**Evaluation of topical application of 10% imidacloprid–1% moxidectin to prevent *Bartonella henselae* transmission from cat fleas**

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**Objective**—To determine whether monthly topical administration of a combination of 10% imidacloprid and 1% moxidectin would lessen flea (*Ctenocephalides felis*) transmission of *Bartonella henselae* among cats.

**Design**—Controlled trial.

**Animals**—18 specific pathogen–free cats housed in 3 groups of 6.

**Procedures**—3 enclosures were separated by mesh to allow fleas to pass among groups yet prevent cats from contacting one another. One group was inoculated IV with *B henselae*, and after infection was confirmed, the cats were housed in the middle enclosure. This infected group was flanked by a group that was treated topically with 10% imidacloprid–1% moxidectin monthly for 3 months and by an untreated group. On days 0, 15, 28, and 42, 100 fleas/cat were placed on each of the 6 cats in the *B henselae*–infected group. Blood samples were collected from all cats weekly for detection of *Bartonella* spp via PCR assay, bacterial culture, and serologic assay.

**Results**—*B henselae* infection was confirmed in the cats infected IV and in all untreated cats after flea exposure; none of the cats treated with the imidacloprid-moxidectin combination became infected.

**Conclusions and Clinical Relevance**—In this setting, monthly topical administration of 10% imidacloprid–1% moxidectin reduced flea infestation, compared with infestation in untreated cats, and thus prevented flea transmission of *B henselae* to treated cats. Regular monthly use of this flea control product in cats may lessen the likelihood of humans acquiring *B henselae* infection. (J Am Vet Med Assoc 2010;236:869–873)

*Bartonella henselae* are intracellular, fastidious, gram-negative aerobic bacilli that have been associated with various human illnesses and are increasingly linked to clinical disease in cats.1–5 In immunocompetent humans, cat scratch disease is the most common syndrome caused by *B henselae*.2 Vascular proliferative diseases such as bacillary angiomatosis and bacillary peliosis are common in *B henselae*–infected, immunocompromised people.3 Endocarditis is another manifestation of *B henselae* infection in both immunocompetent and immunocompromised people and has been detected in a cat.2,3 Vague clinical signs of disease have been associated with *B henselae* infection in immunocompetent people that were likely to have been occupationally exposed.4 Whereas several clinical syndromes in cats may be attributable to *B henselae* infection, fever, uveitis, and lymphadenopathy are the manifestations that have been detected the most convincingly in naturally exposed or experimentally infected cats.2,5–9

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**ABBREVIATION**

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*Ctenocephalides felis* is the vector for *B henselae*, and cats are the natural reservoir for this flea.10 Infection is common in naturally exposed cats and their fleas, and > 30% of cats with fleas are bacteremic.3,10,11 *Bartonella henselae* lives within infected fleas for up to 9 days and, during the infection period, appears to replicate in fleas.12 Cats are known to groom off approximately 50% of fleas placed on their bodies.13 Thus, possible routes of transmission of *B henselae* among cats include flea bites, contamination of open wounds with feces of infected fleas, ingestion of infected fleas or flea feces, and fighting. In an experimental study,10 *C felis* collected from naturally infected cattery cats transmitted *B henselae* when placed on healthy SPF cats but cats with *B henselae* bacteremia without fleas did not transmit infection to SPF cats housed in the same room. Whereas neonatal kittens became infected with *B henselae* after oral inoculation with the agent in 1 study,11 results of another study15 failed to support oral transmission as a route of infection. In the latter study,13 intradermal inoculation of cats with feces from infected fleas, but not flea exposure or ingestion, resulted in infection.

On the basis of the results of these and other studies, several groups interested in human health, includ-
ing the CDC,16 the American Association of Feline Practitioners,3,17 and the Companion Animal Parasite Council,18 recommend routine administration of flea-control products to cats. Although administration of flea-control products is commonly recommended by veterinary practitioners, not all practitioners make such recommendations nor do all cat owners comply. In addition, it is unknown whether the administration of any flea-control product lessens the transmission rate of *B henselae* among cats.

