**Bartonella henselae** infection in British Columbia: Evidence for an endemic disease among humans

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**Abstract**: Human bartonellosis in North America is mainly associated with **Bartonella henselae**, and the availability of laboratory diagnostic tools has significantly heightened awareness of the spectrum of human disease that is caused by this bacterium. We detail herein examples of illness in a pediatric population which serve to confirm that **B. henselae**-associated disease exists in British Columbia. Seroprevalence studies among asymptomatic adults and among children with symptomatic respiratory illness of other causation demonstrated that 36.8% and 18.5% of sera, respectively, had IFA-IgG titres $\geq 1:256$. IFA-IgG titres did not vary significantly whether **B. henselae** ATCC 49793 or a local wild-type **B. henselae** isolate were used as substrate. An assessment of IgM response was consistent with the proposal that endemic seroprevalence is a function of past rather than recent exposure. Both clinical and serological studies are concordant in providing evidence that **B. henselae** is endemic in British Columbia.

**Key words**: **B. henselae**, cat scratch disease, serodiagnosis, seroprevalence.

**Résumé** : La bartonellose humaine en Amérique du Nord est principalement associée avec **Bartonella henselae** et la disponibilité d’outils diagnostiques de laboratoire a augmenté significativement la connaissance de l’éventail des maladies humaines qui sont causées par cette bactérie. Nous détaillons ici des exemples de maladies observées dans une population pédiatrique qui confirmé que les maladies associées à **B. henselae** existent en Colombie-Britannique. Des études de séroprévalence parmi des adultes asymptomatiques et parmi des enfants présentant des symptômes de maladies respiratoires dues à d’autres causes ont démontré que 36.8 % et 18.5 % des sérums avait respectivement des titres IFA-IgG $\geq 1:256$. Les titres IFA-IgG n’ont pas changé de manière significative que ce soit **B. henselae** ATCC 49793 ou un isolat local sauvage de **B. henselae** qui furent utilisés comme substrat. Une évaluation de la réponse IgM fut cohérente avec la proposition que la séroprévalence endémique est fonction d’une exposition passée plutôt que récente. Tant les études cliniques que sérologiques concordent à démontrer que **B. henselae** est endémique en Colombie-Britannique.

**Mots clés** : **B. henselae**, maladie des griffes du chat, sérodiagnostique, séroprévalence.

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**Introduction**

**Bartonella henselae** infection in humans is a complex and fascinating subject which has been explored widely, especially in the last decade (Fournier and Raoult 1998). Although **B. henselae** is recognized mainly as the etiological agent of cat scratch disease (Carithers 1985), an expanding array of clinical manifestations is becoming evident among both immunocompetent and immunocompromised patients. The circumstances surrounding **B. henselae** infection are now complicated by the recognition of other **Bartonella** spp. infections in humans (Anderson and Neuman 1997).

Currently, the major reservoir of **B. henselae** for human infection is felines. Thus, chronically infected animals may harbour the bacterium, which is putatively transmitted to humans by cat scratches, possibly even by fleas. This notion is supported by seroepidemiological studies from the United States (Jameson et al. 1995), which have demonstrated a strong association between feline seropositivity and a high frequency of cat flea infestation. These studies have also demonstrated a relatively high seroprevalence of **B. henselae** antibody in cats from British Columbia, the frequency having been in the 40%–50% range for Vancouver Island and the lower mainland.

From a clinical perspective, suspicion of cat scratch disease has been dependent on the use of a variety of clinical criteria in association with a history of cat exposure (Margileth 1968). Whereas these criteria may well define an illness which is now highly likely to be associated with **B. henselae**, the actual availability of antigen for the purposes of serodiagnosis (Regnery et al. 1992) has facilitated a greater
understanding of the extensive disease spectrum. Anecdotes of *B. henselae*-associated disease continue to emerge in the pediatric community.

Given the combinations of feline seroprevalence and clinically compatible disease, we initiated a series of studies which were designed to further our understanding of infection in this west coast province of British Columbia. We illustrate some of the suspect clinical disease through a portrayal in clinical vignettes, and detail serological studies that examine the background for exposure to the etiological agent.

