Seroprevalence of *Bartonella henselae* and *Bartonella quintana* infections in children from Central and Northern Jordan

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ABSTRACT

Objective: To investigate the prevalence of antibodies to *Bartonella henselae (B.henselae)* and *Bartonella quintana (B.quintana)* among children from central and northern Jordan.

Methods: Sera from 482 children were randomly collected from referenced governmental hospitals in the central and northern parts of Jordan during the period between January 2001 to March 2003. An indirect immunofluorescent assay was used to determine serum antibody titers to *B.henselae* and *B.quintana*. Sera that were reactive at a dilution ≥1:64 were considered positive. In addition, blood from 20 cats belonged to children with high *B.henselae* titers were tested using polymerase chain reaction.

Results: Out of the 482 serum samples examined, 53 (11%) and 20 (4.1%) had positive antibody titers for *B. henselae* and *B.quintana*. *Bartonella henselae* was isolated from 4 cats that belonged to 3 children with high antibody titers to *B.henselae*-IgG. The seroprevalence of IgG antibodies to *B.henselae* was significantly higher ($P<0.05$) in children aged 7-10-years than in younger or older ones. Having a cat in the household and having a history of cat scratches or bites were strongly associated ($P<0.01$) with seropositivity to *B.henselae*-specific IgG. Cats ownership and history of cat scratches or bites had no impact on the prevalence of *B.quintana*. Seropositivity to *B.henselae*-specific IgG was significantly higher ($P<0.01$) in children from northern Jordan than in children from central Jordan.

Conclusion: This study substantiates the presence of *B.henselae* in Jordan, documents the seroreactivity to 2 Bartonella antigens, and suggests that cat ownership and history of cat scratches or bites are important epidemiological risk factors for *B.henselae* infection in Jordan.

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Human infections due to *Bartonella species* are considered emerging zoonotic diseases. These infections include diseases such as Carrion's disease due to *Bartonella bacilliformis (B.bacilliformis)*, trench fever due to *Bartonella quintana (B.quintana)*, and cat scratch disease (CSD) due to *Bartonella henselae (B.henselae)* and *Bartonella clarridgeiae (B.clarridgeiae)*.[1-3] Bacillary angiomatosis and peliosis hepatitis, newly discovered clinical manifestations, were found to be caused by both *B.henselae* and *B.quintana*.[3,4] Cat scratch disease occurs worldwide and is probably the most common *Bartonella* infection. It is a common cause of subacute, regional lymphadenopathy in mostly immunocompetent children and adults.[4,5] Immunocompromised patients are more likely to have systemic infections caused by both *B.henselae* and *B.quintana*.[4] Laboratory methods for the diagnosis of *Bartonella* infections include isolation of the organisms by culture,

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serological assays, histopathological examination, and molecular detection of *Bartonella* DNA in affected tissue.[4,6] However, *Bartonella species* are fastidious, slowly growing bacteria, and routine bacterial culture protocols usually do not allow detection of these organisms.[7] In the last few years, serological tests to detect antibodies to the different *Bartonella species* have been developed and serologic testing for antibodies to *B.henselae* was proposed as an adequate alternative to skin testing.[8-10] The epidemiology of *Bartonella* infections is poorly understood. Humans are most likely to acquire *B.henselae* infections from infected cats.[3,11] No animal reservoir has been implicated yet for *B.quintana*.[3,12] However, *B.quintana* infection can be transmitted by the human body louse.[3] Various reports on the epidemiology of *Bartonella* infections suggested that the problem is distributed worldwide.[4,13-15] We are not aware of any epidemiological reports on human *Bartonella* infections in Jordan or in the Middle East area.

The objectives of this study were to investigate the seroprevalence of *B.henselae* and *B.quintana* antibodies among children from the central and northern Jordan and to study the association between age, gender, cat ownership and history of cat scratches or bites and seropositivity to *B.henselae* and *B.quintana*.

