

Clinical disease in kittens inoculated with a pathogenic strain of *Bartonella henselae*

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Objective—To evaluate disease in kittens inoculated with *Bartonella henselae* strain LSU16.

Animals—Eighteen 12-week-old specific-pathogen-free kittens.

Procedure—Kittens were inoculated with *B henselae* strain LSU16 or saline (0.9% NaCl) solution. Blood samples were collected from kittens on alternate weeks, and bacteremia, clinical signs, and antibody concentrations were monitored for 6 months after inoculation.

Results—Kittens developed raised, erythematous areas at the site of inoculation within 72 hours. Swelling peaked at 14 days and resolved by 28 days after inoculation. Fever had a biphasic pattern, with an episode of 1- to 3-days' duration beginning 6 to 7 days after inoculation followed by an episode of 3- to 8-days' duration beginning 11 to 13 days after inoculation. Kittens were bacteremic by day 14 with peak bacteremia at days 14 to 28. Strong antibody responses to *B henselae* were detected. Clinical disease resolved before bacteremia became undetectable, but signs of disease correlated with the highest degree of bacteremia. Regional lymphadenopathy also was evident.

Conclusion and Clinical Relevance—Clinical disease in kittens was similar to that in adult cats infected with *B henselae* strain LSU16, except that lethargy and anorexia were less severe in kittens, and a biphasic pattern of fever was detected in kittens. Clinical disease after inoculation with *B henselae* may be strain-dependent. To limit transmission of *Bartonella* organisms, appropriate flea prevention should be instituted.

Impact for Human Medicine—Kittens that are febrile, anorectic, lethargic, and that have lymphadenopathy should be tested for *Bartonella* organisms, and contact with immunocompromised owners should be discouraged. (*Am J Vet Res* 2000;61:375–379)

B*artonella henselae* is a zoonotic agent for which cats are natural carriers, and disease in humans is most frequently associated with kittens < 1 year old.¹⁻⁶ In immunocompetent humans, the disease manifests itself as a wide range of clinical disease syndromes, most commonly cat-scratch disease (CSD). In the majority

of immunocompetent patients, CSD is characterized by lymphadenopathy in the nodes that drain the site of a cat scratch. Local lesions such as redness, swelling, and a papule at the site of the scratch also may develop but are not consistently seen. Swelling in the involved lymph nodes usually regresses during a period of weeks to months. Human patients also may be febrile and anorectic and have malaise, headaches 7 to 10 days after exposure, and painful lymphadenopathy in moderate or severe CSD. In approximately 10% of affected humans, lymphadenopathy associated with CSD may become suppurative. Ocular disease, encephalopathy, osteolytic lesions, life-threatening bacillary angiomatosis, and bacillary peliosis may be evident in a small percentage of immunocompetent individuals as well as in a higher percentage of immunocompromised patients.^{7,8}

The pathogenesis of *B henselae* in cats is not clearly understood. Cats naturally infected with *B henselae* may have recurrent periods of bacteremia that may last months to years, and these cats do not have clinical signs of disease during these periods of bacteremia.⁹ In experimentally infected cats, there are conflicting reports with regard to clinical signs of disease, ranging from cats that do not have signs of disease^{10,11} to those with only mild fever^{12,13} to those with reproducible CSD-like disease¹⁴ and CNS abnormalities.^{13,14}

In the study reported here, we determined the pathogenic events after ID inoculation of kittens with the LSU16 strain of *B henselae*, which included bacteremia, antibody responses, and clinical signs of disease, and compared these results with those of another study¹⁴ in which adult cats were infected. Furthermore, we attempted to ascertain the relevance of the observed clinical disease with management of infected kittens.

Materials and Methods

Animals—Eighteen 12-week-old kittens (12 females, 6 males), purchased as specific-pathogen-free kittens,⁹ were used in this study. All kittens had negative results when cultured for *B henselae* and were seronegative for *B henselae* on the basis of results of western immunoblot analysis.¹⁵ Kittens were housed in groups; noninoculated kittens were housed in a separate room from inoculated kittens. All kittens had unlimited access to water and were fed a maintenance diet ad libitum.