**Materials and methods**

**Patient vignettes**

**Patient 1**

A seven-year-old male was referred to British Columbia’s Children’s Hospital from Vancouver Island with persistent lymphadenopathy and thrombocytopenia. The child had been well until two weeks prior to admission when he complained of discomfort in the left groin. Physical examination revealed mild tenderness of the left inguinal area and prominent lymphadenopathy. He was febrile. There were numerous skin lesions on both lower legs which were described as flea bites, and a history of exposure to many cats was elicited. The white blood cell count was 6.0 × 10^9/L, hemoglobin 125 g/L, and platelet count of 191 × 10^9/L. Due to the possibility of infected flea bites, he was given a beta-lactam antibiotic. The inguinal lymphadenopathy regressed slightly, but bilateral axillary adenopathy was noted. A review of blood indices twelve days later revealed a platelet count of 18 × 10^9/L. There was no evidence of bleeding diatheses. When seen on referral, the platelet count had returned to 75 × 10^9/L and no major investigations were undertaken. Indirect immunofluorescence (IFA)-IgG titre for *B. henselae* antibody was 1:8192. This vignette is an example of *B. henselae*-associated lymphadenopathy and immune thrombocytopenia (Billo and Wolfe 1960).

**Patient 2**

A ten-year-old male from the greater Vancouver area was admitted to hospital with complaints relating to sudden loss of vision. Six weeks prior to admission, the child had complained of bitemporal throbbing headaches, occurring almost every two days. Three weeks prior, the child had complained of blurred vision in the left eye. The visual symptoms persisted; the child was able to describe a defect in the centre of the left visual field. The boy had been previously well but he had been investigated for persistent lymphadenopathy (systemic but especially cervical) over the previous year; no cause was determined, although cat scratch disease was not ruled out by laboratory methods. He enjoyed playing with a cluster of household cats. Physical examination showed the patient to be febrile. There was bilateral cervical adenopathy and right inguinal adenopathy. There were no skin lesions. Ophthalmological examination of the left eye revealed retinal exudates, and a severe reduction of visual acuity. Visual evoked responses revealed a delay in the optic nerve conduction. Neurological examination was otherwise unremarkable and there was no pleocytosis of the cerebrospinal fluid. Serological assessment for Epstein-Barr virus, cytomegalovirus, and *Toxoplasma gondii* were not suggestive of infection but the IFA-IgG titre for *B. henselae* was 1:2048. This vignette exemplifies *B. henselae*-associated retinitis (Reed et al. 1998).

**Patient 3**

A five-year-old boy from the Fraser Valley, near Vancouver, presented with complaints of fever and neck pain. Four weeks earlier, he had a tooth extraction as a consequence of investigation of fever and headache, and the dental procedure was followed by oral antibiotic therapy. Eight days after the antibiotic use was completed, fever recurred and another tooth was extracted and again, oral antibiotics were prescribed. Despite these interventions, the child experienced fever, chills, headache, and weight loss. The family had numerous pets and, in particular, the child had been scratched on occasions by a young family cat. Physical examination confirmed fever. A painful torticollis was evident and bilateral tender cervical adenopathy was noted. The liver was enlarged and bilateral inguinal adenopathy was evident. An abdominal ultrasound demonstrated enlarged periportal lymph nodes and hypoechoic foci in the spleen. Tomography of the abdomen confirmed the presence of multifocal lesions within the spleen and as well multiple lesions within the liver. Tomography of the abdomen confirmed the presence of multifocal lesions within the spleen and as well multiple lesions within the liver. A paravertebral lesion at the T10 level was also noted. A bone scan and other radiological investigations were suggestive of a soft tissue mass. Biopsy of the liver lesions demonstrated a necrotic central focus which was surrounded by ill-formed aggregates of histiocytes and other inflammatory cells. Bacterial culture of blood and of the hepatic lesion did not yield any microorganism. Microbiological investigation for tuberculosis, Epstein-Barr virus, cytomegalovirus, toxoplasmosis, and brucellosis were not suggestive of infection. An IFA-IgG titre for *B. henselae* was 1:8192. The child’s fever resolved slowly over a three-week period as did the neck pain. Appetite returned and the child gained weight. Antibiotics had not been particularly focused towards the treatment of cat scratch disease. The child’s illness was consistent with *B. henselae*-associated lymphadenopathy and peliosis hepatitis and other manifestations of systemic cat scratch disease (Arisoy et al. 1999).

In addition to the above vignettes, our pediatric centre has also cared for individuals who have suffered from *B. henselae*-associated neurological illnesses and bacillary angiomatosis.

**Patient sera**

In order to assess *B. henselae* seroprevalence, human sera were obtained from two main sources. Sera were obtained from 142 asymptomatic adult volunteers, ages 18–67, who donated blood samples for transfusion purposes. These samples were obtained from both sexes through seven donation centres in British Columbia (Clearbrook, Kamloops, Prince George, Sechelt, Vernon, Victoria, and Williams Lake; see Fig. 1).

