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Presence of *Bartonella* spp. in Various Human Populations

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Abstract

Bartonella spp. bacteria are significant human pathogens and the agents of bacterial zoonosis acquired from an animal companion. The aim of the study was to determine the seroprevalence of two of the most common *Bartonella* species *B. henselae* and *B. quintana* in various human populations. The studied groups included: alcoholics, intravenous drug users, veterinarians, cats' owners. Blood samples were collected and cultured on chocolate agar plates and in mouse fibroblasts L-929 cell line culture. The levels of *Bartonella henselae* IgM and IgG antibodies were determined by indirect immunofluorescence assay. Specific *B. henselae* IgG were detected in 48.3% of homeless alcoholics, in 45.0% veterinarians and in 53.3% cats' owners. The differences in the prevalence of *B. henselae* antibodies between the studied groups and a control group were statistically supported. No homeless intravenous drug users had specific *B. henselae* and *B. quintana* antibodies. High titers of *B. quintana* IgG antibodies were detected in two homeless alcoholics. *Bartonella* spp. was cultured on chocolate blood agar plates from blood samples from 2 alcoholics. The isolates were identified as *B. henselae* by PCR amplification of the riboflavin synthase gene (*ribC*). The results prove that *B. henselae* and *B. quintana,* emerging human pathogens, are present and widely distributed in Poland in such specific risk groups as: alcoholics, veterinarians and cats' owners.

Key words: *Bartonella henselae*, alcoholics, intravenous drug users, vets

Introduction

Bartonella spp. bacteria are significant human pathogens and the agents of the world's most common bacterial zoonosis acquired from an animal companion. Persistent infections in domestic and wild animals result in the presence of reservoir of *Bartonella* organisms in nature that serve as a source for human infection. The prevalence of *Bartonella* bacteremia can range from 50 to 95% in selected rodent, cat, deer, and cattle populations (Breitschwerdt and Kordick, 2000).

The transmission of *Bartonella henselae* from cats to humans usually occurs *via* scratches (Chomel *et al.*, 1996). It has been reported that dogs infected with *Bartonella henselae* may be associated with human infections (Yamanouchi *et al.*, 2004; Henn *et al.*, 2005). Recently DNA of *B. henselae* was detected in *Ixodes ricinus* ticks removed from humans (Sango *et al.*, 2003).

The most common etiological agent of bartonellosis is *B. henselae*. *B. henselae* bacteria produce two entirely different reactions: nonangiogenic inflammation and angiogenic reactions connected with adherence of the bacteria to vascular endothelium. Nonangiogenic inflammations like a cat-scratch disease (CSD), unilateral regional lymphodenitis concern mainly immunocompetent individuals (Greub and Raoult, 2002; Rolain, 2006). In 5–9% of CSD patients, atypical manifestations may develop, including encephalitis, endocarditis, hemolytic anemia, hepatosplenomegaly, glomerulonephritis, pneumonia, bacteremia and osteomyelitis (Chomel *et al.*, 2006). Diseases connected with angiogenic reactions occur rather in immunocompromised persons. They concern skin (bacillary angiomatosis), liver (peliosis hepatitis), bones, central nervous system or spleen. Moreover, *B. henselae* infections may cause endocarditis in immunocompromised patients, such as HIV positive patients, alcoholics, or organ transplant recipients, (Raoult *et al.*, 1996; Fournier *et al.*, 2001), septicemia (Welch *et al.*, 1993), and neurological disorders (Noah *et al.*, 1995; Noyola *et al.*, 1999).

In immunocompromised individuals and in persons living in poor sanitary conditions, not only *B. henselae*

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variants but even other *Bartonella* species such as *B. quintana* are found. Nowadays, *B. quintana* is known to be responsible for louse-borne bacteremia and endocarditis in homeless people and bacillary angiomatosis in AIDS patients (Guyot *et al.*, 1999; Breitschwerdt and Kordick, 2000).

