

Bartonella henselae Infection in Cats: Evaluation during Primary Infection, Treatment, and Rechallenge Infection

C. E. GREENE,* M. McDERMOTT, P. H. JAMESON, C. L. ATKINS, AND A. M. MARKS

Department of Small Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602

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***Bartonella henselae* infection was established in eight cats of various ages by experimental inoculation. All cats remained persistently bacteremic until they were treated 4 to 7 weeks after primary inoculation. Antibody titers increased and peaked between 4 and 12 weeks for all cats. Treatment with doxycycline for 1 week was effective in suppressing bacteremia in all cats but was effective in clearing infection from only four cats. Amoxicillin, given subsequently, was effective in clearing the infection from three of the remaining cats. One kitten that remained bacteremic was treated unsuccessfully with enrofloxacin, and its bacteremia was finally cleared when it was treated with a clavulanate-amoxicillin combination. After the bacteremia was cleared, with a corresponding reduction in serum antibody titers, all eight cats were rechallenged with *B. henselae*. None of the cats became bacteremic after secondary challenge, and all had higher and more rapid increases in serum antibody titers than after primary inoculation. The cats became resistant to reinfection following recovery from infection, indicating that immunoprophylaxis in cats might be beneficial in helping to reduce their public health risk.**

Bartonella henselae has been associated with a spectrum of human diseases including cat scratch disease, bacillary angiomatosis, visceral peliosis, bacteremia, and endocarditis (1, 5, 7, 9, 15–18). The discovery and isolation of *B. henselae* as the predominant cause of these diseases has accelerated understanding of the epidemiology and pathogenesis of these diseases. Serologic and cultural studies indicate that a high percentage of cats have been naturally exposed to or are infected with this organism (2–4, 8, 9). Seroprevalence varies according to geographic locale and is influenced by climate, probably because of vector maintenance (8). Naturally infected cats appear to remain asymptomatic and bacteremic for many months if they are left untreated (9, 10). Bacteremic cats have been more likely than nonbacteremic cats to be of younger age and to be infected with fleas (9, 19). Seropositive cats either are infected or have recovered from an infection, while seronegativity correlates with the absence of bacteremia (4). Bacteremia can persist in the presence of variably high antibody titers (11). Serologic screening has been suggested as a means of determining a cat's suitability as a pet (4). The objectives of the study were to determine the onset and persistence of bacteremia and seroconversion in experimentally inoculated cats, to see if age had an influence on their course of infection, to determine the efficacies of various antimicrobial drugs in the treatment of these infected cats, and to determine the ability to reinfect successfully treated, previously seroconverted cats.

MATERIALS AND METHODS

Animals. Eight acclimatized cats (five intact females, two males, and one neutered male) from random sources were used in the study. At the onset of the study four of the cats were mature (ages, >1 year), two were adolescent (ages, 3 to 4 months), and two were recently weaned kittens (ages, 8 weeks). The neutered male had been declawed. The cats were matched by age and were then split into two corresponding groups (groups A and B) of four cats each (two adults, one adolescent, and one kitten). They had been prescreened for inclusion in the study by having negative blood culture results and serum antibody titers to *B.*

henselae. The cats' sera tested negative for feline leukemia virus antigen and antibodies to feline immunodeficiency virus and feline infectious peritonitis virus. The cats were housed according to U.S. Department of Agriculture standards in indoor rooms free of ectoparasites and in separate cages with two facing banks of four cages each. They were given food and water ad libitum. The cats were allowed to exercise outside their cages in two groups (groups A and B) of four cats each for 1-h periods daily, but the cats in each group were not allowed to commingle for extended periods.