The combination of 10% imidacloprid and 1% moxidectin is available as a topical spot-on product for use in cats in multiple countries, including the United States. Imidacloprid, an insecticide of the neonicotinoid class with fast-acting anti-flea efficacy, can prevent *C felis* infestation in cats.19 Moxidectin is an avermectin that can prevent infestation of cats with *Dinoflaria immitis* and protect against adult, immature, and L4 larval stages of hookworms and roundworms as well as control *Protodice tes cynitis*.9,20 The objective of the study reported here was to determine whether monthly topical administration of 10% imidacloprid–1% moxidectin would lessen flea (*C felis*) transmission of *B henselae* among cats.

**Materials and Methods**

**Animals**—Eighteen 6-month-old cats of both sexes that were purchased from a commercial research cattery were used in the study. The cats had been vaccinated against feline viral rhinotracheitis, feline calicivirus, feline distemper virus (panleukopenia), and rabies virus, and results of FELV-antigen and FIV-antibody testing were negative. Prior to the study, cats also had negative test results for serum antibody against *Bartonella* spp by use of conventional PCR-assay detection of *Bartonella* spp DNA in blood.9,21 The study design was approved by the Colorado State University Institutional Animal Care and Use Committee and the Animal Care and Use Committees at Bayer Animal Health and at the research facility that housed the cats during the flea infestation portion of the study.9

**Primary *B henselae* inoculation**—Six cats were selected by use of a random number generator for experimental infection via IV inoculation with blood from a naturally infected cat. Blood samples from cats housed at a Florida humane society were collected into EDTA-treated tubes, held at 4°C (39.2°F), and shipped by overnight express on cold packs to Colorado State University. On arrival, DNA was extracted and the samples assessed for DNA of *Bartonella* spp, hemoplasmas, *Ehrlichia* spp, and *Anaplasma* spp by use of conventional PCR assays.12–23 A sample with positive test results only for *B henselae* DNA was selected on the basis of band size and genetic sequencing, screened to ensure absence of FeLV antigen and FIV antibody, and then inoculated IV (0.2 mL) into each of the 6 cats.23 On day 21 after inoculation, all 6 cats had positive PCR-assay results for *B henselae* DNA in blood.

**Flea infestation**—Beginning the morning of day 0, all 18 cats were housed together in 1 room that was divided into 3 sections (R1, R2, and R3). The 6 infected cats were housed together in R2 (middle section), and the other 12 cats were separated into 2 additional groups containing 6 cats each, with 1 group housed in R1 (untreated cats) and the other housed in R3 (treated cats; both sections were adjacent to R2 but not to each other). The sections were separated from each other by mesh so that *C felis* could move among the cats, while body contact between cats or fighting with members of other groups was prevented. Approximately 25% of the floor space in each section was covered in carpet to promote survival and a complete life cycle of *C felis* within the room. Cat perches were placed in the corners of each section adjacent to the mesh dividing the sections to encourage cats from the various groups to be close to each other.

**Ctenocephalides felis** (300 male and 300 female as determined by weight) were purchased and transported to the research facility. The fleas (100/cat) were placed onto the *B henselae*-infected cats in R2. Approximate flea counts were determined intermittently for each cat by research facility staff members by use of a flea comb. However, the staff were not blinded to treatment group identity. An additional 600 fleas (300 male and 300 female) were purchased and placed on the *B henselae*-infected cats housed in R2 on days 15, 28, and 42 of the study. Additional fleas from the same colony were purchased, pooled, and tested to ensure they were free of *Bartonella* spp DNA.