Methods. ***Sample collection.*** Four hundred and eighty-two (267 males, 215 females) serum samples from children under 15-years of age were used in this study. Serum samples were randomly collected during the period between January 2001 and March 2003, from the hematology laboratories of 8 main reference governmental hospitals in the central and northern parts of Jordan. Only blood samples that had normal hematological values were included in this study. Sample size was determined based on an expected seroprevalence of 30% and a confidence interval of 95%. All the studied children were immunocompetent. An informed consent was obtained from each subject's guardian. Information on pet's ownership as well as history of cat scratches or bites, or both were obtained from all participants. The serum samples were divided into 5 age groups: Less than one year old, 1-2-years old, 3-6-years old, 7-10-years old, and 11-15-years old. Serum samples were kept at -20^{o}C until testing. In addition, heparinized blood samples from 20 cats belonged to 15 child with high IFA titer to *B.henselae* were collected for the purpose of single-step polymerase chain reaction analysis.

Immunofluorescence assay (IFA). A commercial tissue-culture *Bartonella* antigen kit were used (BION Enterprises, Chicago, Illinois, United States of America). This kit consists of glass slides that are coated with a mixture of *B.henselae* (Houston strain) or *B.quintana* (Oklahoma strain) infected and uninfected human epithelioid larynx carcinoma cells (HEp-2). The sensitivity of the *B.henselae*-IgG and *B.quintana*-IgG tests were 99 and 88%. The specificity of those 2 tests were 94 and 90%. The HEp-2-*Bartonella*-infected slides were prepared for the IFA assay by washing them with phosphate buffered saline (PBS) (pH, 7.4). Serial dilution of sera were prepared in PBS containing 0.5% bovine serum albumin (BSA) and 0.1% Tween 20 (Sigma, St. Louis, Missouri, United States of America, (USA)). This PBS/BSA/Tween 20 buffer were used as a washing buffer. The slides were overlaid with the serum dilutions and incubated for 30 minutes at 37^{o}C in a humidified chamber. Slides were then washed 3 times with the washing buffer and fluorescein-conjugated goat anti-human IgG antibodies (KPL, Gaithersburg, Maryland, USA) were added. After incubation at 37^{o}C for 30 minutes, slides were washed 3 times with the washing buffer and were air-dried. Negative and positive human sera (BION Enterprises, Chicago, Illinois, USA) were used to evaluate the IFA method. All slides were examined by a fluorescent microscopy (Nikon, Tokyo, Japan). Titers were reported as reciprocals of serum dilutions. Sera with antibody titers of $\geq$1:64 were considered positive as suggested by the *Bartonella* kit manufacturer.

Bacterial cultures. The following strains of bacteria were used: *B.henselae* (ATCC 49882, Houston strain) and *B.quintana* (ATCC 51694) (ATCC, Rockville, Maryland, USA). Bacteria were grown at 37^{o}C for 7 days on chocolate agar plates (BBL, Cockeysville, Maryland, USA) containing 10% defibrinated sheep blood. Bacteria were harvested by rinsing the plates with phosphate buffered saline (PBS, pH 7.4). The bacteria suspension were washed 3 times with PBS and then centrifuged for 10 minutes at 9000 x *g*. The pellets were suspended in 1ml of 0.9% NaCl solution and further diluted to an appropriate concentration and stored at 4^{o}C.

DNA extraction and polymerase chain reaction. DNA for PCR amplification was prepared from bacteria cultures and cats blood using QIAamp DNA preparation kits (QIAGEN Inc., Valencia, California, USA). Polymerase chain reaction for the 16S-23S rRNA intergenic region was performed as described by Jensen et al.[16] The single step PCR was carried out by amplification of the following primers: 5'-CTTCGTTTCTCTTTCTTCA-3'and 5'-AACCAACTGAGCTACAAGCC-3', as forward and reverse primers,. After amplification, the PCR products were electrophoresed on 3% agarose gel and stained with ethidium bromide. *Bartonella species* were identified based on their size.

Table 1 - *Bartonella*-specific antibody titres, as measured by immunofluorescence, of 482 children from central and northern Jordan (2001-2003).