Samples were collected from the cats on a weekly basis by a protocol approved by the Animal Care and Use Committee, University of Georgia. Briefly, the cats were sedated with ketamine hydrochloride (Ketaset; Fort Dodge Laboratories, Fort Dodge, Iowa). Blood (2.5 ml) was collected aseptically from the jugular vein, and 1 ml was inoculated into sterile vacuum tubes containing EDTA (Vacutainer; Becton Dickinson, Rutherford, N.J.) for culture. The remaining blood, 1.5 ml, was placed in a glass tube, and after clotting, the serum was separated by centrifugation and was frozen at -70°C until testing was performed.

Indirect fluorescent-antibody test. The indirect fluorescent-antibody test was conducted as described previously (14) by using the Houston-1 isolate (ATCC 49882) of *B. henselae* cocultivated on Vero cells and measuring specific serum immunoglobulin G titers. Twofold dilutions of serum in phosphate-buffered saline ranging from 1:32 to 1:8,192 were used. For all indirect fluorescent-antibody test measurements, scoring was based on the highest dilution in which intact stained extracellular bacilli were observed. Positive and negative control sera were used each time that the assay was performed. For linear graphing of the data, \log_2 titers were determined.

Blood culture. Anticoagulated whole blood (0.3 ml) was streaked directly onto brain heart infusion (BHI) agar containing 5% bovine blood, and the plates were incubated in a 3% CO_2 environment at 37°C for 21 days. The cultures were examined daily for bacterial growth, and colony numbers were counted at 21 days. This number multiplied by 3.33 was recorded as the number of CFU per milliliter of blood. A sterile inoculating loop was used to place colonial material in a 0.5-ml volume of BHI agar, and these volumes were frozen in separate vials at -70°C for later confirmation of the identities of the organisms. Weekly confirmation of the bacterial isolates from each cat as *B. henselae* was performed by PCR-restriction fragment length polymorphism analysis of the citrate synthase gene as described previously (13).

Challenge inoculum. A first-passage *B. henselae* isolate (isolate Houston-1) cultured from the blood of a febrile patient infected with human immunodeficiency virus (15) was used as the challenge inoculum to infect all cats. This isolate was obtained from Russ Regnery, Centers for Disease Control and Prevention, Atlanta, Ga. A frozen (-70°C) stock suspension was thawed to room temperature prior to use; the inoculum contained a concentration of 10^7 CFU/ml in liquid BHI medium, as determined by culturing serial dilutions at the times of inoculation. An area of skin on the caudal ventral abdomen was clipped and prepared aseptically. A circle was drawn with an indelible pen on the abdominal wall to assist in locating the lesion for subsequent monitoring. Cats were inoculated with 0.14 ml of inoculum, with half given intradermally and half given subcutaneously. Corresponding control cats were inoculated with an equal volume of sterile liquid BHI medium.

* Corresponding author. Mailing address: Department of Small Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602. Phone: (706) 542-3221. Fax: (706) 542-6460.