**Administration of 10% imidacloprid–1% moxidectin**—Three days prior to the initial placement of fleas on the cats (day –3), a commercially available spot-on preparation of 10% imidacloprid and 1% moxidectin was topically administered in the intrascapular region to the cats destined to be housed in R3 while these cats were housed in a separate room to attempt to avoid vapor spread of 10% imidacloprid–1% moxidectin to the other 12 cats. The 10% imidacloprid–1% moxidectin application was repeated in the separate room on days 25 and 33, and the cats were returned to R3 on days 28 and 56. On day 86 of the study, all cats received topical administration of 10% imidacloprid–1% moxidectin.

**Sample collection and assays**—Blood samples were collected via jugular venipuncture by use of a 22-gauge needle from all cats prior to flea infestation (day 0) and then on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84. After collection, blood was transferred into an EDTA-treated tube and an untreated tube to allow the blood to clot prior to serum separation. Each day of sample collection, CBCs were performed at the Clinical Pathology Laboratory at the Veterinary Teaching Hospital at Colorado State University. Samples were prepared for determination of serum titers of IgG against *Bartonella* spp and for PCR assay for *Bartonella* spp on the day of collection.9,21 One aliquot of each blood sample (500 μL) in EDTA was stored at –70°C (–94°F) pending bacterial culture.

After collection of the day 84 samples, all samples were thawed and submitted for culture of *Bartonella* spp. Each sample was used to inoculate a trypticase soy agar plate with 10% sheep blood, and plates were incubated at 37°C (98.6°F) in an environment of 5% carbon dioxide and 100% humidity.1 Results for samples that yielded characteristic *Bartonella* spp colonies were confirmed by *Bartonella* spp PCR assay.
Clinical monitoring—Cats were monitored daily for signs of lethargy or any other obvious abnormalities. When suspicion of illness arose, a full physical examination was performed. If a cat’s rectal temperature exceeded 39.2°C (102.5°F) for >1 day, the cat was removed from the enclosure and administered 10% imidacloprid–1% moxidectin topically once and enrofloxacin at 5 mg/kg (2.27 mg/lb) PO once daily for 14 days. Other necessary supportive care was administered as needed.

Statistical analysis—Commercially available computer software was used to compare results among the 3 cat groups. The CBC data were compared among groups by use of repeated-measures ANOVA. The proportion of infected cats was compared among groups by use of a 2-tailed Fisher exact test. Differences among group mean flea counts were compared with the 2-tailed Student t test. A value of \( P < 0.05 \) was considered significant for all analyses.

Results

*C. felis* infestations—Live *C. felis* were detected on most cats in sections R1 (untreated cats) and R2 (*B. henselae*-infected cats) of the housing room during the experimental period (Figure 1). Detection of fleas on cats in R3 (cats treated with 10% imidacloprid–1% moxidectin) was uncommon, with only 1 flea/cat detected on 3 cats on 4 occasions, beginning on day 70.

Clinical findings—Pruritus or dermatologic disease was not detected in any cat. Whereas cats in R2 and R3 remained clinically normal throughout the study, 3 cats from R1 developed lethargy and a rectal temperature >39.2°C that persisted for at least 2 days. These 3 cats were removed from the study on days 71, 73, and 74, respectively, and received 10% imidacloprid–1% moxidectin, enrofloxacin, and supportive care. One of the 3 cats developed myocarditis, pericardial effusion, pleural effusion, icterus, and ataxia. This cat was euthanized because of its clinical state and poor response to treatment. A necropsy was performed, and results confirmed myocarditis, effusions, and cholangiohepatitis. The remaining 2 cats recovered within 2 days after initiation of antimicrobial treatment. All other cats remained in the study for the full 86 days. After completion of the fleas infestation phase of the study, all cats with positive results for the *Bartonella* spp PCR assay received enrofloxacin for 14 days and ultimately tested negative for *Bartonella* spp DNA in blood for at least 3 months prior to adoption into private homes.

CBCs—No significant difference was found among groups in regard to values of CBC variables.