Antibody titer	*Bartonella henselae* n (%)	*Bartonella quintana* n (%)
1:64	11 (20.7)	6 (30)
1:128	13 (24.5)	10 (50)
1:256	24 (45.3)	4 (20)
1:512	5 (9.4)	0 (0)
Total	**53 (100)**	**20 (100)**

Table 2 - Antibody prevalence of *Bartonella henselae* (*B.henselae)* and *Bartonella quintana* (*B.quintana)* among healthy individuals in Jordan in relation to gender, age, cat ownership, and history of cat scratches or bites.

Variable	N	*B.henselae* N + ve (%)	*B.henselae* N - ve (%)	*P* value	*B.quintana* N + ve (%)	*B.quintana* N - ve (%)	*P* value
Gender							
Male	267	31 (11.6)	236 (88.4)	0.64	13 (4.9)	254 (95.1)	0.52
Female	215	22 (10.2)	193 (89.8)		7 (3.3)	208 (96.7)	
Age							
<1 year	6	5 (7.6)	61 (92.4)	0.04	1 (1.5)	65 (98.5)	0.22
1-2 years	93	6 (6.4)	87 (93.6)		2 (2.2)	91 (97.8)	
3-6 years	105	10 (9.5)	95 (90.5)		4 (3.8)	101 (96.2)	
7-10 years	99	22 (22.2)	77 (77.8)		6 (6)	93 (94)	
11-15 years	119	10 (8.4)	109 (91.6)		7 (5.9)	112 (94.1)	
Cat in house							
Yes	152	33 (21.7)	119 (78.3)	0.01	6 (5.9)	95 (94.1)	0.43
No	330	20 (6)	310 (94)		14 (6.4)	206 (93.6)	
Cat scratch or bite							
Yes	90	18 (20)	71 (80)	0.03	3 (5.7)	50 (94.3)	0.46
No	392	35 (8.9)	349 (91.1)		17 (6.3)	251 (93.7)	

Table 3 - Logistics regression analysis results of factors associated with seropositivity to *Bartonella henselae* (*B.henselae)* among children in central and northern Jordan.

Variable	B	Wald	Sig	Exp (B)	95% CI for Exp (B)
Intercept	3.4	11.1	0.001	-	-
Age (7-10-years-old)	1.3	8.2	0.002	4.2	1.5, 9.2
Cat in the household	2.1	9.5	<0.001	7.5	2.9, 12.0
With cat scratch or bites	1.2	5.4	0.021	3.1	1.9, 11.6

CI - confidence interval, model deviance: 519

Table 4 - Number and percentage of children under 15-years-old with positive antibody titers to *Bartonella henselae (B.henselae)* and *Bartonella quintana (B.quintana)* from central and northern parts of Jordan.

Geographical area	N	*Bartonella species* *Bartonella henselae* N (%)	*Bartonella quintana* N (%)
Northern Jordan	220	31 (14)*	11 (5)
Central Jordan	262	22 (8.4)	9 (3.4)
Total	**482**	**53 (11)**	**20 (4.1)**

* statistically significant ($p<0.05$)

Statistical analysis. The different variables were first screened using the chi-square analysis. To assess the effect of the different variables (gender, age, cats ownership and history of cat scratches or bites) on *B.henselae* and *B.quintana* seropositivity, a multivariable logistic model was constructed. All variables with $P<0.05$ (2-sided) by chi-square analysis were further tested by the model. A backward-stepwise approach was used in construction of the logistic model. Statistical analyses were performed using SPSS software version 10 (SPSS Inc. Chicago, Illinois, USA).