With increasing number of representatives of genus *Bartonella* identified, even more species appear to be associated with human disease like: *B. grahami*, *B. elizabethae*, *B. clarridgeiae*, *B. washoensis*, *B. vinsoni* subs. *arupensis*, *B. vinsoni* subs. *berkhoffi*, *B. koehlerae* (Avidor *et al.*, 2004; Chomel *et al.*, 2004; Rolain *et al.*, 2004).

The aim of the study was to determine the seroprevalence of two of the most common *Bartonella* species *B. henselae* and *B. quintana* in various human populations.

Experimental

Materials and Methods

Patients. One hundred and twenty persons were enrolled in the study. The group included: 29 alcoholics, 20 veterinarians, 15 cats' owners, 6 intravenous drug users. Additionally a group of 50 blood donors, representing healthy population was tested. The presence of *Bartonella* sp. specific antibodies in blood serum and bacteria in blood was examined.

Blood samples were also drawn from seven cats which belonged to the examined persons for blood culture and serological testing.

Blood culture – agar plate, cell line culture. Three hundred microliters of EDTA-treated blood samples of humans or cats frozen at –70°C for at least 24 h were thawed at room temperature before inoculation onto chocolate agar plates (Choc V, Oxoid). The plates were incubated at 37°C in a 5% CO_2 – atmosphere (Genbag CO_2 system; BioMerieux, France) for at least 5 weeks. *Bartonella*-like colonies were tested by the PCR-based methods.

Mouse fibroblasts L-929 (from collection of National Institute of Hygiene, Warsaw, Poland) in Eagle's minimum essential medium (MEM) supplemented with 4% fetal calf serum (Biomed, Poland) were grown in shell-vials (Bibby Sterilin, England) at 37°C for 24 hours until confluent monolayer was obtained. Two hundred microliters EDTA blood collected from humans or cats frozen at –70°C for 24 h and then thawed was inoculated into the cell line monolayer. The cultures were incubated at 37°C in a 5% CO_2 enriched atmosphere for 5 weeks. After that time, the culture medium and cell monolayer were tested to detect *Bartonella* sp. by PCR-based methods.

Serology. The level of *B. henselae* and *B. quintana* IgM and IgG antibodies was determined by indirect immunofluorescent assay (IFA) (Focus diagnostics, USA). Purified *B. henselae* and *B. quintana* cells cultivated in yolk sac of hen eggs and in Vero cells were used as diagnostic antigens to determine IgM and IgG antibodies, respectively. Titers of IgG antibodies ≥64 and IgM antibodies ≥10 were considered positive.

Bound to the antigen (Focus diagnostics, USA) antibodies of cats were developed with anti-cat IgG FITC Conjugate (Sigma, Germany). Titers of IgG antibodies ≥64 were considered positive.

Molecular detection of *Bartonella* spp. from blood samples. Isolated *Bartonella* sp. strains were pelleted by centrifugation for 10 min at 15 000 × g. DNA was extracted with Qiagen columns (QIAamp DNA Mini Kit, QIAGEN, Germany).

PCR assay for detection the *gltA* gene fragment was used for detection of DNA of *Bartonella* genus. PCR amplification of the riboflavin synthase gene (*ribC*) was applied for molecular identification of *Bartonella* species.

The primers used for the *gltA* gene fragment were as follows: 5'GGGGACCAGCTCATGGTGG-3' and 5'AATGCAAAAAGAACAGTAAACA3'. Thermocycling was performed in an MJ Research (USA) apparatus under following conditions: 95°C for 10 min, and 45 cycles of denaturing at 95°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min, followed by 72°C for 10 min. PCR products were separated on 1.5% agarose gel and visualized by staining with ethidium bromide.

Oligonucleotide primers BARTON-1 (5'TAACC GATATTGGTTGTGTTGAAG3') and BARTON-2 (5'TAAAGCTAGAAAGTCTGGCAACATAACG3') described by Johnson *et al.*, 2003) were used for the amplification of the riboflavin synthase gene. PCR cycling consisted of: a single hot-start cycle of 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 1 min. Amplification was completed by an additional cycle at 72°C for 10 min to allow complete extension of the PCR products. PCR products were subjected to digestion with restriction enzyme Taq I or Eam (Fermentas, Lithuania). Restriction enzyme digestion patterns obtained with these enzymes identified *Bartonella* species.