*Bartonella* spp PCR assay and culture—The presence of *B henselae* DNA in the blood of the IV inoculated cats in R2 was confirmed prior to starting the fleas infestation stage of the study. Whereas *B henselae* was cultured and *B henselae* DNA was amplified from the blood of each of the 6 cats in R1 during the study period, infection of cats in R3 was not confirmed by either method. The difference between proportions of infected cats in R1 and R3 was significant (\( P = 0.002 \)). For cats in R1, interval to a positive PCR assay or culture result ranged from 42 to 70 days of flea exposure. By day 84 of the study, *B henselae* bacteremia was present in the 5 surviving cats in R1 but had resolved without treatment in the cats in R2.

After exposure of cats in R2 to fleas, *B henselae* DNA was amplified from 28 of 71 (39%) blood samples and *B henselae* was isolated from 23 of 71 (32%) blood samples. All samples with positive culture results also had positive PCR-assay results. The 5 *B henselae* PCR-assay positive, culture negative samples were considered falsely negative by culture.

Serologic testing—Of the 6 cats in R1, 5 developed detectable anti-*B henselae* IgG titers, with interval to seroconversion ranging from 63 to 84 days of flea exposure. Titers ranged from 1:64 to 1:512, and once positive titers were detected, all cats remained seropositive for the duration of the study. The 1 cat that did not seroconvert was the one that was euthanized; *B henselae* DNA was amplified from some effusions and tissues. All 6 cats in R2 developed detectable anti-*B henselae* IgG titers, with interval to seroconversion ranging from 21 to 35 days after IV inoculation. Titers ranged from 1:64 to 1:1,024, and once positive titers were detected, all cats remained seropositive for the duration of the study. One cat in R3 had an anti-*B henselae* IgG titer of 1:256 on day 21, and the test was repeated on the same sample to confirm this result. However, an anti-*B henselae* IgG was not detected in any other sample from that cat.

**Discussion**

Many studies have revealed an increased risk for *B henselae* infection in cats with versus cats without fleas, and *C. felis* are commonly infected with *B henselae*. The *B henselae*-infected cats housed in R2 (middle section of the room) did not have direct contact with untreated cats in R1 (adjacent to R2 but separated by a screen), and so we believe our results confirm those of others that indicate *C felis* can trans-
mit *B. henselae* among cats. However, the results cannot be used to determine whether infection developed from flea bites, ingestion of fleas, or bites and scratches among cats in R1. In a previous study, *B. henselae*-infected cats without fleas did not transmit the infection to cats housed in the same room. This suggests that the cats in R1 were most likely infected from contact with the infected fleas or their feces.

The detection of *B. henselae* bacteremia by PCR assay or bacterial culture in all untreated cats in R1 combined with the lack of *B. henselae* bacteremia in the cats treated with 10% imidacloprid–1% moxidectin and housed in R3 (also adjacent to R2) suggested that this flea-control regimen blocked the transmission of *B. henselae* among cats. Thus, this protocol should lessen feline and human exposure to *B. henselae* and could potentially lessen the incidence of *B. henselae*-associated illness in cats and people. One cat in R3 had an anti-*B. henselae* IgG titer of 1:256 on day 28, which we suspected was falsely positive because all other test results were negative in this cat and none of the cats in the group developed clinical signs of bartonellosis. However, even if this finding represented a transient *B. henselae* infection, with the statistical evaluation repeated to assume 1 of 6 treated cats was indeed infected, the cats treated with 10% imidacloprid–1% moxidectin in R3 toward the end of the infestation period, the degree of infestation did not appear severe enough to result in *B. henselae* transmission. However, the cats in R3 were housed in a room other than the study room for 6 full days to lessen the risk of vapor spread of 10% imidacloprid–1% moxidectin to untreated cats. It is possible that this housing situation lessened the total duration of contact with *C. felis* and affected the results when compared with the duration of contact for cats housed in R1 and R2. In another study, exposure of cat hair to topical imidacloprid resulted in a significant reduction in *C. felis* feeding, which may explain the lack of *B. henselae* transmission in the group of untreated cats. Alternately, it is possible that infection occurred in these cats but was at a degree lower than the detectable limits of the assays.

