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Editorial Response: Are all *Bartonella henselae* Strains Created Equal?

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Of the five *Bartonella* species associated with disease in humans, *B. henselae* causes the widest spectrum of pathology, including disease with granulomatous features (cat-scratch disease), vascular proliferative features (bacillary angiomatosis-peliosis, BA-BP), and a predominantly intravascular focus (bacteremia-endocarditis). In the wake of the initial detection of *B. henselae* and its laboratory propagation in 1990 [1, 2], investigators have learned that *B. henselae* is distributed worldwide; that cats are reservoirs for the organism; that fleas are vectors for transmission among cats [3, 4]; and that *B. henselae,* like other *Bartonella* species, establishes an intimate and persistent relationship within the bloodstream of its hosts. Yet, we understand little about the diversity and population structure of *B. henselae* and the genetic basis for its pathogenicity. In this regard, the key questions are whether a small number of clonal types account for a disproportionate amount of disease, whether some clonal types are more likely than others to cause a particular type of pathology, whether there is a restricted geographic distribution of specific clonal types, and what is the possible genetic basis of type-specific differences in virulence.

See the article by Arvand et al. on pages 1296–9.

Practical problems often hinder the solution of important theoretical questions. In the case of *B. henselae,* isolation of the organism directly from the host is difficult [5], thereby limiting the number and diversity of cultivated strains available for study. While amplification of the 16S rRNA gene and other phylogenetically reliable sequences directly from clinical specimens offers an alternative to laboratory cultivation, there is no standardized, well-accepted genotypic method for identifying and defining *B. henselae* clonal types. At the same time, cultivation of strains permits correlation of in vitro phenotype with genotype. Thus, the work described by Arvand and colleagues [6] represents a valuable contribution, i.e., the first reported recovery of a clinical *B. henselae* isolate from a vascular proliferative lesion in a European patient.

What do we know about this *B. henselae* isolate and its relation to others? Arvand et al. relied on pulsed-field gel electrophoresis (PFGE) of endonuclease-restricted chromosomal DNA to establish a "match" between this German isolate, Berlin-1, and the Houston-1 type strain, originally isolated from the blood of a febrile, HIV-infected individual in Texas [7]. PFGE chromosomal profiles distinguished between these two strains and *B. henselae* ATCC 49793, originally isolated from the blood of a febrile patient with AIDS in Oklahoma [2], as well as a German feline strain; however, other *B. henselae* strains were not examined in parallel. In fact, PFGE patterns have been determined for only a small number of *B. henselae* strains [8]. Thus, it is difficult to compare Berlin-1 with other isolates from around the world. Recently, Sander and colleagues [9] isolated 13 *B. henselae* strains from 100 domestic cats in Freiburg, Germany [9]; it would be interesting to include these strains in this analysis.

Two other genotypic methods have been proposed for *B. henselae* strain discrimination: restriction fragment length polymorphism (RFLP) analysis of the intergenic transcribed spacer (ITS) region within the rRNA operon [10], and repetitive element PCR [11]. Each method has revealed moderate strain diversity among the small numbers examined so far [12, 13], in contrast with even more limited diversity among *Bartonella quintana* strains.

As *B. henselae* strain typing databases expand and become more standardized, investigators will be able to compare the diversity of strains within cat reservoirs with the diversity of strains that cause various human clinical syndromes. Early studies have revealed a greater number of ITS RFLP strain types in the blood of HIV-infected bacteremic patients than in the cat-scratch-disease lesions of immunocompetent individuals [10, 12], but the role of confounding variables has not been adequately assessed. The possibility that particular *B. henselae* clones or strains are associated with specific clinical syndromes still cannot be excluded. The work of Arvand and colleagues and other groups will eventually provide data with which human-associated and cat-associated isolates can be compared [9, 14, 15]. It is expected that greater strain diversity will be found within the natural reservoir (cats) than within incidental hosts (humans). In most instances, the strains associated with human disease will also be found in the cats with which these patients had direct contact [13].

The geographic distribution of strains remains unclear. Incomplete data indicate that *B. henselae* is endemic in the United States, Europe (France, Germany, Holland, and Denmark), Africa (Zimbabwe and South Africa), Australia, and Japan. These diverse sites provide a rich potential source of genetic data. While repetitive element PCR requires the availability of purified bacterial isolates, in theory ITS RFLP could be performed on cloned genomic fragments amplified directly from clinical specimens with broad-range primers directed against conserved flanking sequences.

Where should these investigations lead? First, standardized, reliable, and discriminatory strain typing methods are needed for *B. henselae.* In general, such methods should provide quantifiable measures of evolutionary distance between any two

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strains, relying on genetically stable markers of genomic ancestry. Primary sequence is a useful, quantifiable character. Although rDNA is a phylogenetically reliable marker, it often fails to provide sufficient resolving power at the strain level. Other genetic loci such as the rRNA operon ITS will need to be evaluated further.

Second, many of the most important virulence-associated genes in microbial pathogens are promiscuous and evolutionarily "unstable;" they are shared among strains by means of plasmids, transposable elements, and phages. These genes need to be targeted separately. However, few virulence-associated genes of *B. henselae* have been identified. It would be expected that the products of such genes subvert host defenses mediated by skin dendritic cells and soluble bloodborne factors, facilitate penetration of tissue stroma and attachment to erythrocytes, elicit angiogenic host cytokines and growth factors, and recognize environmental signals within humans, cats, and fleas. Powerful strategies to identify these genes take advantage of their selective expression within a relevant host environment [16].

In addition, it is now known that many of these genes in general are physically clustered on a plasmid or within a chromosomal "pathogenicity island" and are acquired in "quantum evolutionary leaps" [17, 18]. Might the distribution and composition of pathogenicity islands among extant *B. henselae* clonal populations explain differing associated disease manifestations? Although there is no evidence to support this speculation, the methods that have been used to identify *B. henselae* strains are insensitive to pathogenicity island or virulence gene polymorphisms. With continued efforts to identify disease-associated genes by means of genetic and genomic approaches, the population and genomic structure of *B. henselae* will become more clear. With full genome sequencing for *B. henselae* under way, it may not be long before *B. henselae* isolates and the corresponding host responses are both characterized and compared by using comprehensive DNA microarrays in a semiautomated format.

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