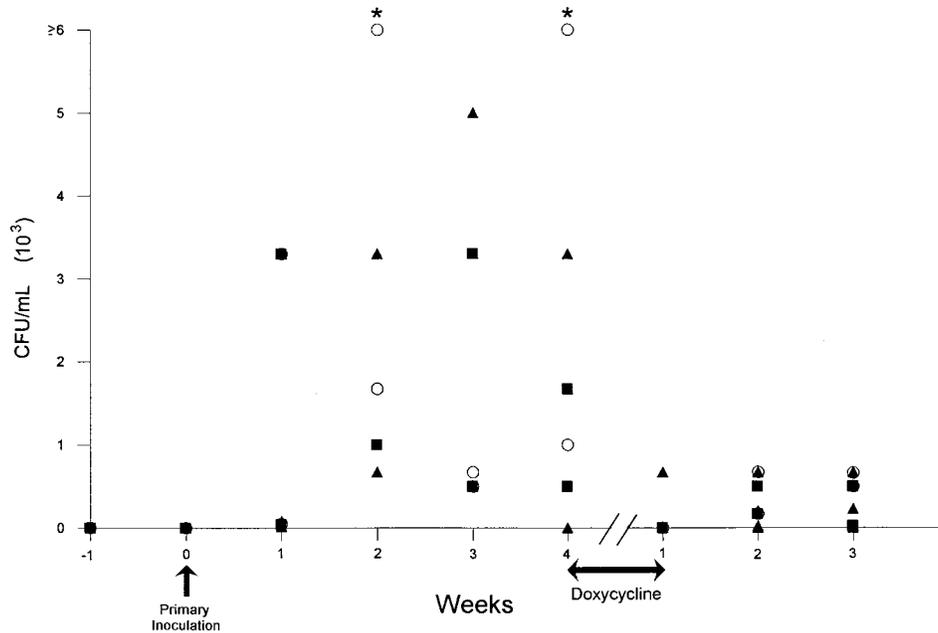


FIG. 1. Blood culture results for eight cats with *B. henselae* infection first inoculated on week zero. Doxycycline was given to each cat for 1 week during the 4- to 7-week p.i. interval. Colony counts (in CFU per milliliter) are shown weekly for 3 weeks, beginning at the end of 1 week of doxycycline therapy. The break along the horizontal scale indicates the variable 4- to 7-week p.i. period prior to treatment. Asterisks indicate counts of $\geq 6 \times 10^3$ CFU/ml. ▲, adult cats; ■, adolescent cats; ○, kittens.

Experimental infections. There were 4 weeks of preliminary acclimatization and baseline sampling for primary inoculation, and then one group of cats was challenged with the *B. henselae* isolate. The cats were bled weekly thereafter for blood culture and serum immunoglobulin G titer measurements. During the primary challenge inoculation and follow-up treatment of group A cats, the uninfected group B cats served as corresponding controls. Cats were subsequently treated with antimicrobial agents to clear their infections and were then given a secondary challenge in a manner and with an inoculum identical to those for the primary challenge. During the secondary challenge reinfection study of group A cats, the primary challenge of group B cats was the corresponding control for the inoculation. When the group B cats received their secondary challenge infection there were no simultaneous controls.

Antimicrobial treatment. Following primary inoculation, persistent bacteremia was established by four positive sequential weekly blood cultures. At that time, infected cats and corresponding control cats were treated for 1 week with oral antibiotics. In the event that the infection was not cleared in an infected cat, as determined by not having at least four successive negative weekly blood cultures, additional antibiotics were tried. Each drug used was given for a 1-week period. At the time of treatment, the cats' weights ranged from 2.3 to 6.8 kg, and dosing was based upon giving appropriate sizes of whole oral tablets as supplied by the manufacturers. The order of drug administration was as follows: doxycycline (50 mg per cat twice daily [BID]; doxycycline hyclate; Schein Pharmaceutical Inc., Port Washington, N.Y.), amoxicillin (50, 100, or 200 mg per cat BID; Amoxi-Tabs, Pfizer Animal Health, Exton, Pa.), enrofloxacin (22.7 mg per cat BID; Miles Inc., Animal Health Products, Shawnee Mission, Kans.), and amoxicillin-clavulanate (62.5 mg per cat three times daily; Clavamox; Pfizer Animal Health). The calculated dosage ranges were 7.3 to 21.7 mg/kg of body weight BID for doxycycline, 18.5 to 43.5 mg/kg BID for amoxicillin, 8.4 mg/kg BID for enrofloxacin, and 23.5 mg/kg three times daily for amoxicillin-clavulanate. Enrofloxacin, a fluoroquinolone antibacterial agent licensed for veterinary use, was selected to test the efficacy of this newer class of drugs against *B. henselae* in vivo.

