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Bacterial tick-borne diseases caused by *Bartonella* spp., *Borrelia burgdorferi* sensu lato, *Coxiella burnetii*, and *Rickettsia* spp. among patients with cataract surgery

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Data Interpretation D
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Background: Clinical data have shown that tick-borne diseases caused by *Borrelia burgdorferi* sensu lato, *Bartonella* spp., *Coxiella burnetii*, and *Rickettsia* spp. can affect the central nervous system, including the eye. The aim of this study was to establish a relationship between the incidence of cataract and evidence of bacterial infections transmitted by ticks.

Material/Methods: Fluid with lenticular masses from inside of the eye and blood from 109 patients were tested by PCR and sequencing. Sera from patients and the control group were subjected to serological tests to search specific antibodies to the bacteria.

Results: Microbiological analysis revealed the presence of *Bartonella* sp. DNA in intraoperative specimens from the eye in 1.8% of patients. Serological studies have shown that infections caused by *B. burgdorferi* sensu lato and *Bartonella* sp. were detected in 34.8% and 4.6% of patients with cataract surgery, respectively.

Conclusions: Presence of DNA of yet uncultured and undescribed species of *Bartonella* in eye liquid indicates past infection with this pathogen. Specific antibodies to *B. burgdorferi* sensu lato and *Bartonella* sp. are detected more frequently in patients with cataract compared to the control group. This could indicate a possible role of these organisms in the pathological processes within the eyeball, leading to changes in the lens. Further studies are needed to identify *Bartonella* species, as well as to recognize the infectious mechanisms involved in cataract development.

Keywords: *Borrelia Burgdorferi* Sensu Lato • *Bartonella* Spp. • *Coxiella Burnetii* • *Rickettsia* Spp. • Cataract

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Background

An increased number of bacterial tick-borne disease cases have been reported in Poland in recent years. These are caused by *Borrelia burgdorferi sensu lato*, *Bartonella* spp., *Coxiella burnetii*, and *Rickettsia* spp. Clinical symptoms of tick-borne diseases can vary, depending on the affected system (osteoarticular, nervous, gastrointestinal, or cardiovascular). Some patients do not notice contact with a tick and the clinical course of acute infection could be mild. The possibility of infection with *Bartonella* spp. can also be associated with a scratch or bite by cats and the penetration of bacteria with the saliva through minor skin damage. This pathogen is detected in approximately 10% of cats in Poland [1]. Uveitis in the course of infection with *Bartonella* spp. was observed in some of these animals, as well as the presence of *Bartonella* spp. DNA in the aqueous humor [2].

Clinical data have shown that tick-borne diseases can affect the central nervous system, including the eye. *Borrelia burgdorferi sensu lato* is a cause of conjunctivitis, keratitis, uveitis, and retinal infection (macular edema and vasculitis). These symptoms are frequent findings in neuroborreliosis patients [3]. Optic neuritis, meningitis encephalitis, and paralysis of the oculomotor nerve have been reported in 3.5% patients with Q fever [4,5]. Retinal vascular involvement may present different clinical pictures: branch retinal artery occlusion, cystoid macular edema, serous retinal detachment, and hypofluorescent choroidal spots in patients with *Rickettsia* spp. infections [6]. The ocular manifestations of CSD (cat-scratch disease) are different: chronic ulcerative conjunctivitis associated with lymphadenopathy (referred to as Parinaud oculoglandular syndrome [POGS], neuroretinitis, sub-retinal lesions, retinitis, and intermediate uveitis. Some of these infections can be self-limiting or asymptomatic illnesses [7,8].

The aim of this study was to establish a relationship between the incidence of cataract and bacterial infections transmitted by ticks caused by microorganisms such as *Borrelia burgdorferi sensu lato*, *Bartonella* spp., *Coxiella burnetii*, and *Rickettsia* spp. Patients with cataract, without ocular inflammation (uveitis, retinitis), and without symptoms of infectious diseases were included in the study group.

Material and Methods

Fluid and lenticular masses were collected from inside of the eye during cataract surgery and stored at -30°C until further research. Blood samples were also collected. Material was obtained from 109 patients (64 women and 45 men) aged 33 to 89 years old (median age 63.5 years). Their past medical history involved general diseases such as hypertension, diabetes,

thyroid disease, cardiac disease, rheumatic disease, asthma, hyperplasia of the prostate, and varices of the lower extremities. In 2 patients, HCV (hepatitis C virus) infection and pulmonary tuberculosis were diagnosed. In 1 man, common variable immunodeficiency was diagnosed. No patients had ocular inflammation. No lenticular masses from the control group were used for medical consideration.

