

Experimental Infection of Young Specific Pathogen–Free Cats with *Bartonella henselae*

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Eighteen 12-week-old specific pathogen–free cats, blood culture– and serum antibody–negative for *Bartonella henselae*, were randomly allocated to groups and were intravenously inoculated with 10^{10} (group 1), 10^8 (group 2), or 10^6 (group 3) *B. henselae* or with saline (group 4) or were not inoculated (group 5). Cats were humanely killed at 2, 4, 8, 16, and 32 weeks after inoculation. All *B. henselae*–inoculated cats were bacteremic by 2 weeks after infection. Bacteremia persisted until 32 weeks after infection in 1 cat. Cats in groups 1 and 2 had fever ($>39.7^\circ\text{C}$) and partial anorexia by 2 weeks after infection that lasted 2–7 days. All infected cats had *Bartonella*–specific IgM and IgG serum antibodies and lymphocyte blastogenic responses. Histopathologic lesions were observed in multiple organs of infected cats through 8 weeks after infection. Cats were readily infected with *B. henselae* by intravenous inoculation, developed histopathologic lesions that apparently resolved, and developed B and T lymphocyte responses to infection.

Bartonella henselae, a fastidious gram-negative bacterium in the family Bartonellaceae of the α -2 subgroup of the class Proteobacteria [1], causes typical and atypical forms of cat scratch disease, systemic and cutaneous angiomatous lesions such as parenchymal bacillary peliosis and bacillary angiomatosis, central nervous system disorders, and prolonged or relapsing fever and bacteremia in human beings [2–7]. Many people affected by the latter four syndromes are immunocompromised, but *B. henselae* also can cause similar diseases in immunocompetent persons [3, 5, 6]. The incidence of cat scratch disease in ambulatory patients in the United States was estimated at 9.3 cases per 100,000 population per year [8]. Cat scratch disease has been described as the most common cause of chronic lymphadenopathy in children and adolescents [2]. Epidemiologic studies indicate that cat ownership and kitten or cat scratches are the strongest risk factors for cat scratch disease and bacillary angiomatosis [9, 10].

B. henselae was recently isolated from the blood of healthy domestic cats; 100% (7/7) of bacillary angiomatosis patients'

cats, 90% (18/20) of cat scratch disease patients' cats, 39.5% (81/205) of pet and stray cats, and 25% (6/24) of a hospital population of healthy cats had *B. henselae* bacteremia [11–14]. Some 5%–81% of cat sera surveyed in the United States, Japan, Portugal, Denmark, Egypt, and Austria and 81% (39/48) of sera from the cats belonging to cat scratch disease patients in a US study were positive for *B. henselae* by immunofluorescent antibody tests [10, 12, 15–18]. Serologically positive cats are often also bacteremic [12–14]. In recent studies, circulating antibodies appeared to prevent reinfection of cats with *B. henselae* [19, 20]. Whether antibodies are actually protective requires further study; some cats with *B. henselae* bacteremia have high circulating antibody levels [21].

No known feline disease has been associated with *B. henselae* infection in cats. Two cats experimentally infected with *B. henselae* developed transient neurologic signs [14]. The means by which cats become infected and transmit the infection to people is incompletely understood, although experimental transmission from cat to cat by fleas has been demonstrated [21]. Domestic cats appear to be both a reservoir and vector for human infections with *B. henselae*. Epidemiologic evidence supports cat scratches as a means of infection of people [9, 10].

The purpose of the study reported here was to describe clinical signs, immune response, and gross and histopathologic lesions in specific pathogen–free (SPF) cats experimentally infected with *B. henselae*.

Materials and Methods

Animals

Eighteen 12-week-old male SPF cats (Harlan Sprague-Dawley, Indianapolis) with negative assays for IgG and IgM antibodies to

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The procedures described in this paper were all in accordance with established animal care and use guidelines and approved in Purdue University PACUC protocol #94-049.

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B. henselae and *Bartonella quintana* were used. The mothers of the cats were also serologically negative for *B. henselae* and *B. quintana*. The vendor certified that the cats were free of antibodies to multiple pathogens, including feline leukemia virus and feline immunodeficiency virus. The cats were placed in individual cages in a negative-pressure high-energy particulate air-filtered room when delivered to our facility at ~3 months of age. No medications or vaccinations were administered. Access to the room was controlled, and people who handled the cats wore protective garments, including barrier gowns, gloves, boots, and surgical masks. The cats were fed a commercial growth diet (Hill's Feline Growth; Hill's Pet Nutrition, Topeka, KS) until the age of 6 months, after which a commercial maintenance diet was fed (Hill's Feline Maintenance).

Experimental Design

Three weeks prior to inoculation, blood was obtained aseptically by jugular venipuncture from each cat for bacterial culture, serology, complete blood count (CBC), and serum biochemical analysis (Ektachem 700; Kodak, Rochester, NY). Urine was obtained by cystocentesis for urinalysis. Blood cultures and CBC were repeated 2 weeks prior to inoculation.

The cats were randomly divided into 4 groups of 4 (groups 1–4) and 1 group of 2 (group 5). Cats in group 1 were given 10^{10} cfu of *B. henselae* in 1 mL of 0.9% NaCl intravenously (iv); group 2 cats were given 10^8 cfu of *B. henselae* iv, group 3 cats were given 10^6 cfu of *B. henselae* iv, and group 4 cats were given 1 mL of 0.9% NaCl iv. Cats were carefully observed immediately following inoculation, and complete physical examinations were conducted twice daily. The clinical examiner was blinded to the inoculation status of the cats through week 16 after infection. Cats in group 5 received no injections but were reserved for use as sentinels to be housed with infected cats once bacteremia was established. One cat from each of groups 1–4 was scheduled for euthanasia and necropsy at 4, 8, 16, and 32 weeks after infection. Cats were humanely killed by intravenous injection of a solution of pentobarbital sodium (BeuthanasiaD Special; Schering-Plough, Kenilworth, NJ).

Blood was obtained by jugular venipuncture 4 days after inoculation, then weekly, for CBC, bacterial culture, and serologic studies during the first month after infection, then every 2 weeks for 1 month, then once monthly and at euthanasia. Blood for bacterial culture was collected into tubes containing a lysing agent (Isolator 1.5 tubes; Wampole Laboratories, Cranbury, NJ). Urine was obtained by cystocentesis for bacterial culture at 6, 8, 10, and 12 weeks after inoculation and at euthanasia. Lymphocyte blastogenesis and immunophenotyping were done at the time of euthanasia.