Results

Prevalence of *Bartonella* sp. infection in homeless alcoholics and in homeless intravenous drug users. Serum samples from 29 homeless alcoholics and 6 homeless intravenous drug users were tested on the presence of specific *B. henselae* and *B. quintana* IgM and IgG antibodies by IFA. Specific *B. henselae* IgG were detected in 14 (48.3%) of alcoholics. The

Table I
Prevalence of seropositive cases of bartonellosis among tested persons

Group	No tested	*B. henselae**				*B. quintana**			
		IgM	IgG	Total	OR; 95%Cl	IgM	IgG	IgM and IgG	Total
Homeless alcoholic	29	0	14	14 (48.3%)	2.9 (1.5–4.3)	0	1	1	2 (6.9%)
Homeless intravenous drug users	6	0	0	0 (0%)	0	0	0	0	0
Vets	20	0	9	9 (45.0%)	2.5 (1.1–3.8)	nt	nt	nt	nt
Cats' owners	15	0	8	8 (53.3%)	3.6 (1.5–5.7)	nt	nt	nt	nt
Blood donors	50	0	12	12 (24%)	1	0	0	0	0
Total	120	0	43	43 (35.8%)	–	–	–	–	–

* titer ≥10 of IgM and titer ≥64 of IgG were considered positive
nt – not tested

differences between prevalence of *B. henselae* antibodies between alcoholics and control group were statistically significant (OR = 2.9; 95%CI 1.5–4.3). None of persons had specific IgM *B. henselae* antibodies. In two individuals IgG *B. quintana* antibodies in high titers 256 and 1024 were detected. The highest titer of *B. henselae* antibodies detected among alcoholics was 256. It occurred in four of 29 (13.8%) tested persons. Nine alcoholics had specific antibodies in titer 128 (31.0%), one in titer 64, in seven individuals antibodies were present in titer 32 and 8 (27.6%) persons from these group were seronegative (Table I, Figure 1).

None of homeless intravenous drug users had specific IgM and IgG *B. henselae* and *B. quintana* antibodies (Table I).

Bartonella spp. strains were cultured on chocolate blood agar plates from blood samples of 2 alcoholics. The isolates were identified as *B. henselae* by PCR amplification of the riboflavin synthase gene (*ribC*).

Prevalence of *Bartonella* sp. antibodies in veterinarians. Serological signs of *B. henselae* infections were found in 9 of 20 (45.0%) veterinarians. In vets *B. henselae* antibodies were present more often than in control group (OR = 2.5; 95% CI 1.1–3.8). One (5%) of 20 tested vets had specific antibodies in titer 128, eight (40%) in titer 64, nine (45%) in titer 32 and 2 (10%) were seronegative (Table I, Figure 1).

Prevalence of *Bartonella* sp. antibodies in cats' owners. Eight of 15 tested cats' owners had IgG *B. henselae* antibodies. *B. henselae* IgM antibodies were not found. Specific *B. henselae* antibodies were detected more frequently in cats' owners than in control group, the difference was statistically significant (OR = 3.6; 95% CI 1.5–5.7). The antibody titers ranged from 64 to 4096 (Table I, Figure 1).

In the case of seven cats' owners simultaneous blood cultures and serological testing of serum samples collected from their pets were done. An examination of blood from a cat which belonged to two children (both of them had high level of *B. henselae* IgG antibodies, one in titer 1024 and the other 4096) was done. The cat of the seropositive owners was not bacteremic and it did not even have specific *B. henselae* antibodies. In another studied pair owner-pet, specific antibodies were present both in the serum of the owner and pet in titers 1024 and 256 respectively. The other examined owners had antibodies to *B. henselae* in titer 256. The cat was nonbacteremic and had *B. henselae* antibodies in titer 64. In one case specific *B. henselae* antibodies were found in a cat, whereas its owner was seronegative (Table II).