A second set of sera were obtained from 54 children with a variety of confirmed respiratory infections; these represent a subset of children studied previously for other purposes and the majority had viral infections (Cimolai et al. 1995). Definitive diagnoses of respiratory infection or other pulmonary illnesses were established by standard diagnostic means. The ages ranged from several months to 16 years (mean 5.5 years). Rather than choose an asymptomatic pediatric population for serum donation, we chose this particular group with active infection or other disease which might be considered to have raised background serological activity, perhaps polyclonal, non-specific, or cross-reactive. Children with respiratory illnesses were chosen because the cat scratch disease spectrum of illnesses does not include respiratory infection. These respiratory illnesses included respiratory syncytial virus infection, influenza, parainfluenza, pertussis, rhinovirus infection, adenovirus infection, mononucleosis, bacterial pneumonias, among others.

**Bacterial isolates**

*Bartonella henselae* ATCC 49793 (Oklahoma) was obtained from the American Type Culture Collection (Manassas, Va.). The passage frequency of this bacterium is unknown. *Bartonella henselae* 129 is
a low passage wild-type isolate that was obtained locally from a patient with lymphadenopathy (Vancouver Island, B.C.).

Immunofluorescence serology

The IFA-IgG assay was performed with agar-cultivated *B. henselae* (Yousif et al. 1996). Bacterium was harvested with cotton applicators and adjusted to a standardized suspension in phosphate-buffered saline (PBS; pH 7.2). Aliquots were acetone-fixed on glass slides bearing pre-formed wells. Sera were diluted with PBS in 2-fold dilutions starting at 1:32. Ten microlitres of diluted serum were incubated with individual wells for forty-five minutes. After washing with PBS, 10 μL of fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (diluted 1:100 in PBS with Evan’s Blue) was added to each well and again incubated for forty-five minutes. Slides were observed immediately under ultraviolet light as maximized for FITC. Positive criteria for IFA varied from a definite but dim yellow-green staining of all bacterial cells to brilliant yellow-green staining of bacteria. The term ‘negative’ was reserved for no staining or barely visible yellow-green staining of all cells, or dim staining of ≤50% of cells. Positive and negative controls were included in each batch of slides, and these controls included an assessment for a low and high titre.

Observer concordance was assessed by blinded comparisons for a large number of observations. IFA-IgG as detailed herein was also compared with a cell-associated antigen substrate-based IFA (Bion, Park Ridge, Ill.; same protocol apart from substrate). For agar-grown substrate, a comparison was also made between the ATCC strain and the low passage wild-type form. Studies were performed to compare anti-IgG and anti-IgG/IgA/IgM conjugates (Jackson ImmunoResearch Lab., Inc. West Grove, Pa.; same protocol), and then also anti-IgM.

Results

Concordance for duplicate blinded readings of IFA-IgG serology using either bacterial substrate was evident; 92.5% of duplicate readings were either equivalent or varied by one dilution; 7.3% and 0.3% of duplicate readings were divergent by four and eight dilutions, respectively.

Significant differences in titres were not strain dependent. A total of 82.4% of sera were found to have equivalent titres or two-fold variation when IFA-IgG on substrates 129 and ATCC 49793 were compared. Only 7.4% and 10.3% showed greater than two-fold higher titres for ATCC 49793 and 129, respectively ($P > 0.1$).

In comparing cell-associated (Bion) and cell-free antigen for IFA-IgG, 78.6% of readings were either equivalent or had two-fold differences. The remainder of the differences were of four-fold variation. Two-thirds of the latter variations were higher for cell-associated antigen ($P > 0.1$).

Among sera from pediatric patients with respiratory illnesses, 18.5% had IFA-IgG titres ≥ 1:256. None of the sera had titres > 1:512. When stratified for school- (> 5 years) and pre-school- (≤5 years) ages, there was no significant difference in the number of sera that were reactive ≥ 1:256 (20.7% and 16.0%, respectively).