RESULTS

Blood cultures. Infection was readily established in cats receiving an inoculum containing live *B. henselae* organisms but not in the corresponding controls. Only cats receiving an inoculum containing organism developed a raised circumscribed lesion at the site of inoculation beginning 4 days postinoculation (p.i.) and lasting for 2 to 4 weeks. Regional (inguinal) lymphadenomegaly was also observed during this interval. All cats were bacteremic (17 to 3,300 CFU/ml) when their blood

was sampled the first week p.i. (Fig. 1). During a 4- to 7-week period p.i., a persistent bacteremia could be documented in all cats (peak count range, 999 to 33,300 CFU/ml), with one kitten showing the highest values. Subsequently, all eight cats received a 1-week course of doxycycline therapy. All except one adult cat had a clearing of the bacteremia within 1 week after doxycycline treatment was instituted (Fig. 1). Four cats (three adults and one adolescent) became abacteremic during a subsequent 4-week post-doxycycline treatment observation interval, and no further antimicrobial therapy was required. Four other cats had increasing levels of bacteremia on subsequent weekly examinations (Fig. 1). The level of recrudescence bacteremia (as determined by the number of CFU per milliliter) never approached that noted during the pretreatment period (peak count range, 6 to 3,300 CFU/ml). The four remaining cats with bacteremia were subsequently treated with amoxicillin for 1 week beginning 4 to 5 weeks after doxycycline was discontinued. Infection was cleared from three of the cats within a period of 1 to 2 weeks after treatment and remained cleared for an additional 5 weeks (Fig. 2). The one remaining cat (a kitten) had a reduction in the level of bacteremia after amoxicillin treatment but remained culture positive, with counts ranging from 3 to 3,300 CFU/ml. This kitten inadvertently received 50-mg tablets instead of the 100-mg tablets that were given to the other kitten and adolescent cat. In comparison, the adult cat had received 200-mg tablets. The kitten receiving the 50-mg tablet was subsequently treated with enrofloxacin, with minimal change in the number of bacteria in its blood (range, 330 to 832 CFU/ml) during 4 weeks of posttreatment observation (data not shown). This kitten was then treated with a clavulanate-amoxicillin combination, and the bacterial concentration in the kitten's blood decreased to <20 CFU/ml for the next 2 weeks and then the bacteremia cleared by the third week (data not shown). The cat was not bacteremic during an additional 4-week period of monitoring. All eight cats were determined to be cleared of bacteremia by having

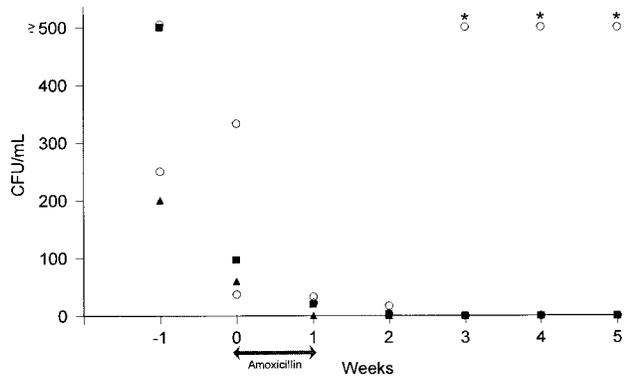


FIG. 2. Blood culture results for four cats with remaining *B. henselae* bacteremia after 1 week of doxycycline treatment. Amoxicillin was administered for 1 week beginning 4 to 5 weeks after doxycycline therapy was completed. The horizontal scale has been adjusted so that the onset of ampicillin treatment coincides with time zero. Asterisks indicate greater than 500 CFU/ml. ▲, adult cats; ■, adolescent cats; ○, kittens.

