Experimental Infection of Domestic Cats with *Bartonella henselae* by Inoculation of *Ctenocephalides felis* (Siphonaptera: Pulicidae) Feces

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ABSTRACT Caged cat fleas, *Ctenocephalides felis* (Bouché), were fed on 6 cats; 3 cats were injected with 5×10^7 colony forming units of *Bartonella henselae* intradermally and 3 cats were injected with an equal volume of saline. After the fleas fed for 4 d, 5 groups of 50 *B. henselae*-exposed fleas were caged and allowed to feed on 5 cats for 6 d. Five cats each were injected intradermally with 1 ml of saline containing 45 mg of feces from *B. henselae*-exposed fleas. Five cats were fed 50 *B. henselae*-exposed fleas and 45 mg of fresh feces from *B. henselae*-exposed fleas. Five cats received all 3 treatments by using fleas and feces collected from cats inoculated with saline (controls). Cats were bled weekly and tested by culture and serology. The cats that were injected with feces from infected fleas were positive by culture for *B. henselae* at 1 or 2 wk after exposure and were the only cats to become bacteremic or seropositive by week 20.

KEY WORDS *Bartonella henselae, Ctenocephalides felis,* flea feces, cat scratch disease

Bartonella henselae is considered the causative agent of human cat scratch disease, and the organism is associated with bacillary angiomatosis and bacillary peliosis in patients with defects in cellular immunity (Tompkins 1997). Infection rates of *B. henselae* in domestic cats in the United States have recently been estimated at ≈30–40% and this incidence of infection may result in health risks for some cat owners (Groves and Harrington 1994). Jameson et al. (1995) reported an overall 27.9% incidence of seropositivity to *B. henselae* in 628 cats in North America and an association between warm, humid environments and feline exposure rates. Epidemiological studies on the risk factors associated with cat scratch disease have established a possible role for fleas in the transmission of *B. henselae* (Zangwill et al. 1993, Koehler et al. 1994). The organism has been detected in cat fleas, *Ctenocephalides felis* (Bouché), by the use of polymerase chain reaction (PCR) techniques (Anderson et al. 1994, Koehler et al. 1994, Relman et al. 1990). Higgins et al. (1996) demonstrated that cat fleas can maintain *B. henselae* and excrete viable organisms in their feces for up to 9 d after feeding on an infected blood meal, which may indicate that infection with *B. henselae* can occur by means of flea feces inoculation at a cutaneous site (Tompkins 1997).

Chomel et al. (1996) recently demonstrated that fleas transferred from *B. henselae* bacteremic cats to specific pathogen free kittens were capable of transferring the infection. Fleas were removed from the bacteremic cats and placed directly on the kittens. Therefore, the route of exposure for the kittens was not established. Although experimental transmission of *Rickettsia typhi* (the causative agent of murine typhus) has been demonstrated by the feeding of oriental rat fleas, *Xenopsylla cheopis* (Rothschild), the normal mode of transmission is considered to be by contamination of broken skin, respiratory tract, or conjunctivae of the host with infected flea feces or flea tissues (Azad 1990). The purpose of this study was to determine if transmission of *B. henselae* could occur directly via flea bite, by cat ingestion of flea and flea feces, or by intradermal injection of flea feces.

Methods and Materials

Cats. Twenty-six cats (4–7 mo old and specific pathogen free cats negative for *B. henselae* by culture and serology) were obtained from Harlan–Sprague–Dawley, Indianapolis, IN, and maintained at the Department of Laboratory Animal Medicine, Louisiana State University. All cats were allowed water and food ad libitum. Cats were current on vaccinations and were housed in individual cages with control cats held in a separate room from experimental cats.

Fleas. Cat fleas were obtained from a flea colony at the Louisiana State University Agricultural Center, St. Gabriel Research Station. Techniques for maintenance of the flea colony were reported in Henderson and Foil (1993). Groups of fleas were obtained from recently emerged adult fleas.

The flea feeding cages were constructed of 450-mm-diameter clear polyvinyl chloride (PVC) pipe

Cats were maintained according to the policies in the "NIH Guide for the Care and Use of Laboratory Animals."

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(Thomas et al. 1996). The end of the cage placed against the cats' skin was covered with 300-μm mesh nylon screening, whereas the opposite end was covered with a 30-μm mesh nylon screening. The lateral thorax of each cat was shaved using electric clippers (#40 blades) (Model A5, Oster, Milwaukee, WI), and the cages were secured under a 20-cm-long piece of tubular stockinette (Tetro, Niles, IL) by using Vetrap dressing (3M Animal Care Products, St. Paul, MN).