Sera from asymptomatic adult donors were more likely than pediatric sera to yield IFA-IgG titres ≥ 1:256 (36.8% overall). Two of these latter sera had titres ≥ 1:1024. When analyzed for source by gender, 45.3% and 26.4% of sera from males and females respectively had titres ≥ 1:256. A slightly greater frequency of seroreactivity at the 1:256 cut-off was observed for ages > 45 years (40.0%) versus those ≤ 45 years (34.7%). Numbers of sera from any individual centre were relatively small ($n = 19–21/centre$) and frequencies of sera ≥ 1:256 ranged from 19.0%–52.4%. The latter included Clearbrook 47.4%, Kamloops 52.4%, Prince George 47.6%, Sechelt 20.0%, Vernon 19.0%, Victoria 45.0%, and Williams Lake 30.0%.

When human antibody was detected by the use of anti-IgG/IgA/IgM conjugate, a minor incremental gain in titre was apparent. Increments in titre for either of the IgG/IgA/IgM or IgG conjugates in contrast to the other for given sera were 48.7% and 24.3% overall, respectively. Most of these differences were of a two-fold variation. For differences of $≥ 4$

**Table 1. Distribution of IFA-IgM titres for sera with IFA-IgG titres above or below the 1:128 dilution.**

<table>
<thead>
<tr>
<th>IgM titre</th>
<th>≤1:128</th>
<th>≥1:128</th>
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<tbody>
<tr>
<td>1:32</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>1:64</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>&gt;1:64</td>
<td>1</td>
<td>0</td>
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fold, 18.9% and 10.8% of sera had greater titres for anti-IgG/IgA/IgM or IgG conjugates, respectively (P > 0.1).

As a marker for acute infection, sera were examined for IgM (Table 1). For sera with IFA-IgG titres <1:128 or ≥ 1:128, there was a similar distribution of IgM titres (P > 0.1). Further analysis of IgM titre distribution as a function of age or substrate strain again did not show significant variation. Three of these sera were from pediatric patients with EBV-related infectious mononucleosis, and titres were ≤1:32 in contrast to an association of EBV infection with elevated IgM as previously detailed (Zbiedtn et al. 1998).

Discussion

Clinical disease compatible with cat scratch disease is recognized in this pacific northwest region of North America. Not only are the classical manifestations of cat scratch disease apparent, but so too are the manifestations of invasive disease. The availability of diagnostic tools has confirmed these associations in the last decade, but has also served to increase the spectrum of recognized B. henselae-associated illnesses. Relatively speaking, the study of human bartonellosis is an evolving science.

Studies of endemic seroprevalence indicate that exposure to B. henselae is common. This background, however, is consistent with the rate of endemic seroprevalence among felines as documented previously (Jameson et al. 1995). Although similar studies from other Canadian provinces are lacking, there is the potential for specific geographic areas to be implicated as having higher frequencies of infected cats, and hence humans. For example, geographic variation of feline seroprevalence is well documented in the United States (Jameson et al. 1995), and it is possible that geographic location, and secondarily climate, may have some impact on cat flea distribution.

The availability of serodiagnostic assays for B. henselae-related illness was clearly a major breakthrough in this field. It is probable that similar lessons will be relearned in relationship to the actual application of these diagnostic tests. Firstly, IFA serology inherently imposes an interpretive dilemma. Although performance in a common and large laboratory will diminish such variability, the dissemination to other centres, which are perhaps more closely affiliated to their association with mammalian disease is emerging (Ellis et al. 1999). In recognition of the above, it would seem prudent to define confirmatory diagnostic markers. The definition of infection by culture positivity or genetic detection (La Scola and Raoult 1999; Sander et al. 1999) is much less likely to be denied, but either mode requires the availability of infected tissue, the latter which is not commonly acquired. Given the ease of collection for blood samples, it would seem best to create serodiagnostic confirmatory assays. Until such a confirmatory assay becomes available, it would be prudent to carefully view diagnostic Bartonella serology and to ensure that positive serology is considered in the context of compatible clinical symptoms much in the same way that has been advocated for Lyme disease (Magnarelli 1989).

As learned from endeavors relating to HIV, hepatitis C, and Lyme serodiagnostics, the development of diagnostic immunoblotting profiles may serve this purpose. Freeland et al. (1999) and McGill et al. (1998) have recently provided some pioneering data in the latter regard. Additional studies will be required to validate any given diagnostic immunoblotting pattern, and a more thorough understanding of cross-reactive antigens, whether of other Bartonella or other bacterial origin, will be required (La Scola and Raoult 1996). Until such studies are available, it remains possible that reactivity in Bartonella IFA could be a function of cross-reactive antigens just as well as true anti-Bartonella antibody. A greater complexity of Bartonella species and their association with mammalian disease is emerging (Ellis et al. 1999).

References


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