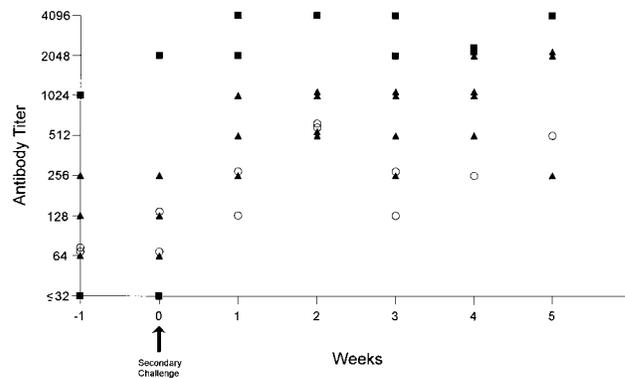


FIG. 4. Antibody titers in cats that were previously infected and treated and subsequently rechallenged with *B. henselae* on week zero. None of the cats became bacteremic after this challenge. ▲, adult cats; ■, adolescent cats; ○, kittens.

negative blood cultures for a minimum of 4 weeks before secondary challenge infection. None of the eight cats became bacteremic after rechallenge when they were thereafter monitored for a minimum of 4 weeks.

Antibody titers. During primary inoculation, mean antibody titers were increased by the second week p.i. (Fig. 3). Mean titers peaked between 3 and 12 weeks after inoculation. The range of peak antibody titers in the cats was 128 to 512 except in one adolescent cat, which had a peak titer of 4,096. By 11 to 14 weeks p.i. and just prior to rechallenge, the titers in five cats were at or below 64, with two cats having intermediate levels of reactivity (titers, 128 to 256) and the adolescent described above having a titer of 1,024 (Fig. 4). Subsequent to rechallenge, the antibody titers increased more rapidly and at a higher level than those after the primary exposure (compare Fig. 3 and 4). The adolescent cats had the highest postchallenge absolute peak titers (4,096), with the adult cats and kittens having lower peak titers (range, 512 to 4,096 and 512, respectively).

DISCUSSION

Cats appear to be very efficient reservoir hosts for infection with *B. henselae*. In screening naturally infected populations, a

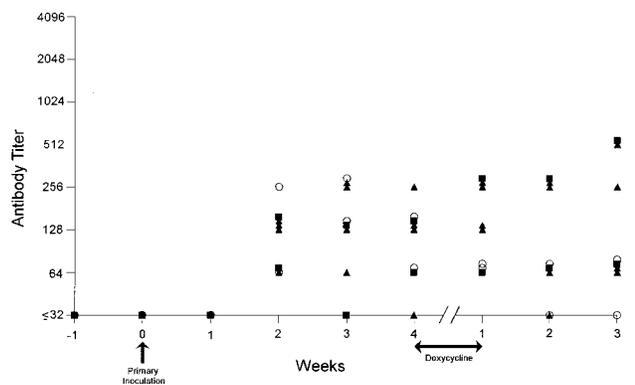


FIG. 3. Antibody titers in eight cats that were first inoculated with *B. henselae* on week zero and subsequently treated with doxycycline. The break along the horizontal scale indicates the variable (4- to 7-week p.i.) period during which 1 week of doxycycline therapy was administered. ▲, adult cats; ■, adolescent cats; ○, kittens.

high percentage of cats are seropositive and asymptotically infected (4, 8, 9, 12). In one study, the blood of 52% of bacteremic cats had colony counts in culture exceeding 1,000 CFU/ml of blood (4). Similar high counts were demonstrated for these experimentally inoculated cats that developed no clinical symptomatology. Younger age of cats has been a predisposing factor noted in epidemiologic studies of *B. henselae* infections of people (4, 9, 19). The kittens in the present study had lower antibody titers than the older cats following secondary challenge; however, evaluation of larger populations of experimentally infected cats will be required to corroborate this finding. Antibiotic therapy may have reduced the rise and persistence of high antibody titers during primary challenge because of the premature clearance of the bacteremia.