Bacterial Culture. *B. henselae* used to inoculate the cats was isolated from a naturally infected cat, confirmed to be *B. henselae* by PCR (Anderson et al. 1994), and was termed LSU16. *B. henselae* was propagated on chocolate agar plates and was harvested from the plates by scraping at 5–7 d into sterile saline. The number of colony forming units (cfu) per milliliter was calculated by plating known volumes of 10-fold dilutions of inocula on blood or chocolate agar.

Experimental Infection. Three cats were injected intradermally with a total of 5×10^7 cfu of *B. henselae* divided among 6 sites on the left or right lateral thorax, and 3 cats were injected with an equal volume of saline, intradermally. Caged fleas (100–200 fleas per cage) were placed on both the right and left lateral thorax of each of the 6 cats at 10 d after injection. Fleas fed for 4 d and feces was collected at days 2 and 4. Feces collected on day 2 was used in a different study. Processing of samples was conducted in 2 separate rooms, one for the control group of fleas and one for the infected fleas. All instruments were washed with soap and water and soaked in sodium hypochlorite (0.01%) before each use. Individual cages of fleas were placed into clean plastic buckets and opened. Then fleas were aspirated into test tubes and transferred to clean cages. Feces was brushed from the cages onto aluminum foil, transferred to sterile 15-ml polypropylene conical tubes, and weighed. Care was taken that the flea feces was not contaminated by any blood or serum from the cats. The fleas from the 3 bacteremic cats were combined, and the feces from the day 4 collections was combined. Similarly, the fleas and their feces from the control cats were separately combined.

At 14 d after infection, the 3 treated cats were bacteremic; the counts were cat #182, 4.63×10^5 cfu/ml; cat #184, 9.53×10^5 cfu/ml; and cat #223, 3.33×10^5 cfu/ml. All 3 cats showed clinical signs consistent with infection with *B. henselae* strain LSU16. Previous studies using LSU16 have shown that cats inoculated in this manner develop fever and lethargy 10 d after infection (K.O., unpublished data). After the fleas were transferred to the recipients, 1 ml of blood from each of the bacteremic cats was injected intradermally in either the right or left thorax of 1 of the 3 saline controls from the 1st part of the study. Control cats had shown no clinical signs and were never bacteremic.

Transmission. The remaining 20 cats were divided into 4 groups of 5 cats by random block design according to sex and age. After feeding for 4 d on the treated cats, 5 groups of 50 *B. henselae*-exposed fleas were caged and allowed to feed on group 1 cats for 6 d. The flea feces from the 3 bacteremic cats was combined, and each cat in group 2 received 45 mg of feces suspended in 1 ml of saline, injected intradermally among 6 sites in the skin of either the right or left thorax. This quantity of feces approximates the amount produced by 10 fleas during a 1-wk period (Rust and Dryden 1997). Group 3 cats were fed 50 *B. henselae*-exposed fleas (held at −8° C for 1 h before feeding to cats) and 45 mg of feces from *B. henselae*-exposed fleas. The fleas and feces were mixed with a small amount of tuna oil, and each cat was observed to ingest all fleas and feces. Group 4 cats received all 3 treatments by using fleas and feces from saline-injected cats; thus, they were fed on by 50 caged fleas for 6 d, fed 50 fleas, fed 45 mg of flea feces, and injected intradermally as above with 45 mg of feces. Cats were bled weekly and tested by culture and serology.

Polymerase Chain Reaction Assay. Procedures for detecting *B. henselae* DNA in flea samples followed Anderson et al. (1994). Samples were triturated with sterile pestles in 1.5-ml centrifuge tubes in 25 μl of sterile water. Samples were then heated to 100°C for 10 min and stored at 0°C. Ten-microliter aliquots were used as template for PCR. Conserved primers were used for amplification of a 414-bp fragment of the 60-kDa stress response protein gene, *htr*A. Samples were amplified for 35 repeated cycles of denaturation at 94°C for 30 s; annealing was at 50°C for 1 min, and the sequence extension step was at 70°C for 45 s. The PCR products were electrophoresed on 1.2% agarose gels and visualized by ethidium bromide staining.