Sera from 101 blood donors (47 women and 54 men) aged 31 to 65 years old (median age 48.5 years) were included as a control in the serologic part of the study.

Blood, fluid, and lenticular masses were subjected to PCR and serological tests to search specific DNA and antibodies (in sera) to the bacteria. The study was pursued following the approval of the Institutional Review Board (IRB) at the National Institute of Public Health – National Institute of Hygiene, issued on 28.06.2012, Approval No: 4/2012.

DNA preparation

A volume of 100 μl of fluid with lenticular masses and blood were taken for DNA extraction. The QIAamp Tissue kit (QIAGEN GmbH, Hilden, Germany) was used according to the manufacturer's recommendations. DNA samples were stored at -20°C .

PCR

Extracted DNA was tested by PCR to detect fragments of genes characteristic for *Borrelia burgdorferi sensu lato*, *Bartonella* spp., *Coxiella burnetii*, and *Rickettsia* spp. using appropriate primer pairs [9–12]. The reaction mixtures of 50 μl contained 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl_2 , 0.1% gelatin, 200 μM dNTPs, 50 pmol of each primer, and 1.5 U Gold Taq DNA polymerase (Perkin-Elmer Cetus, USA). An aliquot of 5 μl of DNA template was added to each reaction mixture. The cycling conditions were as follows: 3 min at 95°C , followed by 40 cycles of 1 min of denaturation at 95°C , 1 min of annealing at 47°C to 59°C (depending on the starters used), 1 min of elongation at 72°C , and finally 7 min of elongation at 72°C . PCRs were performed in a Mastercycler gradient apparatus (Eppendorf AG, Germany). Each run of PCR test included positive (DNA from the collection of strains) and negative controls (water). All amplicons were analyzed by electrophoresis in 1.5% agarose gel stained with ethidium bromide.

Sequencing

For purification of PCR products, the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) was used according to the manufacturer's protocol. All amplicons were sequenced with the ABI 377 DNA Analyzer (Applied Biosystem, USA) according to the manufacturer's recommendations. Sequences were edited using Autoassembler software (Applied Biosystem,

Table 1. Seroprevalence of tick-borne diseases in patients with cataracts.

Pathogen	No. (%) of persons with specific antibodies		p
	Patients with cataract (n=109)	Control group (blood donors) (n=101)	
<i>Bartonella henselae</i> ^a	5 (4.6%)	0	0.06 ^d
<i>Borrelia burgdorferi sensu lato</i> ^b	38 (34.8%)	12 (12%)	<0.001 ^e
<i>Coxiella burnetii</i> ^c	1 (0.9%)	0	1.00 ^d
<i>Rickettsia</i> spp.	0	0	–

^a IgG titer ≥ 256 with IFA; ^b Positive IgM and/or IgG results confirmed with Western-blot; ^c IgG titer ≥ 64 ; ^d F – Fisher's exact test; ^e Chi2 – chi-square test.

USA) and identified using BLAST software by comparison with sequences available in GenBank.

Serology

Antibodies to *B. burgdorferi* IgM and IgG were tested with ELISA test with recombinant antigens (DRG-Medtek, Germany). Positive results were confirmed by Western blot method with Anti-Borrelia-EUROLINE-WB IgM and Anti-Borrelia-EUROLINE-WB IgG (EUROIMMUN, Germany). Results were interpreted as seropositive according to criteria of the German Society of Hygiene and Microbiology (www.dghm.org/red/index.html?cname=MIQ). Levels of *Bartonella* spp. IgG antibodies were determined by indirect immunofluorescence assay (Bartonella IFA IgG; Focus diagnostics, USA) with Vero cells infected with *B. henselae* and *B. quintana* strains as antigens. IgG titer of ≥ 64 to at least 1 of the pathogens was considered as positive.

Levels of serum IgG antibodies to phase I and II *C. burnetii* antigens were assayed by indirect immunofluorescence method with *Coxiella burnetii* I+II IFA IgG/IgM/IgA kit (Vircell, Spain). Serum titer ≥ 64 for IgG was interpreted as positive.