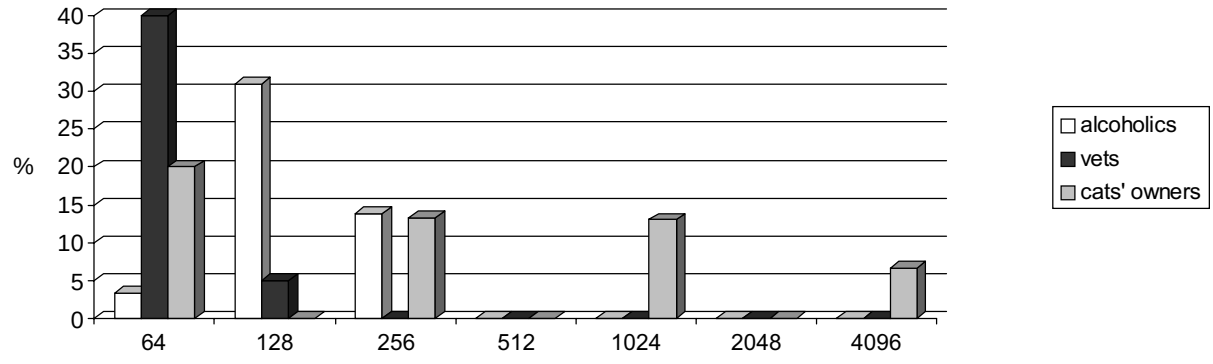

Fig. 1. Titers of IgG antibodies to *B. henselae* among alcoholics, vets, cats' owners
* None of homeless intravenous drug users had specific *B. henselae* antibodies

Table II
Prevalence of *B. henselae* in humans and their cats

No	Human			Cats			kind of contact
	Patient no	*B. henselae* IgG *antibodies*[1]	culture	Cat no	*B. henselae* IgG *antibodies**	culture	
1	41	32	negative	nd	nd	nd	Contact with ill cat
2	43	64	negative	nd	nd	nd	Contact with ill cat
3	44	negative	negative	nd	nd	nd	Bitten by ill cat
4	39	32	negative	nd	nd	nd	Cat's owner
5	40	32	negative	nd	nd	nd	Cat's owner
6	145	32	negative	63	32	negative	Cat's owner
7	151	32	negative	152	negative	nd	Cat's owner
				153	negative	nd	
8	200/05	1024	negative	201/05	256	negative	Cat's owner
9	202/05	1024	negative	204/05	negative	nd	Cat's owner
10	203/05	4096	negative				
11	205/05	256	negative	206	64	negative	Cat's owner
12	1077/05	64	nd	nd	nd	nd	Cat's owner
13	1149/05	256	nd	nd	nd	nd	Cat's owner
14	Bronow	64	nd	nd	nd	nd	Cat's owner
15	1312/05	32	nd	nd	nd	nd	Cat's owner
16	207/05	negative	nd	207	64	nd	Cat's owner
Total	15	8	0	7	2	0	–

* titer ≥64 was considered positive; [1] IgM antibodies were negative; nd – no data

Prevalence of *Bartonella* spp. serum antibodies in blood donors. Fifty blood donors were tested on the presence of serum antibodies to *B. henselae* and *B. quintana.* Specific *B. henselae* antibodies were detected in 12 (24%) of tested people, in 11 (22 %) in titer 64 and in one in titer 128. Specific IgM class antibodies were not found in any of tested donors. None of the tested persons had IgM and IgG *B. quintana* antibodies (Table I).

***Bartonella* spp. isolation.** *Bartonella* spp. strains were isolated from blood of 2 (5.1%) out of 39 persons investigated. The isolates were typed by PCR and identified as: *B. henselae*. Growth of these two strains was detected on chocolate blood agar plates, they did not grow in cell line. In cell line of mouse fibroblasts L-929 none from all patients' tested blood samples cultured were positive for the presence of *B. henselae* strains as detected by PCR.