Results. The true seroprevalence (as adjusted to the test sensitivity and specificity) of *B.henselae* and *B.quintana* positive antibody titers in children was 11% and 4.1%. The seroprevalence of IgG antibodies to B. henselae was significantly higher ($P<0.05$) than that of *B.quintana*. The antibody titers of the 53 serum samples that were positive for *B.henselae*-specific IgG ranged from 1:64-1:512. The majority of the positive sera for *B.henselae*-specific IgG had a titer of 1:256. The titer of IgG antibodies to *B.quintana* varied from 1:64 to 1:256. However, most of the positive sera for B. quintana-IgG had antibody titers of 1:128 **(Table 1)**. *Bartonella henselae* antibodies were detected in all age groups. The percentage of individuals with positive antibodies to *B.henselae* in the age group 7-10-years old was significantly ($P<0.05$) higher than that in the other age groups (Table 2 and 3). No statistical differences in the seropositivity to *B.quintana* were detected between the different age groups **(Table 2)**. Children that had cats in their households' or/and had history of cat scratches or bites were more likely to have positive titers for B. henselae than children with no cats in their households or no history of cat scratches **(Table 3)**. To further emphasize cats as risk factors for seroreactivity to *B.henselae*, blood samples from 20 cats belong to 15 children with positive IFA to B. henselae were tested by PCR. *Bartonella henselae* was isolated from 4 of these cats **(Figure 1)**. Gender had no impact on the seropositivity of children to B. henselae and to *B.quintana*. Ownership of a cat and history of cat scratches or bites had no impact on the seropositivity of childrens' sera to *B.quintana* **(Table 2)**. Out of the 201 children tested from Northern Jordan, 13.9% had positive titers for *B.henselae*-specific IgG. On the other hand, 7% of those tested from central Jordan were positive for *B.henselae*-IgG. This difference in seropositivity to *B.henselae*-specific IgG between children from Northern and central Jordan was statistically significant ($P<0.05$) **(Table 4)**. No geographical differences were detected in the seropositivity to *B.quintana*.

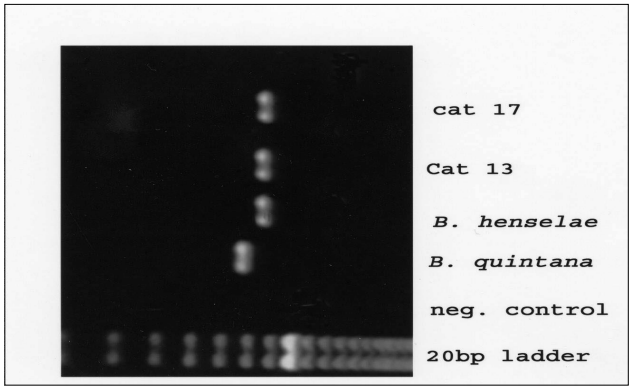

Figure 1 - Polymerase chain reaction based identification of *Bartonella henselae (B.henselae)* from blood of cats belonged to children with high *B.henselae* titers. DNA was extracted from the blood using QIAmp DNA blood kit.

Discussion. The epidemiology of *Bartonella* infections and risk factors for these disorders are not fully understood. The seroprevalence in healthy human blood donors had ranged from 2-6% in the USA,[9,17,18] 4% in Sweden,[15] 30% in Germany[10] and 5.5% in Japan.[19] Infections caused by *B.henselae* and *B.quintana* are frequently reported in younger populations. This study described, for the first time, some aspects on the epidemiology of *B.henselae* and *B.quintana* among Jordanian children. While cats are clearly recognized as the natural reservoir of *B.henselae*, the reservoir of *B.quintana* have not yet been identified.[3,4] Transmission of *B.henselae* from cats to man probably occurs directly via bites and scratches as well as via cat fleas.[20,21] It has been elucidated that contact with cats and presence of cat fleas are among the most important epidemiological risk factors for *B.henselae* infection in man.[20,22] In our study, we found similar results in which a statistically significant ($P<0.05$) number of positive serum samples for *B.henselae*-IgG were found in children with cats in their households. On the other hand, a recent study had suggested that the seroprevalence of IgG antibodies to *B.henselae* is similar for both cat owners and controls.[11] Our laboratory is currently investigating the epidemiology of *B.henselae* in domesticated cats in Jordan. Most scientific reports suggested that cat scratch disease occurs more frequently in children.[4,17,22,23] Other studies have shown that the prevalence is similar in the various age groups.[15,19] In this study, we investigated the seroprevalence of *B.henselae* and *B.quintana* in children under 15-years of age. Our results suggested that children aged 7-10-years old are more likely to have positive *B.henselae*-specific IgG than younger or older children are. Dissecting of this age group revealed that 70% of the *B.henselae*-IgG-positive individuals