IgG *Rickettsia* spp. antibodies were detected with microimmunofluorescence (MIF). A 2-step procedure was used for *Rickettsia* spp. antibody evaluation. For the detection and differentiation of antibodies specific to typhus and spotted fever groups, inactivated *R. typhi* and *R. rickettsii* antigens were used (Rickettsia IFA IgG, Focus diagnostic, USA). Screening was performed at 1:16 dilution.

Statistic analysis

Statistic analysis (chi-square test and Fisher's exact test) was done using the WinPepi 2.69 statistical program (Abramson JH [2011] WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiologic Perspectives

& Innovations 2011, 8: 1 [available on the Internet at www.epiperspectives.com/content/8/1/].

Results

Specific IgG serum antibodies to *Bartonella* sp. antigen were detected in 39 patients (35.8%). Among them, 2 (1.8%) patients had serum antibodies in a titer of 512, 3 patients had antibodies in a titer of 256 (2.8%), 6 patients had antibodies in a titer of 128 (5.5%), and 28 (25.7%) patients had antibodies in a titer of 64.

IgM and IgG antibodies to *B. burgdorferi* sl. were detected in 14 (12.8%) and 19 (17.4%) serum samples, respectively. Both classes of antibodies were detected in 5 samples (4.6%). IgG antibodies to *Coxiella burnetii* in titer of 64 was detected in 1 case (0.9%) (Table 1). IgG antibodies to spotted fever group *Rickettsiae* were not detected.

Specific IgG antibodies to *B. henselae* antigen were detected in 28 (28%) persons from the control group (blood donors) but in low titers of 64 (27 persons) and 128 (1 person). Specific antibodies to *B. burgdorferi* sl. were detected in sera taken from 12 blood donors: IgM were detected in 8 persons and IgG in 4 persons. All sera were seronegative to *C. burnetii* and *Rickettsia* spp. antigens.

DNA of *Bartonella* sp. was detected in the fluids taken from the eyeball from 2 seronegative patients. DNA of this pathogen was not detected in blood samples. These 2 sequences of *gltA* gene showed 99% identity with the *gltA* gene fragment amplicon of uncultured *Bartonella* sp. clone AS050 and clone AS071 (GenBank accession no. JQ695835.1 and JQ695834.1). The closest relative to the officially recognized species is *B. clarridgeiae* strain BCF02 (94% identity). DNA of other studied pathogens was not detected.

No intra- or post-operative complications were observed. Patients with positive serologic or PCR results for infectious agents studied did not have any symptoms of current infection.

Discussion

Diseases of the eye can affect many structures from tissue in the orbital cavity to uveitis and retina. Fungi, bacteria, viruses, protozoa, and systemic and autoimmune diseases may be etiologic agents of inflammation. The diagnosis of abnormalities in the retina and uveitis, involves puncture of the anterior chamber and/or vitreous to search for a specific pathogen, especially in difficult cases. PCR followed by sequencing clearly demonstrates the presence of the infectious factor causing vascular inflammation. Analysis of aqueous humor and/or vitreous is one of the procedures in the diagnosis of ocular inflammation and retinal or vascular membrane diseases [13–16]. It may also be used in experimental studies [17,18]. These are not routinely performed, due to possible complications after puncture of the anterior chamber or vitreous such as hemorrhage, detachment of the retina, and exacerbation of inflammation. Previous reports have suggested that inflammation in the eyeball may occur in the course of infections such as bartonellosis (cat-scratch disease – CSD), Lyme disease, Q fever or rickettsioses [3–8]. In Poland, testing for the presence of these infections is performed sporadically in patients with blurred vision.

Serological studies have shown that antibodies to *B. burgdorferi* sensu lato and *Bartonella* sp. are detected significantly more frequently in patients with cataracts in comparison with the control group. The approximate time of infection in these cases is difficult to determine. Titers of specific IgG antibodies to *Bartonella* sp. may remain positive for 2 years after disease onset [19]. Much longer periods of persistence of IgM and/or IgG antibodies (a few decades) are observed in Lyme disease [20,21]. In the surgical patients there was no evidence of inflammation in the eye and they did not report a history of infectious diseases in the past. Symptomatic cat-scratch disease in humans is rare and is observed more often in sub-clinical infection. Establishment of eye changes in the course of infection is likely to be related to the immune status of the host and the pathogenicity of the strain [22,23].

The ability to collect material from the anterior chamber during cataract surgery from patients without concomitant inflammation allowed us to detect the presence of *Bartonella* sp. DNA in 2 patients (1.8%). Common variable immunodeficiency was diagnosed in 1 of them. In these cases, antibodies to this pathogen were not detected. This indicates that infection could have occurred many years earlier and DNA of bacteria survived in the eyeball tissues.

