

***Bartonella henselae* Biofilm Detected on Catheter of Patient with Persistent Bartonellosis**

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Bartonella spp. can induce long-lasting bacteremia in mammals (1). *Bartonella* spp. are causative agents of cat-scratch disease, endocarditis, bacillary angiomatosis, bacillary peliosis, trench fever, neurocognitive dysfunction and regional pain syndrome (2). Chronic *Bartonella* spp. infection and subsequent manifestations of persistent infection may be the result of the bacteria growing in biofilms. In humans, at least four *Bartonella* species have been associated with infectious endocarditis, a biofilm-associated infectious disease (3). Biofilms are sessile communities of interacting unicellular organisms that adhere to a surface and to each other and create unique, complex structures, covered with a protective layer of extracellular polymeric substances (EPS). Biofilms and their inherent resistance to antimicrobial agents are often key players in many persistent and chronic bacterial infections (4, 5). We acquired skin biopsies from 5 patients with blood PCR-positive *B. henselae*. 60-100 micron-thick sections were immunostained with antibodies to *B. henselae*. Stained sections were imaged using both single- and multi-photon imaging to capture epifluorescence emission and second harmonic generation signal of non-stained collagen I and II (Fig 1). The crystalline triple-helix of native fibrillar collagen (I, II, III and V) generates a strong second harmonic generation (SHG) signal (6). In patients with long-standing bartonellosis, clusters of immuno-reactive *B. henselae* were found intimately associated with fibrillar dermal collagen (Fig 2). Additionally, we found a *Bartonella* spp. biofilm on a peripheral intravenous catheter line removed from a patient with persistent Bartonella (Fig 3). Further, *B. henselae*, cultured 14 or 21 days, on collagen I and II-coated coverslips revealed a *B. henselae* biofilm matrix (Fig 4). Identification and characterization of *Bartonella* spp. biofilms *in vivo* and *in situ* will lead to a better understanding of persistent Bartonellosis and provide direction in treatment modalities.

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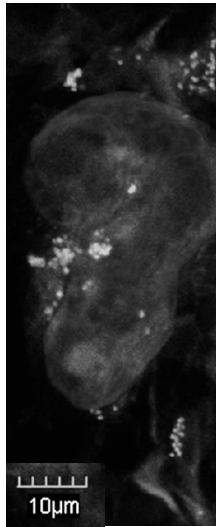


Figure 1: *B. henselae* immuno-reactivity associated with micro-vasculature in dermal layer of non-lesional skin of patient with PCR blood-positive for *B. henselae*, *B. vinsonii*, and *B. spp.* Four-mm punch biopsy from non-lesional calf skin was processed, anti-collagen type IV plus anti-goat secondary antibody conjugated to Cy3 (aqua) and anti-*B. henselae* made in mouse plus secondary antibody conjugated to Cy5.

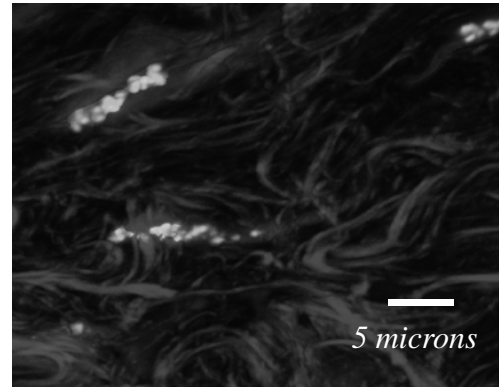


Figure 2: *B.henselae* intimately associated with collagen fibrils of dermis in non-lesional skin of patient with PCR blood-positive for *B. henselae*, *B. vinsonii*, and *B. spp.* Four-mm punch biopsy from non-lesional calf skin was processed, labeled with anti-*B. henselae* made in mouse plus secondary antibody conjugated to Cy5. Imaged with two-photon laser scanning microscopy, 60X oil objective and 5Z electronic zoom. Second harmonic generation signal produces robust image of non-stained collagen type I and II (magenta) and two-photon epifluorescent signal of Cy5 is also captured. (Zeiss multi-photon scope images courtesy of Lens Cezar, PhD, UNICAMP, Campinas, Brazil.)

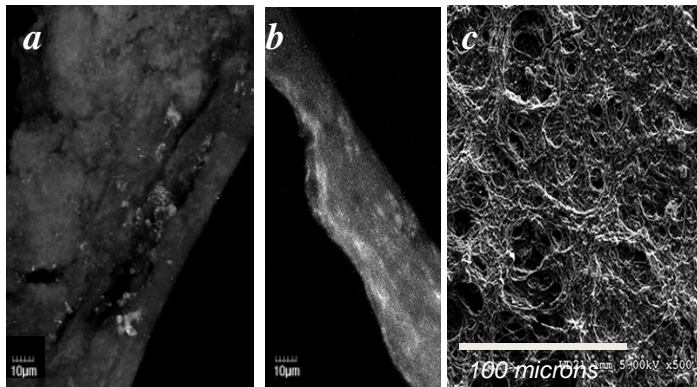


Figure 3. PIC line from patient with persistent Bartonella harbors *Bartonella* spp. biofilm. a: *B. henselae*-ir of pic line. b: Film-Tracer Sypro-Ruby stains PIC biofilm, c: SEM of same PIC line reveals meshwork of biofilm.

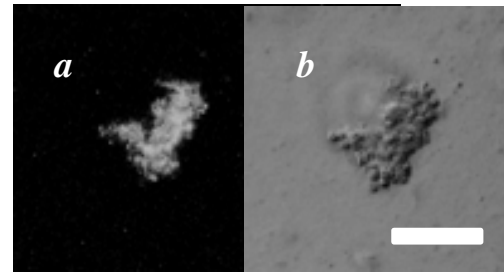


Figure 4: *B. henselae* immunoreactivity of bacteria stain in *B. henselae* cultured cells. *B. henselae* was cultured for 7D, lightly fixed, and stained with combinations of anti-*B. henselae* made in mouse and anti-mouse conjugated to Cy5. All samples imaged using confocal microscopy with PlanApo 60x (oil, NA 1.42). Scale bars = 10 micrometers. a) Immunoreactive *B. henselae* made in mouse, anti-mouse conjugated to Cy5 and b) differential interference contrast.