Discussion

In Poland rates of incidence of Cat Scratch Disease (CSD), the most commonly encountered *B. henselae*-induced syndrom in human, was estimated at 0.05 in 1998 and at 0.14 in 2000 for 100 000 persons (Podsiadly *et al.*, 2003). In the Netherlands, the estimated number of CSD is 2000 cases per year or 12.5 cases per 100 000 persons (Bergmans *et al.*, 1997). For comparison, the annual number of cases in the United States is 9.3 for 100 000 persons, with approximately 2000 cases that require hospitalization (Jackson *et al.*, 1993). The mentioned evidences suggest that *B. henselae* infections in Poland are commonly subclinical or unrecognized and in consequence underreported. Evidence for the presence of *Bartonella* spp. in the Polish environment are the results of serological testing of homeless alcoholics. It has been shown that 48.3% of homeless persons were *B. henselae* seropositive. The studies confirmed that homeless state is a risk factor of exposure to *Bartonella* spp.

Nowadays *Bartonella quintana* infections occur almost exclusively among inner-city alcoholics or homeless populations, drug users and HIV seropositives (Guyot *et al.*, 1999; Boulouis *et al.*, 2005). In our studies, on the base of serological examination, 2 out of 29 tested homeless alcoholics had serological signs of *B. quintana* infection. In Marseilles, 30% of tested homeless patients had high antibody titers to *B. quintana* (Broqui *et al.*, 1999). In the same study isolation of *B. quintana* from the blood of 10 (14%) out of 71 homeless people was described. Five of them had chronic bacteremia for at least several weeks without any signs or symptoms of the disease (Broqui *et al.*, 1999). Although it has been postulated that *B. quintana* is easier to isolate than *B. henselae*

(La Scola and Raoult, 1999) we have been able to culture two *B. henselae* strains from two homeless alcoholics but no *B. quintana* strain.

None of six tested intravenous drug users had specific *B. henselae* or *B. quintana* antibodies. *B. elizabethae* infections seem to be quite prevalent in intravenous drug users and homeless people in various parts of the USA and in Sweden (Comer *et al.*, 1996; McGill *et al.*, 2003). In Stockholm 39% tested drug users had antibodies to *B. elizabethae* and in New York 46% (McGill *et al.*, 2003).

Two studies on *B. henselae* seroprevalence among veterinary professionals from the United States and from Europe reported 7.1 and 51.1% seropositives, respectively (Noah *et al.*, 1997; Nowotny *et al.*, 1997). Our results (45%) confirm the high seroprevalence of *B. henselae* among veterinarians in Europe with application of the same IFA method. In vets *B. henselae* antibodies were detected in lower titers than in cats' owners and alcoholics. In comparison with other tested groups the highest titers of antibodies were detected among cats' owners. Epidemiological studies indicate that cat ownership and kitten or cat scratches are the strongest risk factors for cat scratch disease and bacillary angiomatosis (Tappero *et al.*, 1993; Zangwill *et al.*, 1993; Chomel *et al.*, 2006). In our studies among 15 tested cats' owners more than half of them (53.3%) had *B. henselae* IgG antibodies in high titers. Among blood donors only 24% were seropositive. No cats' owners had any clinical symptoms of CSD or another *Bartonella* infection as the consequence of lack *Bartonella* infection in their pets. Probably the presence of specific *B. henselae* antibodies in cats and their owners is a result of previous contact with the bacteria. In Australia it was found that there is not correlation between pet ownership and presence of specific *B. henselae* antibodies. No difference of seroprevalence in persons with and without domestic pet was detected (26% and 20% respectively) (Dillon *et al.*, 2002).

There are no general procedure optimum for the isolation of *Bartonella* spp. (La Scola and Raoult, 1999). In our study both grow on L-929 cell line as well as chocolate agar plates were used. Our observations suggest that culture on L-929 cell line monolayers is not a sensitive culture system for the isolation of *B. henselae* from human blood samples.

Bartonella spp. exist in wildlife habitats in Poland. Healthy people encounter bacteria belonging to this genus through contact with animals. These contacts results in the presence of specific *Bartonella* serum antibodies, which are visible in the healthy population. Also new groups of people susceptible to *Bartonella* infections are becoming visible, such as urban homeless persons.

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