Isolation and propagation of *B henselae* strain LSU16—An initial isolate of *B henselae* strain LSU16 was expanded twice on chocolate agar, using culture conditions of 5% CO₂ and 37 C. Bacterial colonies were harvested 5 to 8 days after initiation of culture, suspended in heart-infusion broth with 25% glycerol, and stored as aliquots at -70 C. Before inoculation into kittens, these aliquots of *B henselae* were thawed, and the culture was centrifuged to remove cryoprotectant medium. The bacterial pellet then was resuspended in saline (0.9% NaCl) solution and diluted to the appropriate concentration prior to inoculation. Number of colony-forming units (CFU) per milliliter and purity of the

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inoculum was confirmed by culturing serial dilutions on chocolate agar, using 5% CO₂ at 37 C for 7 days. Bacteremia in experimentally inoculated kittens was determined by culturing serial dilutions of blood on chocolate agar, using 5% CO₂ at 37 C for 7 days. Strain LSU16 is a type-II *B henselae*, as determined by use of the method of Bergmans et al¹⁶ and sequencing of the fragment.

Inoculation of kittens—Nine kittens (6 females, 3 males) were inoculated with 2 × 10⁷ CFU of strain LSU16 bacterial culture in 1 ml of saline solution. The inoculum was administered ID; it was divided among each of 6 sites in the skin on the lateral aspect of the trunk. Sites of inoculation were marked with water indelible marker and monitored daily. The remaining 9 kittens were administered 1 ml of sterile saline solution, ID, divided among the 6 sites in the skin on the lateral aspect of the trunk.

Collection of samples—Kittens were anesthetized with tiletamine hydrochloride,^b using the dosage recommended by the manufacturer. To minimize the stress associated with collection of blood samples, the inoculated and noninoculated groups were each allocated into 2 subgroups. Samples were collected from 1 of the inoculated subgroups and 1 of the noninoculated subgroups one week, whereas samples were obtained from the other subgroups the following week; therefore, blood samples were collected from each kitten every other week.

Approximately 6 ml of blood was collected by jugular puncture, using a 10-ml syringe and 21-gauge needle. Blood samples were distributed among 1.5-ml pediatric lysis-centrifugation isolator tubes^c (for bacterial culture), tubes containing EDTA (for a CBC), and evacuated serum tubes^d (for serologic testing). Serum was obtained by centrifuging clotted blood at 800 × g, and harvested serum was frozen in aliquots at -20 C.

Clinical signs of disease—All cats were monitored daily to detect clinical signs of disease. To enumerate clinical disease, each of the following clinical signs was assessed: swelling or redness at the site of inoculation, fever (rectal temperature of 39.5 to 40.5 C) high fever (rectal temperature > 40.5 C), palpable lymphadenopathy (popliteal, prescapular, or submandibular lymph nodes), diarrhea, vomiting, anorexia, aggression, and lethargy. Each kitten was assigned a clinical score that was determined on the basis of 1 point for each of the aforementioned clinical signs observed in the course of 1 week.

Analysis of antibody responses to *B henselae* strain LSU16—The IgG and IgM responses to *B henselae* strain LSU16 were monitored by use of an ELISA, as described elsewhere.¹⁵ Briefly, plates were coated with 100 µl of *B henselae* strain LSU16 at a concentration of 1 µg/ml and incubated overnight at 4 C. Serum from the kittens was diluted 1:100 in 0.25M NaCl, 0.01M tris, 0.001M EDTA (pH 7.5 [high-salt NET buffer]) and 1% bovine serum albumin. After a 30-minute incubation at 22 to 25 C, plates were washed with high-salt NET buffer plus 0.005% Tween 20. Goat anti-cat IgG^e conjugate (1:10,000) or goat anti-cat IgM^f conjugate (1:6,500) was added, and plates were incubated for another 30 minutes at 22 to 25 C. Plates were washed as described previously. 3,3',5,5'-tetramethyl-benzidine substrate solution^f was added, and the reaction was stopped after 10 minutes by addition of 50 µl of 0.1M sulfuric acid. Results were read on a spectrophotometer at 450 nm.

