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OCCURRENCE OF *BARTONELLA HENSELAE* AND *BARTONELLA QUINTANA* IN A HEALTHY GREEK POPULATION

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Abstract. The purpose of this study was to determine the prevalence of IgM and IgG antibodies against *Bartonella henselae* and *B. quintana* in a healthy Greek population using a commercially available immunofluorescent test (Focus test). Five hundred healthy individuals were divided by sex into four age groups and three groups according to contact with cats. IgM antibodies were not detected in any of the subjects examined, while 99 (19.8%) and 75 (15%) were IgG seropositive to *B. henselae* and to *B. quintana*, respectively. No statistical difference in the seropositivity was observed among these groups. The IgG antibody titers ranged from 1/64 to 1/256 for *B. henselae* and from 1/64 to 1/512 for *B. quintana*. A high percentage (12.4%) of cross-reactivity between the two species was observed. Our data show that the prevalence of both *Bartonella* species in Greece is high. However, low IgG antibody levels are not sufficient evidence of active infection.

INTRODUCTION

Members of the genus *Bartonella* are now well recognized as modern pathogens of humans. A wide variety of *Bartonella* species and genotypes has recently been described from non-human vertebrates, and at least some of these can be the source of zoonotic infections.[1] However *Bartonella henselae* and *B. quintana*, the best-characterized members of the genus *Bartonella*, remain the primary sources of *Bartonella*-associated human diseases in Europe and cause a wide range of clinical symptoms.[2] The epidemiology of *Bartonella* infections is poorly understood; most *B. henselae* infections are probably acquired from infected cats,[3] but no animal reservoir has been implicated for *B. quintana*.

In Greece, the cases of seropositivity to *Bartonella* that have been reported involve only children.[4,5] The aims of this study were to determine the seroprevalence of *B. henselae* and *B. quintana* in a healthy Greek population and to identify the epidemiologic factors involved.

MATERIALS AND METHODS

Samples. Between June 2002 and August 2002, serum samples were collected from 500 healthy individuals 2–60 years old living in an urban area of Greece. All adults included in this study were randomly selected from healthy Greek blood donors represented at the local blood bank of the AHEPA University Hospital in Thessaloniki, while the serum samples of the minors were randomly collected during a screening program for β-thalassemia. The ethical committee of the AHEPA University Hospital in Thessaloniki reviewed and approved the study, and informed consent was obtained from each individual, or his or her guardian, before inclusion in the study. Any individual with a clinical history of lymphadenopathy and/or fever in the last six months was excluded. The following information was obtained from each person: sex, age, and contact with cats. The study group was stratified according to this information into two groups by sex: 263 males (mean ± SD age = 29.95 ± 20.95 years) and 237 females (mean ± SD age = 31.04 ± 19.03 years) and four age groups: group 1, 2–14 years old (n = 152); group 2, 15– 29 years old (n = 138); group 3, 30–50 years old (n = 119); and group 4, >50 years old (n = 91). Based on reported contact with cats, the study population was divided in three groups: group A, cat owners (n = 150); group B, those with occasional contact with cats (i.e., in a friend's house), (n = 165); and group C, those who had never had contact with cats (n = 185). The mean ± SD ages were not statistically different between males and females, and there was no significant difference in the distribution of the cat owners in the four age groups.

Serologic technique. All sera were examined using both the *Bartonella* IgG and *Bartonella* IgM indirect immunofluorescent antibody (IFA) test kits (Focus Technologies, Cypress, CA) according to the manufacturer's instructions. The IgM kit includes an absorption step to eliminate free or complexed IgG and uses purified *B. henselae* and *B. quintana* cells diluted in yolk sac fluid. The kit for detecting IgG antibodies uses Vero cells infected with either *B. henselae* or *B. quintana*. The serum samples were initially diluted 1/64 and 1/20 for the detection of IgG and IgM antibodies, respectively. Any serum sample found to be positive at the initial dilution was further titrated. Positive and negative controls were included in each test. Interpretations of the results were made as recommended by the manufacturer; thus, titers of 1/20 for IgM and 1/64 for IgG were considered positive. The serum samples were also examined for *Coxiella burnetti*, *Anaplasma phagocytophila*, and *Chlamydia* species to exclude possible cross-reactivity.

