Development of an Immunoglobulin M Capture-Based Enzyme-Linked Imunosorbent Assay for Diagnosis of Acute Infections with *Bartonella henselae* 

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We describe the development of an immunoglobulin M-specific enzyme-linked immunosorbent assay for the detection of an early antibody response to *Bartonella henselae*, the causative agent of cat scratch disease, bacillary angiomatosis, and endocarditis. This assay discriminates between *B. henselae*-positive and -negative patient samples with sensitivity and specificity values of 100% and 97.1%, respectively.

*Bartonella henselae* is an emerging pathogen of epidemiological and pathological concern. The wide spectrum of diseases caused by this gram-negative bacterium appears to be linked to the state of the host immune system (11). For example, infection with *B. henselae* causes cat scratch disease (CSD) in immunocompetent individuals (14); angio proliferative illnesses, including bacillary angiomatosis, in immunocompromised patients (13); and endocarditis in patients with preexisting heart valve lesions (11).

Most common in children and young adults, CSD is widely accepted as the result of a bite or scratch of a cat infested with cat fleas (*Ctenocephalides felis*) (10, 18) harboring this pathogen. *Bartonella henselae* has also been detected in *Ixodes scapularis* ticks collected in New Jersey (1), supporting the notion that ticks may play a role in the transmission of CSD. Typical disease sequelae include regional lymphadenopathy, low-grade fever, and malaise, with cutaneous lesions occasionally being detected at the site of the bite or scratch (15). Approximately 24,000 new cases of CSD are reported each year, with up to 10% of cases requiring hospitalization resulting from atypical CSD manifesting as neuroretinitis, granulomatous hepatitis, and osteomyelitis (17). Less-well-defined disease conditions possibly associated with *B. henselae* bacteremia are a diverse number of neurological illnesses, such as encephalitis and fatal meningitis (9). In a recent study, *Bartonella henselae* and *B. vinsonii* subsp. *berkhoffii* were detected in six immunocompetent individuals who presented with seizures, ataxia, and memory loss (6). The high rates of carriage by domestic cats, combined with the close proximity in which humans and cats live, increase the likelihood of human exposure to *B. henselae*. In a recent study, an immunoglobulin G (IgG) seroprevalence rate of 53.3% was reported among cat owners in Poland (8).

Current diagnostic tests for infection with *B. henselae* include PCR and serology-based assays, such as immunofluorescence assays (IFAs). IFAs employ whole bacterial cells as the antigen and are currently the most widely used diagnostic tool. However, IFAs are highly reader dependent, generally lack overall specificity, and are not quantitative.

The predicted high seroprevalence to *Bartonella henselae* within the general population as a result of the high rates of cat ownership makes the availability of an assay capable of distinguishing between current or acute infections and prior seroconversion necessary. The availability of a test for the detection of an early response to *B. henselae* antigens would therefore greatly facilitate the better diagnosis and treatment of infected patients.

We recently reported on the use of a recombinant *B. henselae* 17-kDa antigen (the r17-kDa antigen), first described by Anderson et al. (3), for the development of an IgG enzyme-linked immunosorbent assay (ELISA) with a sensitivity and a specificity of 71.1% and 93.0%, respectively (13). In that report, recombinant antigen was coated onto ELISA plates and reacted with patient sera. We have now further characterized this antigen and optimized the conditions for its use in an IgM capture-based ELISA for the detection of an IgM antibody response during the acute stages of infection.

As previously reported, we expressed the r17-kDa protein in a prokaryotic expression system as a histidine-tagged fusion protein which was purified by use of a nickel-agarose column (13). For the IgM capture assay, purified r17-kDa protein (1 mg/ml) was biotinylated with a Sulfo-NHS kit (Pierce, Rockford, IL). MaxiSorp 96-well plates (Nunc, Rochester, NY) were coated with anti-human IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD) at a concentration of 1 µg/ml in coating buffer (0.015 M Na2CO3, 0.035 M NaHCO3 [pH 9.6]) and incubated overnight at 4°C. The plates were washed with phosphate-buffered saline–Tween 20 and blocked with 1% bovine serum albumin (BSA) at room temperature. IgM-positive and -negative patient sera, as initially determined by testing done by Focus Diagnostics and Specialty Laboratory, were confirmed as positive or negative for *B. henselae* in-house by using an IgM IFA kit, according to the manufacturer's (Focus Diagnostics, Cypress, CA) recommended protocol. The sera were diluted 1:100 in 1% BSA with 0.05% Tween 20, prior
Further studies will need to be performed to confirm the coinfection of this particular sample with *B. burgdorferi* and *B. microti* and to determine what effects, if any, this might have on the specificity capabilities of our IgM ELISA. Finally, we acknowledge that the remarkably high sensitivity and specificity values of this assay are unusual for an IgM response. We believe that these values, particularly the specificity value, can be explained at least in part by the antigen used in this study. As a component of the type IV secretion system, the 17-kDa antigen likely plays an important role in the early stages of infection. This protein is a homolog of the VirB5 family, and there is increasing evidence suggesting that these proteins function as specialized adhesins (4). Several key structural and functional studies strongly suggest that VirB5 proteins are likely involved in host recognition by the type IV secretion system pilus, most likely through protein-protein interactions involving amino acids within the C terminus (2, 4, 19). Consistent with a role in the early stages of infection of host cells, this antigen has been shown to be expressed at minimal levels by *B. henselae* organisms cultivated on cell-free laboratory medium but is induced to high levels in the presence of human endothelial cells (16). We believe that our data are consistent with those from studies implicating VirB5 proteins in protein-protein interactions involved in host cell recognition (2, 4, 19). In light of the findings of those studies as well as our own observations (unpublished data) showing the importance of the C terminus in host recognition, the very high specificity obtained with the 17-kDa antigen is perhaps not surprising after all. It is reasonable to speculate that the important role played by the 17-kDa antigen in the type IV secretion system contributes in large part to why it is one of only a few antigens, with BadA being another, shown to be beneficial as a serological marker for *B. henselae* infection.

In conclusion, this is the first demonstration of an ELISA for the laboratory diagnosis of early infection with *Bartonella henselae*. The high sensitivity and the high specificity achievable with this assay make it a diagnostic tool capable of distinguishing between patients with active or very recent infection from those with past exposure to this organism.

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