Statistical analysis—Data were analyzed, using a repeated-measures ANOVA in a split-plot design. Experimental group (inoculated vs noninoculated) and individual cat formed the main plot that was the focus of this study. Week and week-by-group interactions formed the subplots.

Comparisons of the experimental group main-effects means were conducted with a Tukey test. Raw data of each response variable and their respective ranked values were analyzed. All analyses were considered significant at P < 0.05.

Results

Bacteremia in kittens inoculated with strain LSU16—All 9 kittens inoculated with *B henselae* strain LSU16 became bacteremic by the second week after inoculation and maintained a high degree of bacteremia (Fig 1). Peak of bacteremia was between week 1 (4 kittens) and week 3 (3 kittens). Number of bacteria per milliliter of blood began to wane 4 weeks after inoculation, and 5 kittens had negative results by 8 weeks after inoculation. Only 1 kitten had recurrent bacteremia during the 30 weeks of the study, and that kitten had 100 CFU/ml of blood for only 1 week (25 weeks after inoculation).

Clinical disease in kittens inoculated with strain LSU16—Differences in clinical signs observed in the kittens were recorded (Table 1). Mean clinical scores were

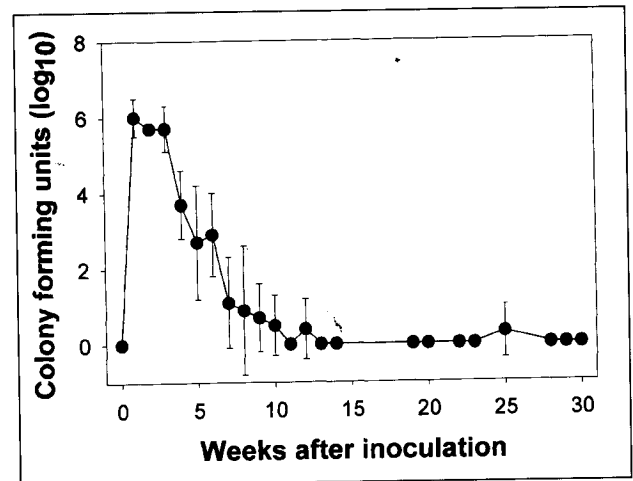


Figure 1—Bacteremia in kittens inoculated with *Bartonella henselae* strain LSU16, as determined by mean (± SD) No. of colony forming units/ml of blood. Organisms were not cultured from blood samples of noninoculated kittens at any time during the course of the study.

Table 1—Clinical signs detected in 12-week-old kittens that were inoculated with saline (0.9% NaCl) solution (noninoculated) or with *Bartonella henselae* strain LSU16 (inoculated)

Clinical signs	Noninoculated kittens* (n = 9)		Inoculated kittens* (n = 9)	
	No.	(%)	No.	(%)
Fever†	0/15	(0)	9/9	(100)
Lethargy	0/15	(0)	9/9	(100)
Swelling or redness at site of inoculation	0/9	(0)	9/9	(100)
Pustule at site of inoculation	0/9	(0)	5/9	(56)
Anorexia	0/9	(0)	6/9	(67)
Anorexia requiring force-feeding or fluids	0/9	(0)	0/9	(0)
Vomiting	0/9	(0)	1/9	(11)
Muscle pain or stiffness	0/9	(0)	3/9	(33)
Abnormal or aggressive behavior	0/9	(0)	7/9	(78)
Lymphadenopathy	2/9	(22)	9/9	(100)

*No. of cats that had the indicated sign of disease at any time during the study; percentages are provided in parenthesis. †Rectal temperature > 39.4 C.