Bartonella is a Gram-negative intracellular parasite that multiplies in erythrocytes and epithelial cells. It may spread to every organ of the host organism with the circulating blood. The presence of DNA in the eyeball suggests unknown route and time of contact with the pathogen (subclinical infection after being bitten by a tick or scratched by a cat). Bacteria reached the eyeball through blood vessels, probably through blood ciliary artery anterior to the anterior chamber of the eye (aqueous humor), without causing inflammation. Lens and aqueous humor are devoid of blood vessels (filtration); therefore, *Bartonella* cannot be eliminated after passing through these structures and most probably can be detected even several years after infection.

Processes of immunological tolerance associated with the phenomenon of anterior chamber-associated immune deviation (ACAID) seem to be the most likely protective mechanism against inflammation in the eye. This cascade of events is induced by introducing antigens into the anterior chamber. ACAID is maintained by stimulating the activity of tissue-specific suppressor cells: lymphocytes CD4⁺, CD8⁺, and CD25⁺ (called T regulatory cells [-T_{reg}]), involvement of several cytokines, MSH, VIP, indoleamine, somatostatin, IL-4, IL-10, and TGF-2. The process of immune tolerance of the eye is also regulated by various organ-building cells, such as pigment epithelial cells of the ciliary body and retinal pigment epithelium. These have been shown to inhibit the activation of Th1 cells. Pigment epithelium releases a number of cytokines involved in ACAID, including TGFβ, thrombospondin and PGE2. Corneal endothelial cells also have a protective role in ACAID, releasing a number of suppressing substances to the lymphocytes (CD46, CD55, and CD59), and also those promoting apoptosis (Fas ligand) [24–27].

DNA of *Bartonella* sp. is stable and can be detected in the tissues long after infection. For example, dental pulp from the teeth of cats and dogs was used for the PCR detection of *Bartonella* sp. in animals buried for a year [28]. Dental pulp has been also proposed as a suitable tissue sample for the identification of pathogenic *B. quintana*. DNA was detected in the dental pulp extracted from the tooth of a homeless patient 6 months after he had been bacteremic [29]. In the literature, the case of 45-year-old man presenting with progressively worsening vitreitis of 1-week duration has been described. Vitrectomy and analysis of the vitrectomy specimens revealed inflammatory cells and necrotic debris 3 years later. PCR analysis of the vitreous fluid sample demonstrated the presence of *B. henselae* DNA [30].

Conclusions

Microbiological analysis revealed the presence DNA of *Bartonella* spp. in intraoperative specimens from the eye in 1.8% of patients. The presence of bacterial DNA in eye tissues indicates

past infection with this pathogen. Serological studies have shown that infections caused by *B. burgdorferi* sensu lato and *Bartonella* sp. are detected more frequently in patients with cataract surgery compared to the control group. These facts

could indicate a possible role of these organisms in the “inflammatory processes” within the eyeball, leading to changes in the lens. Further research is needed to elucidate this topic more completely.

References:

