and perhaps sole, causative agent of CSD, is also a cause of BA and hepatic peliosis, and has been associated with endocarditis, fever, and bacteraemia in adults and children [15, 16]. Isolation of *Bartonella* is typically time-consuming, often requiring 2–6 weeks or longer incubation for primary isolation. The resulting isolation must then be identified by PCR. In general, isolation or detection of *B. henselae* from blood is not successful for CDS patients who have no evidence of systemic disease. Conversely, isolation of *Bartonella* spp. from blood of immunocompromised patients, or patients with evidence of systematic disease is usually possible. PCR offers a rapid and specific means to detect the organism directly from clinical samples and is more sensitive than isolation [17]. In humans, serological testing is the reference test for diagnosis of CDS and other infections by *Bartonella* [18]. An IgG anti-*B. henselae* antibody titre of $\geqslant 1:64$ is considered as positive for infection.

Nineteen people (8·71%) had antibodies against *B. henselae*. This seroprevalence is slightly lower than those reported by other investigators: 30% by Sander *et al.* [18] in Germany, 19·8% by Alexiou-Daniel *et al.* [19] in Greece, and 24·7% by Garcia-Garcia *et al.* [20] in Sevilla, Spain. However, similar or lower seroprevalence have been found in healthy populations from Sweden (3·2%) or La Rioja, Spain (5·88%) [21, 22]. We are able to assume that this seropositivity may indicate a past infection with *Bartonella* spp. However, we could not exclude unspecified serological cross-reactivity with other heterologous antigens. It is well known that *Bartonella* can cross-react with other genera, such as *Coxiella burnetti* or *Chlamydia* [23, 24]. Therefore, we compared these data with those obtained in a previous study (M. Vila *et al.*, unpublished data). All 218 individuals included in this study were examinated by an IFA test for *Chlamydia* [*Chlamydophila pneumoniae* IFA IgG (Vircell, S.L., Santa Fé, Granada, Spain), cut-off 1/64]. Eight (3·66%) serum samples had antibodies against *B. henselae* exclusively; three serum samples reacted against *C. pneumoniae* and *Bartonella* at the same titre levels; six sera showed higher titres against *B. henselae* (1/128 *vs.* 1/32, 1/512 *vs.* 1/32 and 1/256 *vs.* 1/32); and in two other samples titres against *Chlamydia* were higher (1/256 *vs.* 1/64 and 1/128 *vs.* 1/64). These last two sera should not be considered positive for *B. henselae*. These results indicate *B. henselae* may be present in Catalonia and its seroprevalence might range from 6·42% to 7·9%.

The only statistically significant association observed was that between *B. henselae* seropositivity and age. *B. henselae* seropositivity was significantly more prevalent in subjects aged 30–44 years. This observation agrees with a study carried out in Greece, which also showed higher *B. henselae* seropositive rates in children aged <14 years (non-significant). Moreover, those children had greater titres [19]. Taking into account that the cat is the main *Bartonella* reservoir, most seropositive subjects had

contact with pets. Highest titres in children could be due to greater contact with pets. Seropositives rates differ from one study to another due to the different epidemiological and climatic environments. Therefore, we carried out a seroprevalence study of *B. henselae* in cats from the same region and over the same period of time [25]. Seroprevalence of *B. henselae* in cats was 26% and there were 7% of cats with bacteraemia. If cats aged <1 year were considered, incidence of bacteraemia rises to 12·1%.

As shown in Sander *et al*. [18], most titres were not high. Thus, it seems that subjects did not have recent infection. In our study 1·37% of the healthy population examined had IgG antibodies to *B. henselae* at a titre 1:128, and 0·91% had IgG antibodies at a titre of 1:256 and 0·45% had IgG antibodies at a titre of 1:512.

Because bartonelloses are a newly recognized class of infection in Spain, it is important to identify certain potential risk factors. It is reasonable to assume that as reliable, validated, and safe methods for serological diagnosis of *Bartonella* infections become a routine procedure in many clinical laboratories, the spectrum of *Bartonella*-associated diseases will continue to expand. The present study therefore provides an epidemiological and serological framework for future *Bartonella* studies.

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DECLARATION OF INTEREST

None.

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