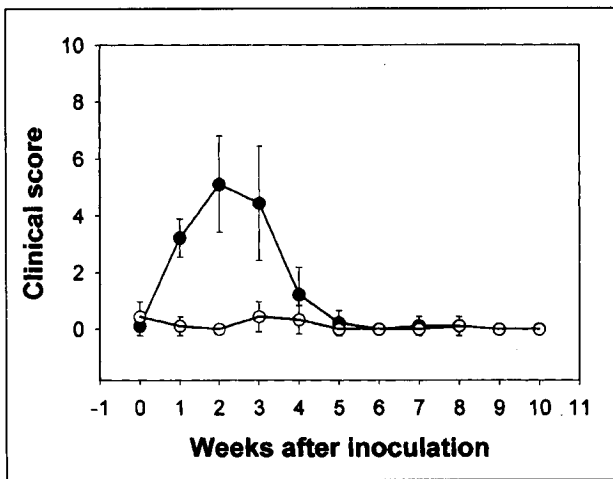


Figure 2—Clinical scores (mean \pm SD) for 9 kittens inoculated with *B henselae* strain LSU16 (●) and 9 noninoculated kittens (○).

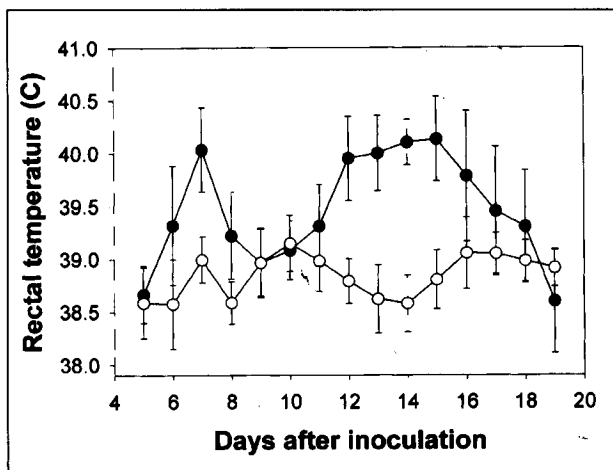


Figure 3—Characterization of fever in 9 kittens inoculated with *B henselae* strain LSU16 (●) and 9 noninoculated kittens (○). Values are mean (\pm SD) rectal temperature.

determined (Fig 2) and were significantly different between the groups (mean clinical score, 1.2 for inoculated vs 0.1 for noninoculated; $P < 0.05$; clinical score ranking, 114.47 for inoculated vs 84.53 for noninoculated; $P < 0.05$). All *B henselae*-inoculated kittens developed lesions at the sites of injection. These lesions started to develop by 72 hours after inoculation, became red and hard by 10 days after inoculation, and gradually regressed during the subsequent 14 to 18 days. All kittens exposed to *B henselae* developed fever. The fever had a biphasic pattern, with an episode of 1- to 3-days' duration beginning 6 to 7 days after inoculation, followed by a second episode of 3- to 8-days' duration beginning 11 to 13 days after inoculation (Fig 3). The rectal temperatures were significantly different between the 2 groups (mean rectal temperature 39.5 C for inoculated vs 38.8 C for noninoculated, $P < 0.05$; rectal temperature ranking 165.5 for inoculated vs 93.5 for noninoculated; $P < 0.05$). All 9 kittens became relatively sluggish and lethargic between 6 and 21 days after inoculation. In addition, all 9 inoculated kittens were observed to have a decrease in appetite, but none required forced feeding or SC administration of replacement fluids. Amount of