At necropsy, bone marrow, spleen, liver, kidney, and salivary gland for routine aerobic and *Bartonella* cultures and spleen, bone marrow, and peripheral and mesenteric lymph node for lymphocyte isolation were collected by use of sterile technique. Eyes were placed in Bouin's fixative, and other tissues were placed in 10% neutral buffered formalin. Samples of selected tissues were frozen in liquid nitrogen. Five-micron paraffin sections were stained with hematoxylin-eosin. Selected tissues were also stained with Warthin-Starry and Steiner silver stains.

Bacteriology

Bacteria used for inoculation of SPF cats. The *B. henselae* used was isolated from the blood of a cat owned by a patient with cat scratch disease. It was characterized by standard culture techniques, including gas-liquid chromatography and immunofluorescence, which together are specific for identification of *Bartonella* to the species level [22]. Bacteria used as inoculum were derived from the original isolate after minimal subculturing. The bacteria were grown on chocolate agar (Becton Dickinson, Cockeysville, MD) to confluence, then washed from the agar by use of sterile saline. Bacteria were washed three times and resuspended in sterile saline at 10^{10} , 10^8 , and 10^6 cfu/mL.

B. henselae sarcosine-insoluble outer membrane protein antigen. *B. henselae* *n*-lauroylsarcosine-insoluble outer membrane protein (OMP) antigen preparation was produced as previously described [22]. Protein concentration was determined (Bio-Rad Protein Assay; Bio-Rad Laboratories, Richmond, CA), and aliquots of the OMP antigen preparation were frozen at -20°C until needed. Antigen used for lymphocyte blastogenesis and ELISPOT assays was prepared from the *B. henselae* isolate used to inoculate the cats. Antigen used for EIAs was prepared from *B. henselae* 87-66 (49793; American Type Culture Collection, Rockville, MD).

Culture of tissue specimens. Lysed blood and urine from experimental animals was centrifuged at 16,000 g at 4°C for 10 min. One-tenth milliliter aliquots of pellet material were spread onto each of two chocolate agar plates by use of a sterile glass rod. Quantitative cultures of blood and tissue specimens were done at the time of necropsy for all cats, and of all blood samples collected between 5 and 8 months after inoculation.

Tissue samples collected for culture were placed in sterile plastic bags containing nutrient broth. The bags were placed in a homogenizer (Stomacher-80 Lab-Blender; A. J. Seward, London), and the resulting homogenate was streaked onto chocolate agar plates. Plates were incubated at 35°C with 5% CO_2 and were held for at least 4 weeks before being coded as negative for growth of *B. henselae*. Samples were also inoculated onto blood agar and MacConkey agar plates for routine aerobic culture. Identity of *B. henselae* colonies was verified by colony morphology and Gram's-staining characteristics. Periodically, confirmation by the indole and catalase tests and labeling with polyclonal goat anti-*B. henselae* serum and fluorescein isothiocyanate (FITC)-conjugated rabbit anti-goat IgG (Southern Biotechnology Associates, Birmingham, AL) was also performed.

Immunology

Peripheral blood mononuclear cell separation. Heparinized whole blood was diluted 1:1 with PBS, then layered onto a ficoll-hypaque solution (specific gravity, 1.077; Histopaque-1077; Sigma, St. Louis) and centrifuged at 400 g for 40 min at room temperature. Cells collected from the interface were washed twice with PBS and resuspended in complete medium (RPMI 1640 [Mediatech, Herndon, VA] supplemented with 1% glutamine, 100 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, and 0.25 $\mu\text{g}/\text{mL}$ amphotericin B [Sigma] and 10% bovine serum product [FetalClone I; HyClone Laboratories, Logan, UT]). Cell concentrations were adjusted to 1×10^6 or 2×10^6 cells/mL. Cells were >98% viable as assessed by trypan blue dye exclusion.

Lymphocyte blastogenesis. Peripheral blood mononuclear cells were prepared as described above. Ninety-six-well half-area tissue culture plates (3696; Costar, Cambridge, MA) were prepared with triplicate wells containing concanavalin A (5 or 1 $\mu\text{g}/\text{mL}$), pokeweed mitogen (1 $\mu\text{g}/\text{mL}$), *B. henselae* *n*-lauroylsarcosine-insoluble OMP antigen preparation (10 or 2 $\mu\text{g}/\text{mL}$), or complete medium only. Peripheral blood mononuclear cell suspensions were added to each well (25- μL volume; 2.5 or 5×10^4 cells/well). Plates were incubated for 72 h (concanavalin A and pokeweed mitogen) or 120 and 168 h (*B. henselae*) at 37°C with 5% CO₂. Cells were labeled for the final 18 h of incubation via addition of 0.25 μCi of [³H]thymidine in 25 μL of complete medium per well. Cells were harvested onto glass fiber filters by use of a semiautomated harvester (PHD Cell Harvester; Cambridge Technology, Watertown, MA) and counted in a scintillation counter (Tri-Carb 1500; Packard Instrument, Downers Grove, IL). Stimulation indices (SIs) were calculated by dividing mean counts per minute (cpm) of triplicates of mitogen- or antigen-containing wells by mean cpm of wells containing medium only. Stimulation ratios (SRs) were calculated to facilitate comparison of responses to *B. henselae* antigen among groups. The SRs were calculated by dividing the SI of each cat by the SI of the control cat tested at the same time point.

Lymphocyte immunophenotyping. B lymphocytes in peripheral blood mononuclear cells were identified by use of FITC-conjugated goat anti-cat IgG (Southern). CD4-positive lymphocytes were identified by use of FITC-conjugated mouse anti-cat CD4 (Southern). CD8-positive lymphocytes were identified by use of biotin-conjugated mouse anti-cat CD8 (Southern) and streptavidin-phycoerythrin (Life Technologies GIBCO BRL, Gaithersburg, MD). Samples were analyzed by flow cytometry (EPICS Elite; Coulter, Miami).

ELISPOT assays for antibody secreting cells. Spleen, lymph node, and bone marrow were disrupted by mincing and pipetting to yield single-cell suspensions. Cell suspensions from lymph nodes and bone marrow were washed with complete medium, and mononuclear cells were counted. Spleen cell suspensions were layered over a ficoll-hypaque solution as described for peripheral blood mononuclear cells. Cells from the interface were washed twice with complete medium and counted. Cell viability was >98% as assessed by trypan blue dye exclusion.