Western Blot Analysis. *B. henselae* cultured as above was adjusted to a final O.D. 600 = 1 and a bacterial lysate was prepared. Western blot analysis was performed with modifications as described for other bacteria (Sambrook et al. 1989). Briefly, 75 μl of lysate was electrophoresed in 12% polyacrylamide mini-gels. The electrophoretically separated antigens were transferred to nitrocellulose. The blots were blocked overnight with 10% nonfat dry milk in 50 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 7.4 (NET) and washed in NET-Tween 20 (0.05%) for 20 min. Feline sera diluted to 1:10 in 10% nonfat dry milk in NET was applied to the blots and incubated at room temperature for 1 h. The blots were washed with NET-Tween 20 and incubated with peroxidase-conjugated, affinity pure F(ab') fragment goat anti-cat IgG (Jackson Immunoresearch, Avondale, PA) diluted at 1:5,000 in 10% milk-NET for 1 h. Antigen-bound conjugate was detected using ECL Western Blot Detection System (Amersham Life Science, Arlington Heights, IL) diluted 1:2 in distilled water.

Results and Discussion

The 3 cats injected with 1 ml of blood from one each of the bacteremic cats on day 14 showed clinical signs consistent with infection with *B. henselae* strain LSU16 and were culture positive, which demonstrated that the blood of the injected cats was infectious. Feces collected 14 d after injection (day 4 of flea feeding) from fleas fed on the 3 *B. henselae*-positive cats were

culture and PCR positive for 2 out of 3 cats (#182 and #184). Three out of 5 cats receiving feces intradermally from infected fleas were bacteremic by 1 wk after exposure. The remaining 2 cats were bacteremic by 2 wk. At 10 wk after exposure the cats injected with feces from infected fleas were the only cats that had become culture positive. In addition, none of the cats in groups 1, 3, and 4 were seropositive for *B. henselae* antibodies by week 20.

Guptill et al. (1997) reported that they were able to infect 1- to 2-wk-old kittens by oral inoculation of *B. henselae*. Up to 15% of adult *C. felis* have been shown to move between cat hosts (Rust 1994). Because it has been shown that up to 49.5% of the fleas on a cat are later found in its feces (Wade and Georgi 1988), the ingestion of infected fleas or their feces was tested as a route of exposure for uninfected cats. Although this mode of transmission may occur, we did not obtain transmission via this route in our study. We did not test the tuna oil for antibacterial activity but the fleas and flea feces were suspended in the tuna oil for less than 1 min, which we considered unlikely to affect infectivity of *B. henselae*.

Azad (1990) demonstrated that *X. cheopis* fleas that were infected for >21 d were capable of transmitting *R. typhi* by bite but transmission of the organism in the feces can occur within a week. In our study, fleas fed on bacteremic cats for 4 d and then were transferred to specific pathogen free cats and allowed to feed for 6 more days, which should have allowed ample time for bacterial growth within the fleas, if it occurs. The incubation period from inoculation with feces until expression of clinical signs was 1 wk in 3 of the 5 recipients. Colonies of *B. henselae* can take as long as 4 wk to become obvious in blood agar cultures, but LSU16 is visible in 5–7 d. If propagation of *B. henselae* in fleas was important for oral transmission due to blocking in the digestive system similar to plague (Harwood and James 1979) or release from the gut cells into the gut lumen similar to *R. typhi* (Azad 1990), up to 21 d could be required for oral transmission of *B. henselae*. Therefore, the results of our study do not preclude the possibility of propagation of *B. henselae* in fleas nor the oral transmission of the organism by fleas. We suggest that even if such mechanisms do exist, the exposure of hosts to infected flea feces should be considered to be a major route for the transmission of *B. henselae*.

The only method of transmission of *B. henselae* that we were able to demonstrate was via the intradermal injection of flea feces from fleas fed on cats for 4 d; at which time amplification of *B. henselae* within the fleas would have been unlikely. Abbott et al. (1997) found that cats are susceptible to intradermal exposure of *B. henselae* when compared with intravenous exposure, and they concluded that this information along with the fact that they could not demonstrate cat to cat transmission supports a role for possible arthropod vectors. Considering the amount of feces produced by feeding cat fleas (Rust and Dryden 1997), there could be considerable exposure of humans or other cats to infected feces from fleas feeding on bacteremic cats. Higgins et al. (1996) presented qualitative evidence that *B. henselae* propagates in the flea, but more rigorous studies will be required before the cat flea is considered a biological vector of *B. henselae*. The bacteremia in cats in our study was shown to exceed 1×10^6 cfu/ml. Therefore, the simple passage of the bacteria in the flea feces and subsequent infection of a 2nd host by penetration of the feces through a compromised skin barrier due to a scratch or other abrasion could occur and would be considered a type of mechanical transmission by an arthropod. In subsequent studies, the quantity of bacteria that is passed in the feces, as well as the length of time that infectious bacteria survive in flea feces should be determined.

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