Cats remained bacteremic in the present study until treatment was instituted. Various antibiotics appeared to be effective in reducing bacterial counts in blood. In most cats, blood cultures were either negative or the titers were low when the cats were examined after 1 week of therapy. By following these treated cats for several weeks after therapy was discontinued, subsequent bacteremia ensued. We attribute the resurgence of bacteremia to ineffective clearing of infection and not reinfection. Control cats housed with the infected cats in vector-free environments in these and our other studies (6) have not become infected after 3 to 5 months of cohabitation. Koehler et al. (9) reported successful clearing of bacteremia in two naturally infected cats given doxycycline for 30 days and in one cat given doxycycline and lincomycin intermittently because the doxycycline caused vomiting. One remaining cat receiving only two doses of doxycycline was not effectively treated.

Unfortunately, the duration of follow-up to determine the effective clearing of the bacteremia was not reported by those investigators. The cats in the present study were treated with each antimicrobial agent for 1 week. The reason for our use of a shorter treatment interval and longer monitoring period than those described by Koehler et al. (9) was to allow us to compare the in vivo efficacies of several drug regimens. If doxycycline had been administered for longer than 1 week, the treatment may have been more beneficial. Doxycycline was used in lieu of tetracycline in the present study because it is more lipid soluble and thus has greater bioavailability when it is administered orally. In the present study, whole tablets were given for convenience of dosing, and higher than recommended dosages were used to facilitate treatment of the bacteremia. The dosages used may be an important consideration, because in the

one kitten that inadvertently received 50- instead of 100-mg amoxicillin tablets, treatment was unsuccessful.

All cats in our study were eventually treated, because one of our main objectives was to follow the immune responses of cats after they had successfully eliminated their bacteremia. A disadvantage of our model is that we could not compare the natural course of elimination of bacteremia because of treatment in our cats. From previous studies, it is apparent that experimentally infected, untreated cats can remain asymptomatic and bacteremic for 1 year or longer (9, 10, 12). Using the same Houston-1 strain inoculum to experimentally infect untreated cats, we have found that the bacteremia persists for at least 12 to 14 weeks (6). Further evidence that the bacterial counts were reduced because of treatment rather than because of recovery from infection with time is the dramatic and consistent reduction in the bacterial count noted immediately after each treatment. Only enrofloxacin, which was used to treat the persistently infected kitten, did not seem to be at all effective in reducing or eliminating infection. Since all cats in the present study received the drugs in succession in a predictable order, it is possible that the drugs used later appeared to be more effective because of the repeated courses of treatment that were used.

All eight cats appeared to be resistant to rechallenge infection by developing a much higher and more rapid antibody response than that which occurred following primary infection and by not becoming bacteremic. One potential explanation would be a residual effect that could have resulted from antimicrobial treatment interfering with subsequent reinfection. This possibility is dismissed because the group B cats were given antimicrobial therapy along with the group A cats. When they were challenged simultaneously, the group A cats, which were receiving their secondary exposure, did not become reinfected, but the corresponding group B cats developed bacteremia during their primary inoculation. Judging from the resistance of treated cats to reinfection, the potential for immunization of cats appears feasible. By preventing bacteremia in the feline reservoir host, the risk of transmission from cats to people through insect vectors or by traumatic exposure could be minimized.