Statistical analysis. Statistical analysis was performed using statistical package SPSS for Windows, Release 10.0.1 (standard version; SPSS, Inc., Chicago, IL). The Student's *t*-test was used to compare mean ages of subjects seropositive and seronegative for *B. henselae* and *B. quintana*. The chi-square test (with or without Yates' continuity correction) and Fisher's exact method for small samples were used for comparison of prevalence rates in the subgroups. The chi-square Mantel-Haenszel procedure was used to evaluate categorical variables.

RESULTS

Of the 500 subjects examined, 99 (19.8%) and 75 (15%) were seropositive for IgG antibodies to *B. henselae* and *B. quintana*, respectively. Sixty-two (12.4%) had IgG antibodies to both *Bartonella* species. IgM antibodies were not detected in any of the samples examined. Titers of 1/64, 1/128, 1/256, and 1/512 for IgG antibodies to *B. henselae* were found in 13.4%, 4.2%, 2.2%, and 0% of the subjects examined, respec-

TABLE 1
Titers of antibodies to *Bartonella henselae* according to sex, age, and exposure to cats

	<1/64	1/64	1/128	1/256	Total positive (%)	Total
Sex						
Males	206	36	16	5	57 (11.4)	263
Females	195	31	5	6	42 (8.4)	237
Age (years)						
2–14	118	13	14	7	34 (6.8)	152
15–29	105	10	2	2	14 (2.8)	119
30–50	107	25	4	2	31 (6.2)	138
>50	71	19	1	0	20 (4)	91
Contact						
None	155	25	4	1	30 (6)	185
Occasional	135	19	5	6	30 (6)	165
Cat owner	111	23	12	4	39 (7.8)	150
Total (%)	80.2	13.4	4.2	2.2	19.8	100

tively. These titers for IgG antibodies to *B. quintana* were found in 6%, 5.4%, 2%, and 1.6% of the subjects, examined, respectively. The overall prevalence of *B. henselae* and *B. quintana* did not differ significantly between males (21.7% and 15.6%, respectively), and females (17.7% and 14.3%, respectively). No statistically significant differences were found in the prevalence of antibodies to both *Bartonella* species among the four age groups (Tables 1 and 2). However, there were statistically significant differences in titers to both *B. henselae* ($P < 0.001$) and *B. quintana* ($P < 0.01$), which were higher in age groups 1 and 3. Significantly ($P < 0.05$) higher titers to *B. henselae* were also found in cat owners, but this difference was not observed for *B. quintana.*

Cross-reactions were observed between the two *Bartonella* species, although most of them ($P < 0.01$) occurred for *B. quintana* (Table 3). A significantly higher percentage of cross-reactions appeared in age groups 1 ($P < 0.001$) and 3 ($P < 0.05$). Moreover, cat owners showed more cross-reactions between *B. henselae* and *B. quintana* than those who never had close contact with cats.

The 500 individuals included in this study were examined by the IFT test for *C. burnetti*, *A. phagocytophila*, and *Chlamydia* species. All were seronegative for these microorganisms.

DISCUSSION

Bartonella quintana, the historical agent of louse-borne trench fever in World War I, is at least one source of bacillary angiomatosis and has been associated with endocarditis, fever, and bacteremia in both immunocompetent and immunocompromised hosts.[6,7] *Bartonella henselae*, now regarded as the primary, and perhaps sole, causative agent of cat scratch disease, is also a cause of bacillary angiomatosis and bacillary peliosis, and has been associated with endocarditis, fever, and bacteremia in adults and children.[6,8]

Laboratory diagnosis of these microorganisms is difficult because isolation is time-consuming and use of the polymerase chain reaction requires specific laboratory facilities and equipment. Since serologic diagnosis by the IFA test is easy to perform, serology is the diagnostic method used most often.[9,10] However, interpretation of the results of this test should consider the seroprevalence in the healthy population because this it shows a wide variation in different countries.[11–14] This diversity may be due to the fact that different diagnostic reagents have been used to estimate the seroprevalence. In Germany,[15] the seroprevalence of antibodies in children without cat scratch disease was different when determined by two IFA tests, and in France[16] in a control group, specificity was lower with the Focus test (87%) than with an in-house test (98%). Our results are consistent with those of Zbinden and others,[11] who using the same test, reported a low (50%) specificity in Swiss urban individuals, but in contrast to those of Harrison and others[13] in the United Kingdom, who found a specificity of 99%. These differences highlight the importance of determining the seroprevalence in each population of interest and ensuring that the cut-off antibody levels selected are locally appropriate.

