

## Immunofluorescent Detection of Intraerythrocytic *Bartonella henselae* in Naturally Infected Cats

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Received 21 March 2001/Returned for modification 18 April 2001/Accepted 8 May 2001

**To determine the presence of *Bartonella henselae* bacteremia in six cats, we compared isolation using blood culture with direct immunofluorescence on blood smears. Three cats that were positive by blood culture were also positive by direct immunofluorescence, and laser confocal microscopy confirmed the intraerythrocytic location of *B. henselae*.**

*Bartonella henselae* is an agent of cat scratch disease and can be responsible for other human diseases such as bacillary angiomatosis, peliosis hepatitis, and endocarditis (12). The natural host of *B. henselae* is the domestic cat, and its vector is the cat flea. The organism can be isolated from the blood of 4 to 70% of apparently healthy cats depending on the cat population concerned and its geographic location (2, 5, 7). In infected cats, the target cells of *B. henselae* seem to be the erythrocytes (8, 14), although this has been refuted in a recent study (4). The detection of *B. henselae* in feline erythrocytes using immunological techniques has not been described, and the sensitivity of such methods is thus unknown. In our study we compared the use of blood cultures and a monoclonal antibody (MAb) against *B. henselae* in direct immunofluorescence tests to detect the organism in cat blood. Also, we used laser confocal microscopy to determine the position of *B. henselae* in blood smears that were positive by immunofluorescence.

The study was performed in June 2000 during an exercise to eradicate stray cats from a French military base. Six cats were captured using oral Dexetanol (Instituto DEX, Seville, Spain) in fresh meat according to the manufacturer's instructions. Once sedated, the cats were anesthetized with Imalgène (Meriel, Lyon, France), and 3 ml of blood was collected aseptically using intracardiac puncture. Cats were euthanized by intracardiac inoculation of Dolethal (Vétoquinol SA, Lure, France) according to the manufacturer's directions. Thin blood smears were made from the fresh blood and stored at room temperature until the direct immunofluorescence assays were carried out. The remaining fresh blood was stored at  $-20^{\circ}\text{C}$  until it was thawed at room temperature and 1-ml aliquots were inoculated onto Columbia 5% sheep blood agar plates (BioMerieux, Marcy l'Etoile, France). The plates were placed in polyethylene bags to prevent desiccation and incubated at  $37^{\circ}\text{C}$  under 5%  $\text{CO}_2$  (Genbag  $\text{CO}_2$  system; BioMerieux). For 3 months the plates were examined weekly for evidence of bacterial growth. When *Bartonella*-like colonies were observed, they were characterized using the sequences of their 16S rRNA genes and the BLAST 2.0 program (National Center for Bio-

technology Information) as described previously (6). The blood smears were examined for evidence of *B. henselae* infection using two specific MAbs prepared in our laboratory: the first (B3D4) was *Bartonella* genus specific (11) and the second (H2A10) (10) was *B. henselae* specific using standard immunofluorescence techniques (9). MAb H2A10 was of the immunoglobulin G2a (IgG2a) subclass and reacted with a 43-kDa epitope present only in *B. henselae* strains. This MAb does not cross-react with other *Bartonella* species, including *B. quintana*, *B. elizabethae*, *B. clarridgeiae*, *B. bacilliformis*, *B. taylori*, *B. doshiae*, and *B. vinsonii*, or with any of the bacteria already tested for MAb B3D4 (11).

Positive smears were examined under a laser scanning confocal microscope with an excitation wavelength of 488 nm and an emission wavelength of 617 nm to determine the location of the organisms in the erythrocytes. Sections of the erythrocytes were made in increments of 0.5  $\mu\text{m}$  as previously described (13).

*Bartonella* organisms at  $10^5$  CFU/ml were isolated from the blood of three of six (50%) cats, and their 16S rRNA gene sequences were identical to that of *B. henselae* URLLY8 strain Marseille (3). The blood smears made from these cats had positive immunofluorescence when assayed either with MAb B3D4, specific for the *Bartonella* genus, or with MAb H2A10, specific to *B. henselae* strains (Fig. 1). Three to 8% (mean, 5%) of the erythrocytes were found to be infected, with most of these cells containing only one bacterium (average, 1.08 bacteria/erythrocyte). These results are consistent with previous reports that the prevalence of bacteremia in cats is 22 to 59% (5) and that the percentage of infected red blood cells varies from 1 to 20% (1, 2, 7). The average red cell count in the cat is  $5 \times 10^9/\text{ml}$ , and with the 5% level of infection we detected, there were around  $2.5 \times 10^8$  infected erythrocytes per milliliter of blood. In our blood cultures, however, we detected only  $10^5$  CFU/ml, and we conclude that a large number of bacteria were killed during the processing of the blood and/or its storage at  $-20^{\circ}\text{C}$  and thus that 1 in 2,500 bacteria was cultivable.