Ninety-six-well flat-bottom EIA/RIA plates (Costar 3590) were incubated overnight at 4°C in a humid chamber with 100 $\mu\text{L}/\text{well}$ 2 $\mu\text{g}/\text{mL}$ *B. henselae* *n*-lauroylsarcosine-insoluble OMP antigen preparation diluted in PBS. Plates were washed three times with PBS-0.05% Tween 20 (PBST), blocked by incubation with 100 $\mu\text{L}/\text{well}$ PBS and 0.1% bovine serum albumin for 1 h at room temperature in a humid chamber, then washed twice with PBST and once with PBS. Cells from each tissue were added at 10^5 cells/well. Plates were incubated at 37°C with 5% CO₂ for 5 h and washed three times with PBST; next, 75 μL of alkaline phosphatase-conjugated goat anti-cat IgG (Southern) diluted 1:2000 in PBS was added to each well. Plates were incubated overnight in a humid chamber at 4°C and washed three times with PBST and once with PBS, followed by addition of 5-bromo-4-chloro-3-indolyl phosphate (Sigma), in agarose at 100 $\mu\text{L}/\text{well}$. Plates were held at 4°C for 4–24 h for color development before spots were counted. Results were expressed as number of antibody-secreting cells per 10^6 cells for each tissue examined.

EIA for serum antibodies. Ninety-six-well EIA/RIA plates (Immuno Plates, Maxisorp type; Nunc, Roskilde, Denmark) were coated with 100 $\mu\text{L}/\text{well}$ *B. henselae* OMP antigen (1 $\mu\text{g}/\text{mL}$) and incubated overnight at 4°C in a humid chamber. Plates were then washed three times with PBST and blocked with 0.2% bovine serum albumin-PBST for 1 h at room temperature. Plates were washed three times with PBST, after which cat sera diluted 1:100 in PBST were added at 50 $\mu\text{L}/\text{well}$, and plates were incubated for 1 h at room temperature. After four washings with PBST, 50 $\mu\text{L}/\text{well}$ goat anti-cat IgG or IgM (Kirkegaard & Perry, Gaithersburg, MD) was added at 1:2000 and incubated for 1 h at room temperature. Plates were washed four times with PBST; next, alkaline phosphatase-labeled rabbit anti-goat IgG (Sigma), diluted 1:15,000, was added at 50 $\mu\text{L}/\text{well}$, and plates were incubated for 1 h at room temperature. After four additional washes, *p*-nitrophenol phosphate (Sigma, 2 mg/mL) in buffer was added as substrate, and plates were incubated for 45 min for color development before determination of optical density (OD) at 405 nm by an automated reader (Dynatech, Chantilly, VA). The average OD of triplicate determinations was calculated. At each postinoculation time point, the postinoculation OD measurement for each cat was divided by the preinoculation OD for that cat to yield an index. The mean index for each treatment group was also calculated.

To facilitate comparison of OD values with conventional titers, the following was done: On the basis of the EIAs performed as described above, the postinfection serum from each animal with the highest OD at the 1:100 dilution was selected for comparison. For IgG assays for each animal, the baseline serum from immediately before infection was assayed at a dilution of 1:100, and the serum with the highest OD for each cat was assayed at dilutions of 1:100, 1:1000, 1:10,000, and 1:100,000. The ODs obtained with each of these dilutions were plotted on the *y* axis and the log₁₀ of each dilution (i.e., 2, 3, 4, 5) was plotted on the *x* axis to yield a standard curve for each peak serum specimen. By use of the linear relationship so established, the logarithm of the peak serum dilution that would yield an OD equivalent to that of the baseline serum at a titer of 100 was determined. The antilog of this value yielded the calculated titer of the peak serum that yields an OD equivalent to that of the baseline serum at a titer of 100. The same procedure was carried out for IgM data, except that serial 2-fold dilutions were used to establish the standard curves.

Immunohistochemistry

Paraffin sections 5 μm thick mounted on positively charged glass slides (SuperFrost Plus; Fisher Scientific, Pittsburgh) were stained by use of a kit (Vectastain; Vector Laboratories, Burlingame, CA). Briefly, sections were deparaffinized by use of xylene, rehydrated through an ethanol series, washed in PBS, and blocked with 1.5% normal rabbit serum. Polyclonal goat anti-*B. henselae* serum (adsorbed with normal SPF cat blood cells and diluted 1:3000) was applied, followed by biotinylated rabbit anti-goat IgG (Vector) at 1:200. Avidin-biotinylated alkaline phosphatase (Vector) was used at 1:111, and Vector Red (Vector) was used as the substrate for alkaline phosphatase. Slides were counterstained with hematoxylin. Negative controls included tissue sections incubated with PBS with 1% normal rabbit serum, normal goat serum adsorbed with normal SPF cat blood cells diluted 1:3000 in place of

goat anti-*B. henselae* serum, or sections of tissue obtained from control cats. Positive controls were sections of human spleen containing *B. henselae* and smears of formalin-fixed pure cultures of *B. henselae*.

Frozen sections 4 μm thick were mounted on positively charged glass slides (Fisher), dried overnight at 4°C, and fixed in cold acetone for 5 min. The remaining procedure was the same as described for paraffin sections, including negative control slides. Positive control slides were smears of pure cultures of *B. henselae* fixed in acetone. Frozen sections were also stained with adsorbed goat anti-*B. henselae* serum (1:3000) followed by FITC-labeled rabbit anti-goat IgG (Southern) diluted 1:100.

Results

Clinical Signs and Physical Examination Findings

All group 1 cats and 3 cats in group 2 developed fever (rectal temperature $>39.7^\circ\text{C}$) and lethargy within 2 h of inoculation that subsided by 5 h after inoculation. All cats in group 1 also developed fever on day 9 or 10 after infection that persisted for 5–7 days, and 2 cats in group 2 developed fevers on days 13 or 16 after infection that persisted for 2 days. During the second febrile period, cats exhibited mild anorexia but remained alert, responsive, and playful. One cat in group 3 developed fever and partial anorexia on day 22 after infection, which persisted for 4 days. A liver abscess was found at necropsy of this cat on day 28 after infection. No other clinical signs of disease were observed.

Because all cats in group 1 developed fever and anorexia, a group 1 cat and 1 cat that had been reserved for use as a sentinel were necropsied on day 14 after inoculation. Data from the sentinel cat killed 2 weeks after inoculation are reported with control cat data. This left only 1 cat for use as a sentinel. This sentinel was housed with a group 1 cat during weeks 8–32 after infection.