Our results show that 22.4% of the healthy population examined had IgG antibodies to *B. henselae* and/or *B. quintana*. A total of 12.4% had IgG antibodies to both *Bartonella* species, 7.4% only to *B. henselae*, and 2.6% only to *B. quintana*. This cross-reactivity, which has also been observed in other patient groups,[17] indicates that this serologic analysis is genus specific, but not species specific. Therefore, we can assume that this seropositivity may indicate a past infection with these *Bartonella* species, although we cannot exclude unspecified serologic cross-reactivity with other heterologous antigens. It is well known that *Bartonella* spp. cross-react with other genera and species, such as *C. burnetii, A. phagocytophila*, and *Chlamydia*.[17–19] However, all 500 individuals included in this study were examined by an IFA test and found to be seronegative for these microorganisms.

No difference in seropositivity was observed between males

TABLE 2
Titers of antibodies to *Bartonella quintana* according to sex, age, and exposure to cats

	<1/64	1/64	1/128	1/256	1/512	Total positive (%)	Total
Sex							
Males	222	17	13	6	5	41 (8.2)	263
Females	203	13	14	4	3	34 (6.8)	237
Age (years)							
2–14	133	5	2	8	4	19 (3.8)	152
15–29	107	4	6	0	2	12 (2.4)	119
30–50	112	11	12	2	1	26 (5.2)	138
>50	73	10	7	0	1	18 (3.6)	91
Contact							
None	158	16	9	2	0	27 (5.4)	185
Occasional	140	6	11	5	3	19 (3.8)	165
Cat owner	127	8	7	3	5	15 (3)	150
Total (%)	85	6	5.4	2	1.6	15	100

TABLE 3
Cross-reactivity of sera positive for *Bartonella henselae* and *B. quintana* according to sex, age, and exposure to cats

	B. henselae	B. quintana
Sex		
Males	34/57	34/41
Females	28/42	28/34
Age (years)		
2–14	19/34	19/19
15–29	9/14	9/12
30–50	19/31	19/26
>50	15/20	15/18
Contact		
None	20/30	20/27
Occasional	20/30	20/25
Cat owner	22/39	22/23

and females and among the four age groups, although the levels of IgG antibodies were higher in the age groups 1 (2–14 years old) and 3 (31–50 years old) with titers reaching 1/256 for *B. henselae* and 1/ 512 for *B. quintana* (Tables 1 and 2). Most of the titers in age group 1 were 1/128 or higher, whereas most of the titers in age group 3 were low (1/64). This is most likely due to exposure to *B. henselae* by children playing with kittens, and the persistence of low level of antibody into adulthood. A similar but less distinct trend was observed with *B. quintana*; this is most likely due to cross-reactivity.

No difference in seropositivity was also found among the cat owners and those who never had close contact with cats. These data are consistent with those of Sander and others,[12] who using the same test, suggested that *B. henselae* does not infect cat owners more frequently. However, the cat owners had significantly higher antibody titers only to *B. henselae* ($P < 0.05$) and not to *B. quintana*. Titers of 1/64, 1/128, 1/256, and 1/512 to *B. henselae* were found in 15.3%, 8%, 2.7%, and 0% of the cat owners respectively, while the range of seropositivity to *B. quintana* in this group was extremely lower: 5.3%, 4.7%, 2%, and 3.3%, respectively. This may be due to the fact that one of the examined groups consisted of cat owners, who are infected more often by *B. henselae*.

As shown in other studies[11,12,15] low levels of antibody are not sufficient evidence of active infection. In our study, 2.2% of the healthy population examined had IgG antibodies to *B. henselae* at a titer of 1/256 and 1.6% had IgG antibodies to *B. quintana* at a titer of 1/512, while IgM antibodies were not detected in any of the samples examined. Thus, an IgG titer of 1/256 for *B. henselae* and 1/512 for *B. quintana* and an IgM titer of 1/20 or higher for both *Bartonella* spp. could indicate current or recent infection.

It is reasonable to assume that as reliable, validated, and safe methods for serologic diagnosis of *Bartonella* infections become a routine procedure in many clinical laboratories, the spectrum of *Bartonella*-associated diseases will continue to expand. The greatest value for validated laboratory testing appears to exist in atypical cases or in the differential diagnosis of serious lymphoproliferative syndromes. Our data indicate that the prevalence of antibodies to *B. henselae* and *B. quintana* is rather high in our region and that physicians should evaluate serologic results in combination with clinical manifestations.

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