By laser scanning confocal microscopy *B. henselae* was seen within the erythrocytes, particularly near the cell membrane but also in the center of the cell (Fig. 1). These findings using immunological techniques confirm the intraerythrocytic location of *B. henselae* in naturally infected cats. Also, our findings show that direct fluorescence with a specific MAb is a sensitive,

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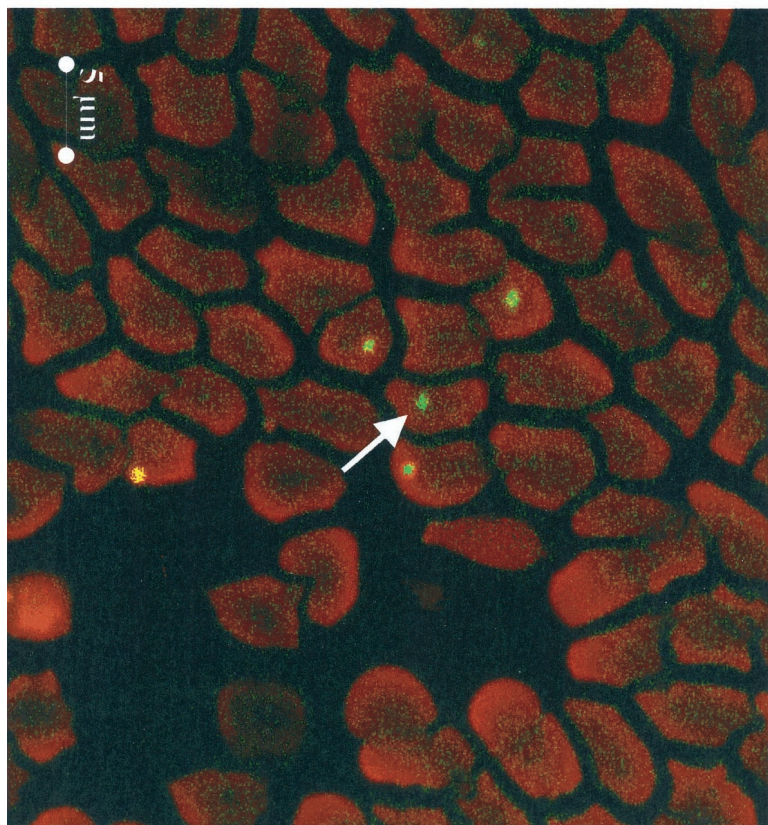
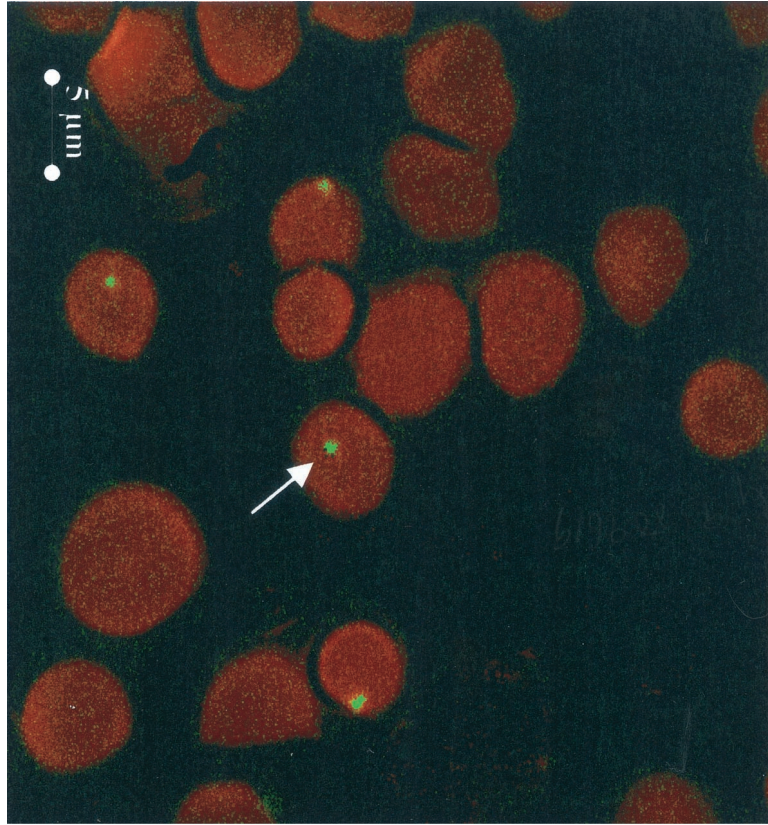


FIG. 1. Digital sections of feline red blood cells infected with *B. henselae* as viewed by laser scanning confocal microscopy. Sections were taken in 0.5- $\mu$ m increments from top to bottom. *B. henselae* organisms were revealed with a specific MAb. Arrows indicate intracellular *B. henselae*.

rapid, and simple technique which may replace blood cultures for detecting *Bartonella* infections in cats.

We thank Patrick Kelly for review of the manuscript and Robert Pistoressi for technical assistance.

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