Enlarged mandibular or popliteal lymph nodes were palpable in 11 of 12 cats in groups 1–3 between weeks 2 and 6 after infection. Lymphoid hyperplasia was diagnosed on cytologic evaluation of fine needle aspirates of enlarged lymph nodes.

Clinical Pathology

The group 3 cat with a liver abscess had a mild neutrophilia 2 days after its fever resolved. Neutrophilia was otherwise notably absent. There were no other clinicopathologic abnormalities.

Bacteriology

All cats inoculated with *B. henselae* became bacteremic within 2 weeks. Bacteremia persisted until 16 weeks after infection, when all remaining cats had blood cultures negative for *B. henselae*. The single cat remaining in group 1 after 16 weeks after infection had blood cultures positive for *B. henselae* be-

ginning again at week 20 after infection and persisting through week 32 after infection. Cats in groups 4 and 5 never developed bacteremia. There were no consistent differences in colony counts recovered from the blood of cats in different dose groups. The number of colony-forming units per milliliter of blood within each group decreased over time (table 1).

B. henselae were cultured from liver, kidney, spleen, and bone marrow of the group 1 cat 2 weeks after infection and from liver of the group 3 cat 4 weeks after infection. No bacteria were recovered from any tissues collected at necropsy at any other time points. Urine cultures were negative.

Immunology

Lymphocyte blastogenesis. Blastogenic responses to concanavalin A and pokeweed mitogen were similar among cats in all groups and throughout the study. The blastogenic responses were comparable to reported values for SPF cats [23, 24]. Blastogenic responses to *B. henselae* *n*-lauroylsarcosine-insoluble OMP antigen showed no consistent differences between 5- and 7-day cultures. The average of these culture periods is presented in figure 1.

Lymphocyte immunophenotyping. The CD4:CD8 ratios were similar within and among groups 1–4 at each time point. CD4 cell numbers ranged from 611 to 4352/ μL (median, 1665) and CD8 cell numbers ranged from 148 to 1070/ μL (median, 435). These CD4:CD8 ratios were similar to those previously reported for SPF cats of the same age [25, 26].

ELISPOT assay. *Bartonella*-specific antibody-secreting cells were detected in spleen, bone marrow, and peripheral lymph nodes of all infected cats and not in tissues of control and sentinel cats. The greatest number of antibody-secreting cells was in the spleen (figure 2). Occasional spots detected in wells from control cats are included in figure 2 for comparison but are considered insignificant relative to the large numbers detected in tissues from infected cats. Smaller numbers of antibody-secreting cells in bone marrow ($13\text{--}30/10^6$ mononuclear cells) and peripheral lymph node ($5\text{--}19/10^6$ mononuclear cells) were observed at 16 and 32 weeks after infection. Peripheral lymph node cells were not included in the assay until 8 weeks after infection, and antibody-secreting cells were not detected in cells from mesenteric lymph nodes at any time.

EIA for serum antibodies. IgM and IgG responses to *B. henselae* were seen in all infected cats, and their serum anti-*B. henselae* IgG and IgM indices remained consistently higher than those of group 4 cats (figure 3). The sentinel cat had indices similar to those of control cats (data not shown).

Silver stains and immunohistochemical labeling. Few bacteria were seen on Warthin-Starry and Steiner silver stains of the spleen of the group 1 cat examined 2 weeks after infection. Some *B. henselae* were identified on immunohistochemical labeling of spleen and liver of this cat by use of both FITC and alkaline phosphatase methods, as well as in liver of the group 2 cat examined 4 weeks after infection and in spleen of

Table 1. Numbers of cats with blood cultures positive for *B. henselae*.

Dose	Weeks after inoculation							
	2	4	8	12	16	21	25	32
Control	0/4	0/4	0/3	0/2	0/2	0/1	0/1	0/1
10 ⁶	4/4	4/4 (1.1 × 10 ⁵)	3/3 (1.5 × 10 ⁴)	1/2 (3 × 10 ²)	0/2	0/1	0/1	0/1
10 ⁸	4/4	4/4 (1.7 × 10 ⁵)	3/3 (1.7 × 10 ³)	1/2 (1 × 10 ²)	0/2	0/1	0/1	0/1
10 ¹⁰	4/4	3/3	3/3 (6.5 × 10 ³)	2/2 (6 × 10 ³)	0/2	1/1 (1 × 10 ¹)	1/1 (3 × 10 ¹)	1/1 (3 × 10 ¹)

NOTE. Data are no. bacteremic/total. Nos. in parentheses are cfu/mL of blood. Values given through week 8 are for 1 cat/group; quantitative culture was done only for cats necropsied through that time. Values given for weeks 12–32 are averages from 2 cats or values from single cat with positive blood culture.

the group 2 cat examined 32 weeks after infection. In all of these tissues, bacteria were present extracellularly either singly or in small clusters. Intracellular bacteria were never observed in tissue sections or in blood of infected cats.

Pathology

A variety of lesions were seen only in infected cats and were therefore attributed to *B. henselae*. There was mild to marked lymphoid hyperplasia of the spleen of the infected cats, with development of prominent germinal centers. Expansion of the marginal zone was evident in the spleen of the group 1 cat at 2

weeks after infection. Circumscribed aggregates of neutrophils (microabscesses) were present throughout the red pulp of the spleen of this cat (figure 4), and an increased number of neutrophils was present in the red pulp of the group 2 cat 4 weeks after infection. Peripheral lymph nodes of the infected cats were macroscopically enlarged 4 and 8 weeks after infection. Histologically, there was marked lymphoid hyperplasia characterized by an expanded paracortex and secondary follicles with germinal centers. Medullary cords were thickened with lymphocytes and plasma cells. Two small coalescing necrotizing granulomas were present in a lymph node of the group 2 cat 8 weeks after infection (figure 5). There was a moderate number