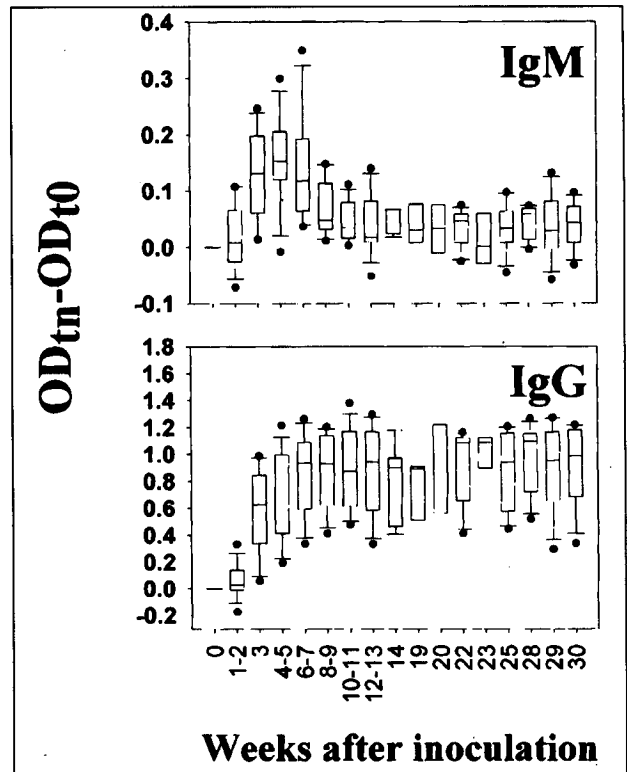


Figure 4—The IgM (top) and IgG (bottom) antibody responses of 9 kittens inoculated with *B henselae* strain LSU16. Antibody indices for each kitten were calculated by subtracting the optical density determined for samples obtained before inoculation (OD_{t0}) from the optical density determined for samples obtained at various times after inoculation (OD_{tn}). Index range (25th to 75th percentile) is indicated by boxes. Lower and upper bars indicate 10th and 90th percentiles, respectively. Mean index value for each week is indicated by the center bar. Black dots represent values outside calculated ranges. Each bar represents samples obtained from the 9 kittens. Some samples, as indicated on the x-axis, were obtained during a 2-week period.

weight gain did not differ between inoculated and noninoculated kittens. Similarly, differences were not detected between inoculated and noninoculated kittens for percentage or absolute number of RBC, WBC, lymphocytes, monocytes, or neutrophils.

Palpable enlargement of regional lymph nodes was evident in all 9 inoculated kittens. The enlargement of the lymph nodes followed the onset of bacteremia and resolved within 3 weeks. Kittens were not remarkably sensitive in the area of nodal enlargement. Transient enlargement of lymph nodes was observed in some noninoculated cats (Table 1).

Most of the inoculated kittens had various additional clinical signs, including muscle tenderness or evidence of pain, particularly along the vertebral column. Mild signs, consistent with CNS involvement, included unusual aggressiveness, unusual sensitivity to noise and touch, and reduced responsiveness to external stimuli in 7 inoculated kittens. These signs were evident approximately 7 days after inoculation and were completely resolved in all kittens by 21 days after inoculation. Fever, lethargy, anorexia, or lymphadenopathy were not observed in 1 kitten during the episode of recurrent bacteremia 25 weeks after inoculation.

Antibody responses to inoculation with strain LSU16—Antibody responses to inoculation with strain LSU16 were monitored by use of an ELISA. All kittens inoculated with *B henselae* strain LSU16 had high concentrations of IgG by 4 weeks after inoculation, which were maintained for the duration of the study (Fig 3). We also examined the IgM response to strain LSU16, and observed measurable concentrations of IgM by 3 weeks after inoculation, which began to wane by 6 weeks after inoculation (Fig 4).

Discussion

Until recently, cats were considered to be inapparent carriers of *B henselae*. In the study reported here, *B henselae* strain LSU16 caused reproducible, clinically characteristic disease in kittens. In all 9 kittens, we observed redness and swelling at the site of inoculation. Additionally, all kittens were febrile, anorectic, and lethargic, and myalgia, behavioral or neurologic changes, and lymphadenopathy were evident in most of the kittens. These signs are compatible with those reported in adult cats after infection with strain LSU16¹⁴ and are also similar to those reported in humans with moderate to severe CSD.⁶ Similar to results for our study of adult cats,¹⁴ we did not observe tenderness in the palpably enlarged lymph nodes of those kittens with lymphadenopathy. This may have been attributable to the fact that the accessible lymph nodes (submandibular, prescapular, and popliteal) were not the nodes draining the site of inoculation.

Antibody responses in kittens inoculated with strain LSU16 also were similar to those of adult cats, although the response appeared to be somewhat slower in onset in kittens than adult cats.¹⁴ The IgM concentration decreased during the course of the study but did not return to baseline values, suggesting continued antigen stimulation. Inoculated kittens had increased concentrations of IgG during the second week after inoculation, which continued to increase through week 8 after inoculation and then were maintained throughout the duration of the study.