had at least one cat in their households. This further highlights cat ownership as an important risk factor for *B.henselae* infection. In addition, we have isolated *B.henselae* from cats that belonged to children with high IFA titers to *B.henselae*. Currently, our laboratory is studying the phenotypic and genotypic relationship of this *B.henselae* isolate with reference strains. Previous studies have shown that gender has no impact on the prevalence of *B.henselae* infection in man.[15,19] Similarly, this study suggested that gender is insignificantly associated with seroprevalence to the different *Bartonella* antigens. However, other studies have indicated that male individuals are more likely to acquire CSD than female individual.[24] Studies performed in the different parts of Europe and North America had shown geographical variation in *Bartonella* infections.[15,25] Warm and humid environments were found to be associated with higher seroprevalence to the different *Bartonella species*, suggesting the theory of a possible arthropod vector such as fleas.[26] Although the climate in Jordan is hot and dry throughout most of the year, the prevalence of B. henselae was almost the same as the prevalence in other countries.[22,27,28] A higher seroprevalence of B. henselae-IgG was found in Northern Jordan, suggesting a different geographical distribution. The precipitation level and temperature are slightly higher in Northern Jordan than in the central part, which complies with the fact that the seropositivity to *Bartonella species* is associated with warm and humid environments. Reports of *B.quintana* infections became rare since the 1940's until the description of the infection in patients infected with human immunodeficiency virus (HIV).[4] In addition to the presence of body louse, alcoholism was found to be closely associated with the acquisition of *B.quintana* infection.[4,29] In this study, no association was found between seropositivity to *B.quintana* and gender, cat ownership, or history of cat scratches.

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References

1. Jerris RC, Regnery RL. Will the real agent of cat-scratch disease please stand up? ***Annu Rev Microbiol*** 1996; 50: 707-725.
2. Kordick DL, Hilyard EJ, Hadfield TJ, Wilson KH, Steigerwalt AJ, Brenner et al. *Bartonella* clarridgeiae, a newly recognized zoonotic pathogen causing inoculation papules, fever, and lymphadenopathy (cat scratch disease). ***J Clin Microbiol*** 1997; 35: 1813-1818.
3. Maurin M, Birtles RJ, Raoult D. Current knowledge of *Bartonella species*. ***Eur J Clin Microbiol Infect Dis*** 1997; 16: 487-506.
4. Anderson BE, Neuman MA. *Bartonella spp*. as emerging human pathogens. ***Clin Microbiol Rev*** 1997; 10: 203-219.
5. Bass J, Vincent MJ, Person DA. The expanding spectrum of *Bartonella* infections. II. Cat-scratch disease. ***Pediatr Infect Dis J*** 1997; 16: 163-179.
6. Sander A, Posselt M, Bhm N, Ruess M, Altwe M. Detection of *Bartonella henselae* DNA by two different PCR assays and determination of the genotype in histologically defined cat scratch disease. ***J Clin Microbiol*** 1999; 37: 993-997.
7. Sander A, Schmidt A. editors. In: Contributions to microbiology. Vol. 1. *Bartonella* and Afipia species emphasizing *Bartonella henselae*. (Microbiological diagnosis of *Bartonella species* and Afipia felis. (CH): Karger; 1998. p. 98-112.
8. Raoult D, Tissot Dupont H, Enea-Mutillod M. Positive predictive value of Rochalimaea henselae antibodies in the diagnosis of cat-scratch disease. ***Clin Infect Dis*** 1994; 19: 335-340.
9. Regnery RL, Olson JG, Perkins BA, Bibb W. Serological response to "Rochalimaea henselae" antigen in suspected cat-scratch disease. ***Lancet*** 1992; 339: 1443-1445.
10. Sander A, Posselt M, Oberle K, Bredt W. Seroprevalence of antibodies to *Bartonella henselae* in patients with cat scratch disease and in healthy controls: evaluation and comparison of two commercial serological tests. ***Clin Diagn Lab Immuno*** 1998; 5: 486-490.
11. Rath PM, Von recklinghausen G, Ansorg R. Seroprevalence of immunoglobulin G antibodies to *Bartonella henselae* in cat owners. ***Eur J Clin Microbiol Infect Dis*** 1997; 16: 326-327.
12. Haimerl M, Tenter AM, Simon K, Rommel M, Hilger J, Autenrieth B. Seroprevalence of *Bartonella henselae* in cats in Germany. ***J Med Microbiol*** 1999; 48: 845-856.
13. Jalava J, Kotilainen P, Nikkari S, Skurnik M, Vanttinen E, Lehtonen OP et al. Use of the polymerase chain reaction and DNA sequencing for detection of *Bartonella quintana* in the aortic valve of a patient with culture-negative infective endocarditis. ***Clin Infect Dis*** 1995; 21: 891-896.
14. Blomgren M, Hardt-Madsen M. Cat-scratch disease an overlooked disease in Denmark? ***Ugeskr Laeger*** 2002; 159: 2876-2877.
15. Holmberg M, McGill S, Ehrenborg C, Wesslén L, Hjelm E, Darelid J et al. Evaluation of Human Seroreactivity to *Bartonella Species* in Sweden. ***J Clin Microbiol*** 1999; 37: 1381-1384.
16. Jensen WA, Fall MZ, Rooney J, Kordick DL, Breitschwerdt EB. Rapid identification and differentiation of *Bartonella species* using a single-step PCR assay. ***J Clin Microbiol*** 2000; 38: 1717-1722.
17. Jackson LA, Perkins BA, Wenger JD. Cat scratch disease in the United States: an analysis of three national databases. ***Am J Public Health*** 1993; 83: 1707-1711.
18. Jackson LA, Spach DH, Kippen DA, Sugg NK, Regnery R, Sayers MH et al. Seroprevalence to *Bartonella quintana* among patients at a community clinic in downtown Seattle. ***J Infect Dis*** 1996; 173: 1023-1026.
19. Maruyama S, Boonmar S, Morita Y, Sakai T, Tanaka S, Yamaguchi F et al. Seroprevalence of *Bartonella henselae* and Toxoplasma gondii among healthy individuals in Thailand. ***J Vet Med Sci*** 2000; 62: 635-637.
20. Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K, Roberts-Wilson J et al. Experimental transmission of *Bartonella henselae* by the cat flea. ***J Clin Microbiol*** 1996; 34: 1952-1956.
21. Maruyama S, Nakamura Y, Kabeya H, Tanaka S, Katsube Y. Prevalence of *Bartonella henselae*, *Bartonella* clarridgeiae and the 16 rRNA gene types of *Bartonella henselae* among pet cats in Japan. ***J Vet Med Sci*** 1999; 62: 273-279.