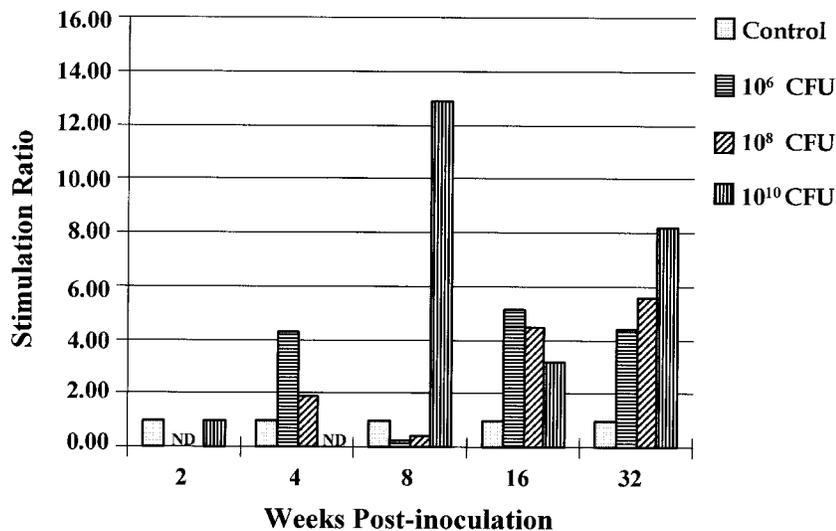
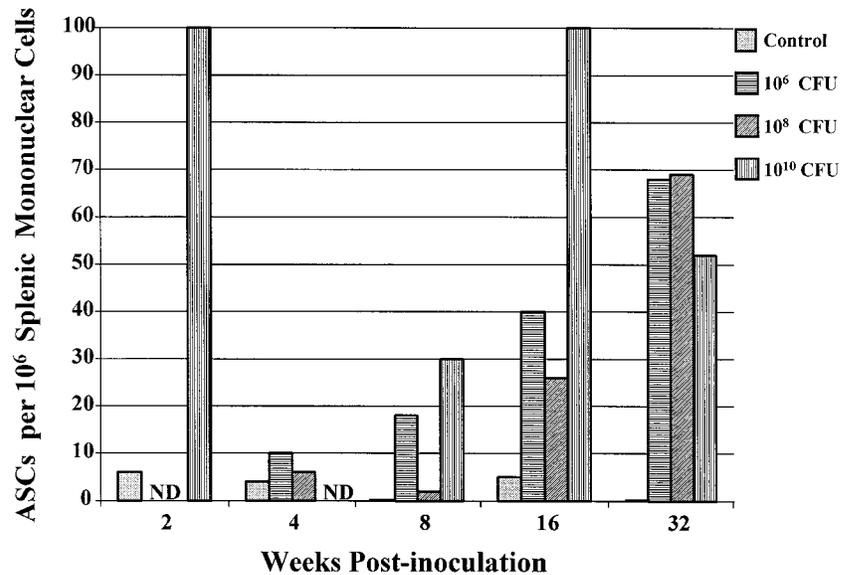


Figure 1. Stimulation of lymphocyte blastogenesis by *B. henselae* antigen. Each point represents single cat from each group. Stimulation indices (SIs) were calculated by dividing mean counts per minute (cpm) of triplicates of mitogen- or antigen-containing wells by mean cpm of medium-only wells. Stimulation ratios (SRs) were calculated to facilitate comparisons among groups by dividing SI of each cat by SI of control cat tested at same time point. Graph depicts averages of SRs calculated at 5 and 7 days of incubation by use of 10 μ g/mL *B. henselae* sarcosine-insoluble outer membrane protein antigen preparation. SIs of control cats ranged from 0.43 to 1.6 after 5 days of incubation and 0.6 to 1.4 after 7 days of incubation. Mean cpm \pm SD for unstimulated cells of control cats ranged from 42 \pm 15 to 360 \pm 18 after 5 days of incubation and 43 \pm 13 to 684 \pm 238 after 7 days. Mean cpm \pm SD for unstimulated cells of cats inoculated with 10⁶ cfu ranged from 56 \pm 23 to 250 \pm 73 after 5 days of incubation and 29 \pm 18 to 312 \pm 91 after 7 days. Mean cpm \pm SD for unstimulated cells of cats inoculated with 10⁸ cfu ranged from 42 \pm 10 to 354 \pm 218 after 5 days of incubation and 26 \pm 17 to 1208 \pm 810 after 7 days. Mean cpm \pm SD for unstimulated cells of cats inoculated with 10¹⁰ cfu ranged from 23 \pm 2 to 348 \pm 139 after 5 days of incubation and 39 \pm 9 to 112 \pm 20 after 7 days. ND = not done.

Figure 2. Antibody-secreting cells (ASC) in spleen. More were detected in spleen at later time points than at early time points in cats inoculated with 10^8 and 10^6 cfu. Only 1 cat in each group was tested at each point in time; therefore, no statistical tests were applied. ND = not done.



of neutrophils in the cortex and medulla of lymph nodes of the group 1 cat 8 weeks after infection.

There were small foci of necrosis in the parenchyma and an increased number of neutrophils in sinusoids of the liver of the group 1 cat 2 weeks after infection. A 5-mm abscess protruded from the capsule of the liver of the group 3 cat 4 weeks after infection, and there were small foci of necrosis throughout the liver of this animal. There were 2 small foci of granulomatous inflammation in the liver of the group 1 cat and a single neutrophil aggregate in the liver of the group 2 cat 8 weeks after infection.

Focal pyogranulomatous nephritis and interstitial myocarditis were present in the group 2 cat 4 weeks after infection.

Certain pathologic phenomena were seen in both control and infected cats and therefore not attributed to *B. henselae*. These included a mild multifocal histiocytic pneumonia in both control cats and 4 of 5 infected cats 4 and 8 weeks after infection and eosinophilic granuloma of the lip in 2 of 4 control cats and 2 of 12 infected cats over the 32 weeks of observation.

Discussion

Cats can be infected by intravenous inoculation with 10^6 to 10^{10} cfu of *B. henselae* and may remain bacteremic for at least 32 weeks after inoculation. This report is the first to describe generalized peripheral lymphadenopathy, fever with anorexia and lethargy, and histopathologic lesions in cats infected with *B. henselae*. Naturally infected cats may develop similar clinical signs and lesions. Clinical signs would likely dissipate quickly, as they did in the cats in this study, and may not be noticed by cat owners.

Intravenous transmission is not likely in the natural setting, but because bacteremia is a consistent aspect of feline *B.*

henselae infections, the iv route was chosen for the experimental inoculations. We have since successfully inoculated cats intradermally, and similar clinical signs occur (unpublished data). The pathogenesis of *B. henselae* infections in cats inoculated by infected fleas or another natural route may differ from that reported for the iv-inoculated cats in this study. The pathogenesis of *Borrelia burgdorferi* infections in dogs inoculated by needle injection differs from that of *B. burgdorferi* infections in dogs inoculated by infected ticks [27]. Examination of tissues of cats experimentally infected with *B. henselae* by use of fleas is necessary to determine whether such a difference exists.