Clinical signs observed in kittens following inoculation with *B henselae* strain LSU16 are consistent with the timing and clinical signs seen in adult cats,¹⁴ with the following 2 exceptions: lethargy and anorexia were less severe in kittens than adult cats, and kittens developed fever that had a biphasic pattern, in contrast to the single period of fever observed in adult cats. Whether the decrease in lethargy and anorexia in kittens, compared with that for adult cats, is a real phenomenon or merely reflective of differences in typical amounts of activity is problematic. In fact, although a decrease in food consumption was observed in all inoculated kittens, they continued to gain weight, and their weight gain was not significantly different from that of noninoculated kittens.

One interesting observation was that the kittens reported here had fever with a biphasic pattern (Fig 3), which differed from the single episode of fever seen in adult cats.¹⁴ In both studies, bacteremia was uniformly high during the febrile period. Although there has not been a direct comparison of fever patterns of kittens and adult cats, a biphasic pattern similar to that observed in

the kittens in this study also was observed in 14- to 16-week-old kittens infected with feline infectious peritonitis virus.¹⁷ Proinflammatory cytokines interleukin-1, tumor necrosis factor α , and interleukin-6 are endogenous pyrogens that lead to the production of fever-inducing prostaglandins.¹⁸⁻²⁰ It is possible that kittens produce more of these cytokines or are more sensitive to the activity of these cytokines than adult cats. The mechanisms responsible for the biphasic pattern of fever observed in these kittens after infection with *Bartonella* organisms and other agents should be investigated.

Analysis of the results of this study confirms the pathogenic potential of the LSU16 strain of *B henselae* in kittens and documents that the observed disease is not dependent on age. Furthermore, if the disease we observed in association with strain LSU16 is characteristic of many strains of *B henselae*, this observation could have important implications in veterinary and human medicine. In veterinary medicine, fever, lymphadenopathy, anorexia, and lethargy of unknown origin commonly are observed in kittens. We suggest that many of these cases may be the result of primary infection of kittens with *B henselae*. It has been documented that *B henselae* infection is common in kittens, with serologic surveys revealing infection rates as high as 91%.²¹ Analysis of our results suggests that clinical disease is observed when bacteremia is highest and that once a kitten recovers from the primary infection, bacteremia can recur without clinical signs. A role for fleas in the biological characterization of *B henselae* has been established²²; in that study, our laboratory group determined that feces from fleas feeding on bacteremic cats can serve as an infective source of *B henselae*.

In human medicine, immunocompromised patients are at risk of developing serious diseases, including encephalopathy, bacillary angiomatosis, and bacillary peliosis, if exposed to *B henselae*. We believe that it is during the period of bacteremia that cats are most likely to transmit the organism to humans and other cats. We have suggested that the potential for transmission of *B henselae* from cats to humans could be reduced by adequate flea control.²² On the basis of the results of the study reported here and other related studies, we suggest that kittens and cats with fever, anorexia, and lethargy of unknown origin be screened for *B henselae* bacteremia and that interactions between affected cats or kittens and susceptible people be limited. We believe this approach, combined with rigorous flea control, may provide a means for decreasing the incidence of CSD and its sequelae in humans.

Finally, it is difficult to clear *B henselae* from chronically infected kittens by using antibiotics.¹⁰ It is possible that antibiotic intervention may be more effective during the primary bacteremia, before the establishment of chronic infection. Fever, anorexia, and lethargy in kittens are detectable clinical indications that could suggest early *Bartonella* infection and may provide a landmark for therapeutic intervention. We suggest that the effectiveness of treatment with antibiotics early in the course of infection needs to be assessed.

^aHsdCpb: CADS(APD), Harlan Sprague-Dawley, Madison, Wis.

^bTelazol, Fort Dodge, Ames, Iowa.

^cIsolator 1.5-ml Microbial Tubes, Wampole Laboratories, Cranbury, NJ.

^dVacutainer, Becton-Dickinson, Franklin Lakes, NJ.

^eGoat anti-cat IgG (Fc Fragment)-horseradish peroxidase conjugate and goat anti-cat IgM (μ -chain specific)-horseradish peroxidase conjugate, Bethyl Laboratories Inc, Montgomery, Tex.

^fTMB Microwell Peroxidase Substrate System, Kirkegaard & Perry Laboratories, Gaithersburg, Md.

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