Introduction

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

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 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...
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. grahalmi*, *B. elizabethae*, *B. claridgeiae*, *B. washoensis*, *B. vinsoni* subs. *arupensis*, *B. vinsoni* subs. *berkhoffii*, *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% CO₂ – atmosphere (Genbag CO₂ 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% CO₂ 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* 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 Qiangen 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’GGGGACGAGCTCATGTTG-3’ and 5’ AAT GCA A A A G A C A G T A A A C A 3’. 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’TAAACC GATATTGTTGTTGGAAG-3’) and BARTON-2 (5’TAAAGCTAAGCTTGCGAACATAACGG-3’) were 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
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).

---

Table I

<table>
<thead>
<tr>
<th>Group</th>
<th>No tested</th>
<th>B. henselae*</th>
<th>OR; 95%CI</th>
<th>B. quintana*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>Total</td>
</tr>
<tr>
<td>Homeless alcoholic</td>
<td>29</td>
<td>0</td>
<td>14</td>
<td>14 (48.3%)</td>
</tr>
<tr>
<td>Homeless intravenous drug users</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Vets</td>
<td>20</td>
<td>0</td>
<td>9</td>
<td>9 (45.0%)</td>
</tr>
<tr>
<td>Cats’ owners</td>
<td>15</td>
<td>0</td>
<td>8</td>
<td>8 (53.3%)</td>
</tr>
<tr>
<td>Blood donors</td>
<td>50</td>
<td>0</td>
<td>12</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>0</td>
<td>43</td>
<td>43 (35.8%)</td>
</tr>
</tbody>
</table>

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

*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
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 syndrome 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 seropositive (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*

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

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

**Table II**

Prevalence of *B. henselae* in humans and their cats
(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 of 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 result 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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**Literature**