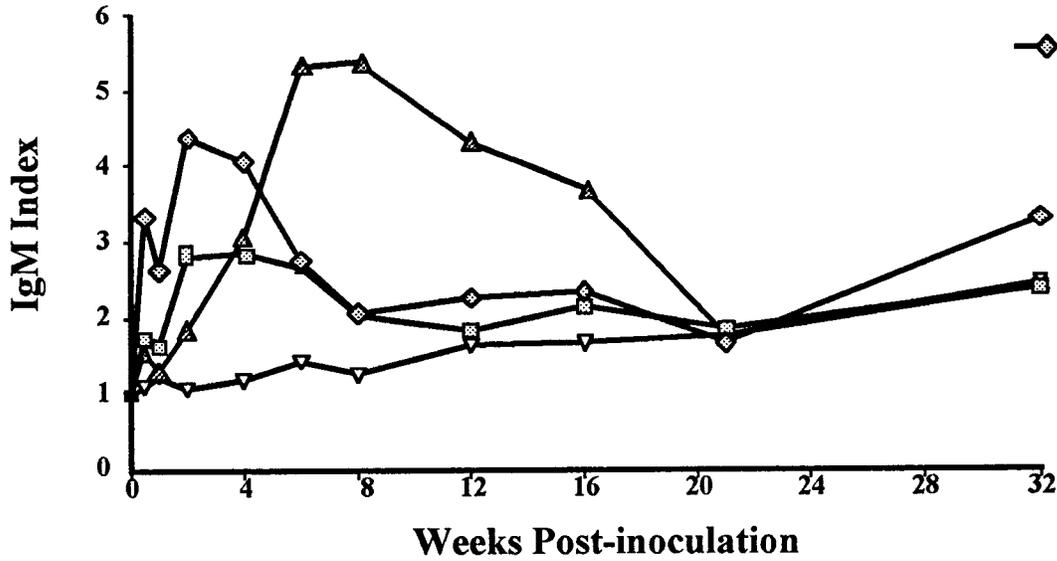
The duration of bacteremia in cats experimentally infected in this study is similar to that published for other experimental infections [19, 20] and shorter than what appears to occur in naturally infected cats [28]. The difference in duration may be a result of a phenotypic difference in *B. henselae* that cats are exposed to naturally and a resultant difference in cats' immune responses. One cat in this study had a negative blood culture at 16 weeks after infection, followed by two positive cultures at 20 and 32 weeks after infection. The cat may have been truly abacteremic at the time of the negative culture, or bacteremia below the limit of detection of bacterial culture may have been present. Other investigators have also reported intermittent bacteremia in cats experimentally infected with *B. henselae* [29].

Generalized lymphadenopathy was observed in all infected cats. Coalescing necrotizing granulomas are typical lesions in lymph nodes of cat scratch disease patients, and similar lesions were observed in the lymph node of 1 experimentally infected cat 8 weeks after infection. While generalized lymphadenopathy has not been previously reported in cats infected with *B. henselae*, idiopathic generalized lymphadenopathy of pet cats has been described [30–32]. Argyrophilic organisms were seen

cats
per group:

4 4* 3 2 2 1 1 1
*3 cats in high dose group

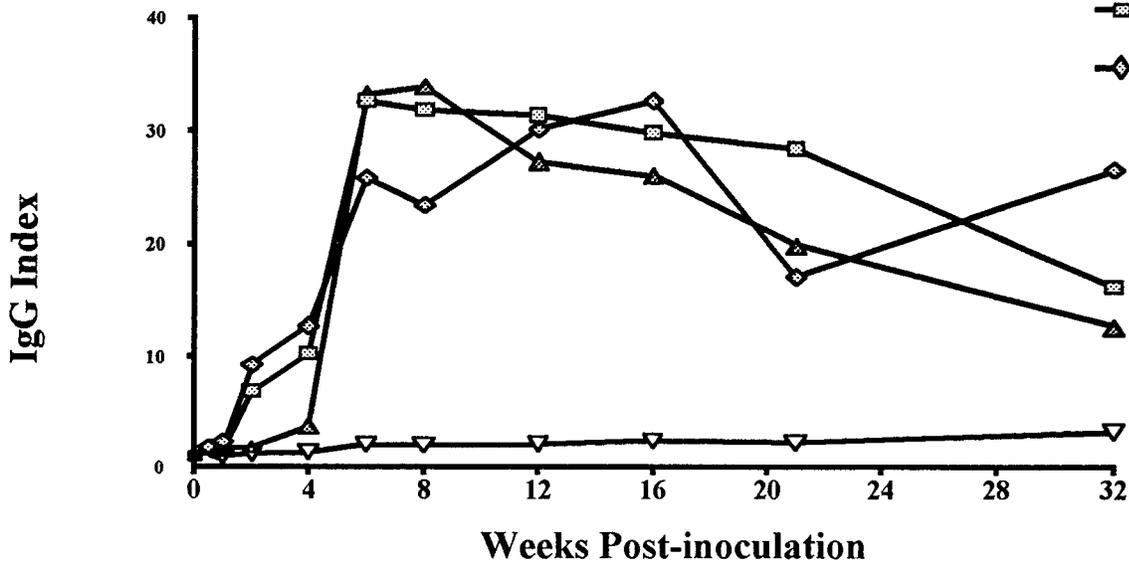
- ▽— Control
- ▲— 10^6 CFU
- 10^8 CFU
- ◇— 10^{10} CFU



cats
per group:

4 4* 3 2 2 1 1 1 1
*3 cats in high dose group

- ▽— Control
- ▲— 10^6 CFU
- 10^8 CFU
- ◇— 10^{10} CFU



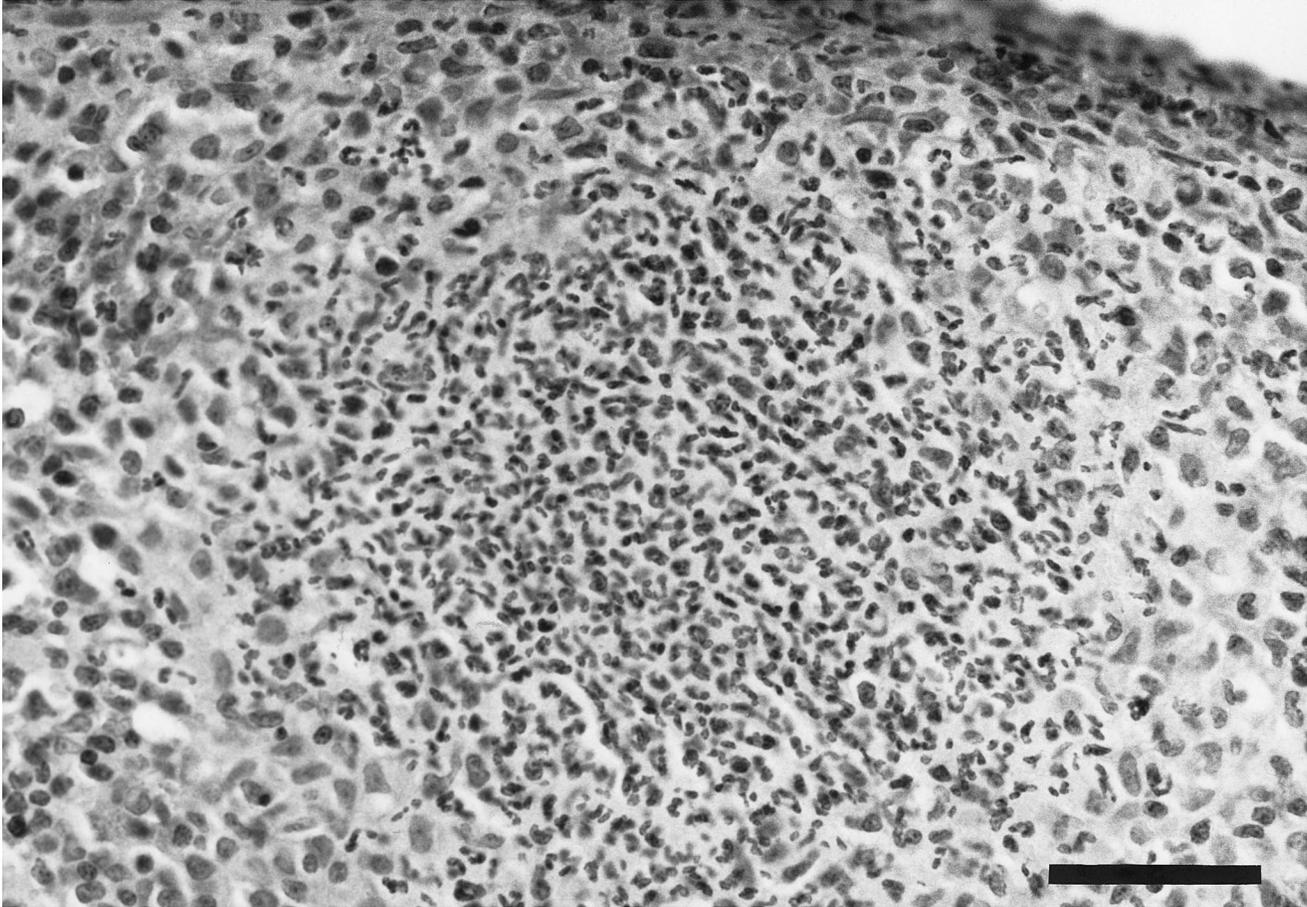


Figure 4. Splenic microabscess, group 1 cat (10^{10} cfu), 2 weeks after infection. Magnification, $\times 400$. Bar = 50 μm .

in lymph nodes of 11 (31%) of 35 young cats with idiopathic generalized lymphadenopathy [30]. Kirkpatrick et al. [30] speculated that the argyrophilic organism may be the agent that causes cat scratch disease in human beings. Some cats in these reports may have been infected with *B. henselae*, but because no bacterial culture, immunohistochemical labeling, or microbial gene amplification or identification data were reported, the *B. henselae* status of those cats remains unknown.

Infected cats in the current study had both IgM and IgG antibody responses to *B. henselae*. The antibody response of cats in group 3 lagged behind that of group 1 and 2 cats by ~ 2 weeks. This may be a result of the lower dose of bacteria

these cats received. The increase in IgM and IgG in the group 1 cat at week 32 after infection may have been a consequence of a recrudescence of bacteremia after 16 weeks after infection.

Bacteremias decreased as IgG indices increased. Decreasing bacteremia coincident with increasing IgG was also observed by another investigator [33]. The role of antibody in pathogenesis of *B. henselae* infections of cats and human beings has not been determined. *Bartonella*-specific antibody appears to enhance phagocytosis of *B. henselae* by human neutrophils in vitro [34]. However, complement-mediated killing of *B. henselae* occurs in nonimmune human sera in vitro and is not enhanced by addition of sera with *Bartonella*-specific antibodies [34].

Figure 3. Antibody responses after experimental infection of cats with *B. henselae*. IgM (top) and IgG (bottom) indices (y axis) are mean values of all animals within given group at multiple points in time (x axis). Number of cats in each group decreased by 1 at weeks 4, 8, and 16, so that values reported after week 4 and through week 8 represent means of 3 cats, values reported after week 8 and through week 16 represent means of 2 cats, and values reported after week 16 represent values of only 1 cat per group. Serum was diluted to 1:100 for IgM and IgG assays, and optical densities (ODs) were recorded. Indices were calculated by dividing OD for each postinfection time point for individual cat by mean preimmune OD value for that cat. Means of indices were calculated for construction of graphs. Maximum IgM OD for infected cats corresponded to titer of 1:3981 and occurred at 2 weeks after inoculation in cat inoculated with 10^{10} cfu of *B. henselae*. Similar IgM titers also occurred at 5 weeks after inoculation in 2 cats inoculated with 10^6 cfu of *B. henselae*. Maximum IgG OD for infected cats corresponded to titer of 1:37,584 and occurred at 16 weeks after inoculation in cat inoculated with 10^6 cfu of *B. henselae*.