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REFERENCES

- Anderson, B., K. Sims, R. Regnery, L. Robinson, M. J. Schmidt, S. Goral, C. Hager, and K. Edwards. 1994. Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J. Clin. Microbiol.* **32**:942-948.
- Childs, J. E., J. G. Olson, A. Wolf, N. Cohen, Y. Fakile, J. A. Rooney, F. Bacellar, and R. L. Regnery. 1995. Prevalence of antibodies to *Rochalimaea* species (cat-scratch disease agent) in cats. *Vet. Rec.* **20**:519-520.
- Childs, J. E., J. A. Rooney, J. L. Cooper, J. G. Olson, and R. L. Regnery. 1994. Epidemiologic observations on infection with *Rochalimaea* species among cats living in Baltimore, Maryland. *J. Am. Vet. Med. Assoc.* **204**:1775-1778.
- Chomel, B. B., R. C. Abbott, R. W. Kasten, K. A. Floyd-Hawkins, P. H. Kass, C. A. Glaser, N. C. Pedersen, and J. E. Koehler. 1995. *Bartonella henselae* prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. *J. Clin. Microbiol.* **33**:2445-2450.
- Clarridge, J. E., III, T. J. Raich, D. Pirwani, B. Simon, L. Tsai, M. C. Rodriguez-Barradas, R. Regnery, A. Zollo, D. C. Jones, and C. Rambo. 1995. Strategy to detect and identify *Bartonella* species in routine clinical laboratory yields *Bartonella henselae* from human immunodeficiency virus-positive patient and unique *Bartonella* strain from his cat. *J. Clin. Microbiol.* **33**:2107-2113.
- Greene, C. E. Unpublished data.
- Jackson, L. A., B. A. Perkins, and J. D. Wenger. 1993. Cat scratch disease in the United States. *Am. J. Public Health* **83**:1707-1711.
- Jameson, P., C. Greene, R. Regnery, M. Dryden, A. Marks, J. Brown, J. Cooper, B. Glaus, and R. Greene. 1995. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. *J. Infect. Dis.* **172**:1145-1149.
- Koehler, J. E., C. A. Glaser, and J. W. Tappero. 1994. *Rochalimaea henselae* infection: a new zoonosis with the domestic cat as reservoir. *J. Am. Vet. Med. Assoc.* **271**:531-535.
- Kordick, L. A., and E. B. Breitschwerdt. 1995. Itraerythrocytic presence of *Bartonella henselae*. *J. Clin. Microbiol.* **33**:1155-1156.
- Kordick, D. L., and E. B. Breitschwerdt. 1995. Blood transmission of *Bartonella henselae* in kittens, abstr. B-352, p. 226. *In* Program and abstracts of the 95th General Meeting American Society Microbiology 1995. American Society for Microbiology, Washington, D.C.
- Kordick, D. L., K. H. Wilson, D. J. Sexton, T. L. Hadfield, H. A. Berkhoff, and E. B. Breitschwerdt. 1995. Prolonged *Bartonella* bacteremia in cats associated with cat scratch disease patients. *J. Clin. Microbiol.* **33**:3245-3251.
- Norman, A. F., R. Regnery, P. H. Jameson, C. E. Greene, and D. C. Krause. 1995. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J. Clin. Microbiol.* **33**:1797-1803.
- Regnery, R., M. Martin, and J. Olson. 1992. Naturally occurring "*Rochalimaea henselae*" infection in domestic cat. *Lancet* **340**:557-558.
- Regnery, R. L., B. E. Anderson, J. E. Clarridge III, M. C. Rodriguez-Barradas, D. C. Jones, and J. H. Carr. 1992. Characterization of a novel *Rochalimaea* species, *R. henselae* sp. nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. *J. Clin. Microbiol.* **30**:265-274.
- Relman, D. A., J. S. Loutt, T. M. Schmidt, S. Falkow, and L. S. Tompkins. 1990. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. *N. Engl. J. Med.* **323**:1573-1580.
- Roux, V., and D. Raoult. 1995. Inter- and intraspecies identification of *Bartonella* (*Rochalimaea*) species. *J. Clin. Microbiol.* **33**:1573-1579.
- Slater, L. N., D. F. Welch, D. Hensel, and D. W. Coody. 1990. A newly recognized fastidious gram-negative pathogen as a cause of fever and bacteremia. *N. Engl. J. Med.* **322**:1587-1593.
- Zangwill, H. M., D. H. Hamilton, B. A. Perkins, R. L. Regnery, B. D. Plikaytis, J. L. Hadler, M. L. Cartter, and J. D. Wenger. 1993. Cat scratch disease in Connecticut: epidemiology, risk factors, and evaluation of a new diagnostic test. *N. Engl. J. Med.* **329**:8-13.