1. Podsiadły E, Tylewska-Wierzbowska S: Occurrence of *Bartonella* spp. in selected reservoirs and vectors in Poland. *Post Mikrobiol*, 2008; 47: 275–81 [in Polish]
2. Lappin MR, Kordick DL, Breitschwerdt EB: *Bartonella* spp antibodies and DNA in aqueous humour of cats. *J Feline Med Surg*, 2000; 2: 61–68
3. Mora P, Carta A: Ocular manifestations of Lyme borreliosis in Europe. *Int J Med Sci*, 2009;6: 124–25
4. Shaked Y, Samra Y: Q fever meningoencephalitis associated with bilateral abducens nerve paralysis, bilateral optic neuritis and abnormal cerebrospinal fluid findings. *Infection*, 1989;17: 394–95
5. Bernit E, Pouget J, Janbon F et al: Neurological involvement in acute Q fever: a report of 29 cases and review of the literature. *Arch Intern Med*, 2002; 162: 693–700
6. El Matri L: Ocular manifestations of rickettsiosis: Retinal involvement an treatment. *Int J Med Sci*, 2009; 6: 128
7. Ormerod LD, Dailey JP: Ocular manifestations of cat-scratch disease. *Curr Opin Ophthalmol*, 1999; 10: 209–16
8. Chi SL, Stinnett S, Eggenberger E et al: Clinical characteristics in 53 patients with cat scratch optic neuropathy. *Ophthalmology*, 2012; 119: 183–87
9. Podsiadły E, Karbowski G, Tylewska-Wierzbowska S: Presence of *Bartonella* spp. in *Ixodidae* ticks. *Clin Microbiol Infect*, 2009; 15: 328–29
10. Roux V, Rydkina E, Ereemeeva M et al: Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. *Int J Syst Bacteriol*, 1997; 47: 252–61
11. Fournier PE, Raoult D: Comparison of PCR and serology assays for early diagnosis of acute Q fever. *J Clin Microbiol*, 2003; 41: 5094–98
12. Chmielewski T, Fiett J, Gniadkowski M et al: Improvement to laboratory recognition of Lyme borreliosis with the combination of culture and PCR methods. *Mol Diagn*, 2003; 7: 155–62
13. Muthiah MN, Michaelides M, Child CS et al: Acute retinal necrosis: a national population-based study to assess the incidence, methods of diagnosis, treatment strategies and outcomes in the UK. *Br J Ophthalmol*, 2007; 91: 1452–55
14. Moshfeghi DM, Dodds EM, Couto CA: Diagnostic approaches to severe, atypical toxoplasmosis mimicking acute retinal necrosis. *Ophthalmology*, 2004; 111: 716–25
15. Bhoombunchoo C, Ratanapakorn T, Sinawat S et al: Infectious endophthalmitis: review of 420 cases. *Clin Ophthalmol*, 2013; 7: 247–52
16. Stunf S, Petrovec M, Žigon N et al: High concordance of intraocular antibody synthesis against the rubella virus and Fuchs heterochromic uveitis syndrome in Slovenia. *Mol Vis*, 2012; 18: 2909–14
17. Noma H, Funatsu H, Mimura T et al: Aqueous humor levels of vasoactive molecules correlate with vitreous levels and macular edema in central retinal vein occlusion. *Eur J Ophthalmol*, 2010; 20: 402–9
18. Cheung CM, Vania M, Ang M et al: Comparison of aqueous humor cytokine and chemokine levels in diabetic patients with and without retinopathy. *Mol Vis*, 2012; 18: 830–37
19. Metzker-Cotter E, Kletter Y, Avidor B et al: Long-term serological analysis and clinical follow-up of patients with cat scratch disease. *Clin Infect Dis*, 2003; 37: 1149–54
20. Feder HM Jr, Gerber MA, Luger SW et al: Persistence of serum antibodies to *Borrelia burgdorferi* in patients treated for Lyme disease. *Clin Infect Dis*, 1992; 15: 788–93
21. Hilton E, Tramontano A, DeVoti J et al: Temporal study of immunoglobulin M seroreactivity to *Borrelia burgdorferi* in patients treated for Lyme borreliosis. *J Clin Microbiol*, 1997; 35: 774–76
22. Fontenelle JP, Powell CC, Hill AE et al: Prevalence of serum antibodies against *Bartonella* species in the serum of cats with or without uveitis. *J Feline Med Surg*, 2008; 10: 41–46
23. Resto-Ruiz S, Burgess A, Anderson BE: The role of the host immune response in pathogenesis of *Bartonella henselae*. *DNA Cell Biol*, 2003; 22: 431–40
24. Taylor AW, Kaplan HJ: Ocular immune privilege in the year 2010: ocular immune privilege and uveitis. *Ocul Immunol Inflamm*, 2010; 18: 488–92
25. Streilein JW: Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. *J Leukoc Biol*, 2003; 74: 179–85
26. Taylor AW: Ocular immune privilege. *Eye*, 2009; 23: 1885–89
27. Gregory MS, ReppAC, Hawlina M et al: Membrane Fas ligand activates innate immunity and terminates ocular immune privilege. *J Immunol*, 2002; 169: 2727–35
28. Aboudharam G, Vu DL, Davoust B et al: Molecular detection of *Bartonella* spp. in the dental pulp of stray cats buried for a year. *Microb Pathog*, 2005; 38: 47–51
29. Aboudharam G, Fournier PE, Drancourt M et al: Molecular detection of *Bartonella quintana* DNA in the dental pulp of a homeless patient. *Eur J Clin Microbiol Infect Dis*, 2004; 23: 920–22
30. Goldstein DA, Mouritsen L, Friedlander S et al: Acute endogenous endophthalmitis due to *Bartonella henselae*. *Clin Infect Dis*, 2001; 33: 718–21