22. Zangwill KM, Hamilton DH, Perkins BA, Regnery RL, Plikaytis BD, Hadler JL et al. Cat scratch disease in Connecticut. Epidemiology, risk factors, and evaluation of a new diagnostic test. ***N Engl J Med*** 1993; 329: 8-13.
23. Carithers HA. Cat-scratch disease: an overview based on a study of 1,200 patients. ***Am J Dis Child*** 1985; 139: 1124-1133.
24. Klein JD. Cat Scratch Disease. ***Pediatr Rev*** 1994; 15: 348-353.
25. Relman DA. Are all *Bartonella henselae* stains created equal? ***Clin Infect Dis*** 1998; 26: 1300-1301.
26. Jameson P, Greene C, Regnery RL, Dryden M, Marks A, Brown J et al. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. ***J Infect Dis*** 1995; 172: 1145-1149.
27. Amerein MP, De Briel D, Jaulhac B, Meyer P, Monteil H, Piemont Y. Diagnostic value of the indirect immunofluorescence assay in cat scratch disease with *Bartonella henselae* and Afipia felis antigens. ***Clin Diagn Lab Immuno*** 1996; 3: 200-204.
28. Demers DM, Bass JW, Vincent JM, Person DA, Noyes DK, Staege CM et al. Cat-scratch disease in Hawaii: etiology and seroepidemiology. ***J Pediatr*** 1995; 127: 23-26.
29. Spach DH, Kanter AS, Dougherty MJ, Larson AM, Coyle MB, Brenner DJ et al. *Bartonella (Rochalimaea) quintana* bacteremia in inner-city patients with chronic alcoholism. ***N Engl J Med*** 1995; 332: 424-428.