

Running title: *Bartonella henselae* in central nervous system

## ***Bartonella henselae* and *Borrelia burgdorferi* infections of central nervous system**

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**Key words:** mixed infection, central nervous system, *B. henselae*

To investigate the role of *B. henselae* in patients with symptoms suggesting neuroborreliosis, serum and cerebrospinal fluid samples were tested with serological and PCR methods. Among 17 examined patients, in 12 cases *B. burgdorferi* infections were detected, in 1 case *B. henselae* infection was ascertained and in two patients mixed *B. burgdorferi* and *B. henselae* infections were found. These results indicate that mixed infections should be taken into consideration in establishing diagnosis of neurological disorders. The conclusion needs further studies.

### **Introduction**

The aim of our study was to investigate the role of *B. henselae* in central nervous system disorders among patients with recognized neuroborreliosis. Since *B. henselae* and *B. burgdorferi* sensu lato shared the same tick vector – *Ixodes sp.* mixed infections should be considered in the differential for patients who present with neurological symptoms such as seen with borreliosis. Lyme disease is the most frequent tick-borne disease in the world. Neurologic abnormalities are prominent in this disease. During the first stage lymphocytic meningitis with cranial or peripheral nerve palsy and

radiculoneuritis accompanied by stiff neck and headache may occur. In some cases a wide spectrum of clinical manifestations including encephalopathy, polyneuropathy or encephalomyelitis in course of disease are present (1).

In 11% of patients with cat-scratch disease neurological symptoms are observed. They include encephalitis, cerebral arteritis and radiculitis (2). Mixed infection of the central nervous system by *B. burgdorferi sensu lato* and *B. henselae* were described (3).

## **Material and methods**

Seventeen paired serum and cerebrospinal fluid samples were studied. Materials from patients with various clinical symptoms suggesting neuroborreliosis were sent from various Polish hospitals. Among them, 9 patients had symptoms of meningitis (headache, nausea, vomiting and mild neck stiffness), two persons - sclerosis multiplex, two ones had a headache. In the remaining persons, in one case double vision with difficulty walking, in the second mediastinal lymphadenitis with pulmonary interstitial changes, in third depression with paresis of face muscles, in the last one bilateral facial nerve palsy were observed.

Specific antibodies to *B. burgdorferi sensu lato* in serum were tested with recombinant antigens p21 (OspC), p41i (inner part of flagellin) for IgM and p21, p41i, p18, p100 for IgG class in ELISA test (BIOMEDICA, Austria). Serum samples were also tested for the presence of *B. henselae* and *B. quintana* specific antibodies. Levels of serum IgM and IgG immunoglobulins were measured with indirect immunofluorescence test (MRL Diagnostic, USA).

DNA was extracted from the cerebro-spinal fluid samples with QIAamp tissue kit (QIAGEN, Germany) according to manufacturer recommendations. Extracted DNA was subjected to PCR amplification of the 16S rRNA for *B. burgdorferi sensu lato* (5) and 16S-23S rRNA fragment gene characteristic for *Bartonella* species (4).

Reactions were performed in a final volume of 50 µl containing: 10 mM Tris-HCl, 50 mM KCl, 3,5 mM MgCl<sub>2</sub>, 0.1% gelatin, 200 µM dNTPs, 50 pmol of each primer and 2 U of Taq DNA polymerase (Perkin-Elmer Cetus, USA). Aliquots of 5 µl of DNA template were added to each reaction mixture. PCR was run in a Mini Cycler apparatus (MJ Research, USA).

## Results

Fourteen of 17 examined patients had IgM or IgG antibodies to *B. burgdorferi*. Three patients with clinical symptoms suggesting neuroborreliosis were seronegative. The 16S rRNA *B. burgdorferi* gene fragment was not detected in CSF samples.

*B. henselae* infection was detected in three patients.

## Patient No. 1

She suffered from meningitis. DNA of *B. henselae* was found in CFS, he had antibodies to *B. burgdorferi*. Although specific antibodies to *B. henselae* were not found in his serum, it may be regarded as a mixed infection.

## Patient No. 2

Patient suffered also from meningitis. He had IgG antibodies to *B. henselae* and IgM antibodies to *B. burgdorferi* at borderline level. What might indicate a probable mixed infection.

## Patient No. 3

Patient complained of headache as a lasting symptom after meningitis due to neuroborreliosis. Two months before neuroborreliosis was recognized serologically and ceftriaxone regimen was applied. At the time of testing he was seronegative to *B. burgdorferi*, but antibodies to *B. henselae* were found in titer 64. It might suggest a single *B. henselae* infection.

## Conclusions

These results confirm that *B. henselae* can be an etiological agent of a central nervous system infection with symptoms resembling neuroborreliosis. Therefore *B. henselae* infection should be considered on the differential when evaluating patients suspected of having neuroborreliosis. Mixed infections of central nervous system with *B. burgdorferi* and *B. henselae* should be taken into consideration especially in patient group with incomplete resolution of Lyme borreliosis symptoms after treatment. The conclusion needs further investigation.

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