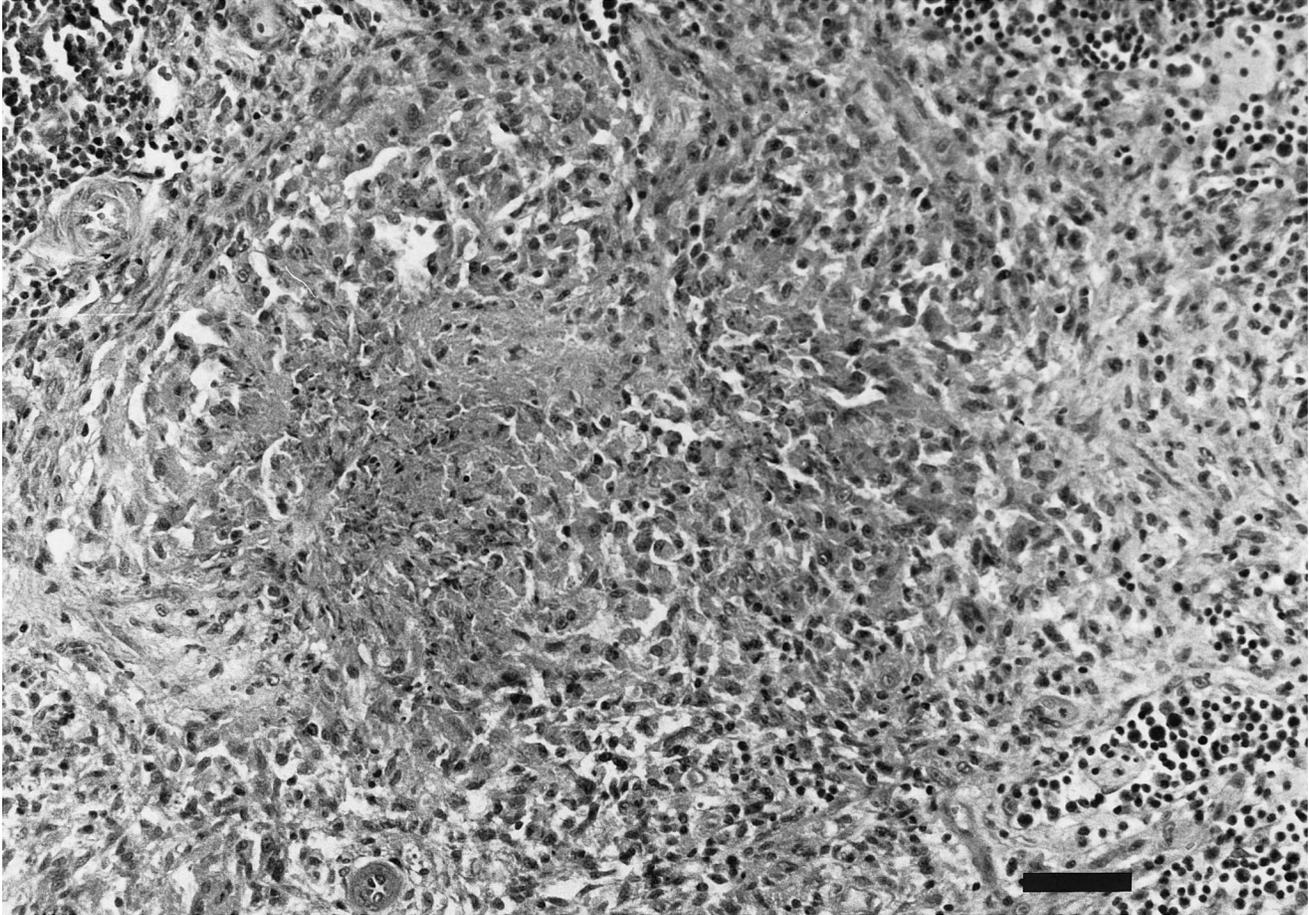


Figure 5. Necrotizing granuloma, peripheral lymph node, group 2 cat (10^8 cfu), 8 weeks after infection. Magnification, $\times 200$. Bar = 50 μm .

The lymphocyte blastogenesis data demonstrate a clear T cell response by *B. henselae*-infected cats to stimulation of lymphocytes with concanavalin A and pokeweed mitogen. These data, combined with the results of EIAs for serum antibodies, ELISPOT assays for antibody-secreting cells in tissues, and lymphocyte immunophenotyping, indicate that *B. henselae* is not immunosuppressive for cats.

Some means by which *B. henselae* may evade the cat's immune response include antigenic variation by *B. henselae* or inhibition of NK cell function, as may occur in *B. burgdorferi* infections in other species [35]. *B. henselae* may also be sequestered in relatively immunologically protected sites in cats. One such site may be the central nervous system, in light of reports of mild central nervous system signs in some infected cats [14]. In addition, *B. henselae* may possess mechanisms for avoiding phagocytosis by immune cells, or methods of promoting uptake by nonimmune cells [36], and thereby be sequestered from uptake and processing or attack by the immune system.

The sentinel cat housed with a bacteremic group 1 cat between 8 and 32 weeks after infection had persistently negative

blood cultures, and all tissues were culture-negative at necropsy. These findings, taken together with those of other investigators [19, 21], indicate that infection is unlikely to be readily transmitted among cats by direct contact, including scratches, bites, normal grooming behavior, or shared litter pans or food dishes.

The exact tissue location of *B. henselae* was not determined in the experimentally infected cats. Bacteria were never seen within intact erythrocytes, contrasting with a previous report of intraerythrocytic *B. henselae* [37]. *B. henselae* were seen infrequently on immunohistochemically labeled sections of spleen and liver of infected cats at various time points throughout the course of infection. The organisms were extracellular, were not associated with inflammatory lesions, and may have been present in blood that was in the tissues. Therefore, finding organisms in liver and spleen may not represent localization but rather reflect the vascular nature of these tissues. Conversely, absence of organisms at sites of inflammation may signify a local host response effective in damaging bacterial cells so that they are not visible or labeled by antiserum. In immunocompetent human beings with cat scratch disease, bacteria may be

found at sites of lymph node abscesses, usually extracellularly. Bacteria have also been reported within histiocytes and neutrophils in affected lymph nodes [38]. In some cases, bacteria are not found in lymph nodes of people with cat scratch disease, particularly in tissues examined >1 month after onset of disease, possibly because of destruction of organisms within abscesses [38–40]. A recent application of Warthin-Starry staining and immunohistochemical labeling identified *B. henselae* organisms in one-third of cat scratch disease-associated lymph nodes [41].

Cats experimentally infected with *B. henselae* may develop bacteremia of several months' duration but show few clinical signs. This is consistent with what has been reported for naturally infected cats [14]: Cats develop persistent infections and there are few or no clinical signs of disease. The results of this study indicate that intravenous inoculation of cats can be used as a reliable challenge model in which to test vaccine efficacy at preventing persistent bacteremia. This study also provides useful information about primary *B. henselae* infection in a natural host, and the system described may also be useful for examining mechanisms of host-bacteria interaction.

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