All cats that had been inoculated IV with *B. henselae* and were housed in R2 had positive PCR-assay results for detection of *B. henselae* in blood within 21 days after inoculation, whereas the cats in R1 exposed to fleas had positive results between days 42 and 70. In addition, all cats in R2 seroconverted within 35 days after IV inoculation. The cats in R1 that seroconverted developed the first detectable anti-*B. henselae* IgG titers between days 56 and 77. These differences between groups likely merely reflected the dose of *B. henselae* given by IV inoculation (0.2 mL of blood from an infected cat) versus the dose delivered via flea exposure. However, our findings differ from those of another study in which transmission of *B. henselae* among cats via *C. felis* was evaluated. In that study, most kittens exposed to *B. henselae*-infected fleas collected from infected cattery cats developed bacteremia within 14 days after exposure and became seropositive for *B. henselae* infection within 28 to 35 days. In the study described here, the *C. felis* were deemed free of *B. henselae* when placed on the cats in R2. Presumably, the fleas had to acquire *B. henselae* infection from the cats in R2, magnify the infection, and then infect cats in R1, explaining the difference in the intervals to bacteremia and seroconversion between the 2 studies. Alternately, responses to *B. henselae* may vary by the strain of the organism.

Although none of the *B. henselae*-inoculated cats in R2 developed noticeable signs of illness, half (n = 3) of the cats in R1 infected after flea exposure became clinically ill. Fever, inappetence, and lethargy were detected in these 3 R1 cats, with 1 cat developing pleural effusion, pericardial effusion, supplicative myocarditis, and cholangiohepatitis. Differences in virulence between strains of *B. henselae* have been reported to more severe myocarditis did not develop an anti-*B. henselae* antibody response, which may have played a role in the development of clinical illness.

Whereas all cats in R2 were infected with *B. henselae* after IV inoculation, the intervals to bacteremia and seroconversion appeared to be slower than those in another study of 2 different strains of *B. henselae*. However, these observations may reflect differences in *B. henselae* strains. Furthermore, in the previous study, the inoculum was derived from a bacterial culture, which may have resulted in a large number of organisms in each inoculum. The *B. henselae*-inoculated cats in R2 had negative results of both bacterial culture and PCR assay by day 84 of the study. Although persistent and relapsing bacteremia is common with *Bartonella* spp, it is possible that the degree of bacteremia may vary with *B. henselae* strain. The present study was designed such that bacteremic cats were to be treated with enrofloxacin at the end of the study. Thus, we were unable to gather additional information concerning duration of bacteremia following flea transmission.

All cats in the present study with positive results of bacterial culture concurrently had positive results for the PCR assay. However, we believe the 5 cats with positive PCR-assay results but false-negative culture results likely reflected a low number of *B. henselae* per microliter of blood. In future studies, a larger amount of blood or a more sensitive technique could be used for bacterial culture.

The present study revealed evidence of flea transmission among cats, and the experimental method used provided evidence that monthly topical administration of 10% imidacloprid and 1% moxidectin in cats lessens *B. henselae* transmission among cats, at least in a controlled setting. However, findings may be different in household settings in which conditions are not tightly controlled, and additional studies evaluating prevention of *B. henselae* transmission in household and other settings are necessary.

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a. SNAP FIT/FelV Combo, IDEXX Laboratories, Portland, Me.
b. High Quality Research, Fort Collins, Colo.
c. Heska Corp., Loveland, Colo.
Advocate, Bayer AG, Leverkusen, Germany.
f. Beeton-Dickinson, Franklin Lakes, NJ.
g. Baytril, Bayer AG, Leverkusen, Germany.
h. SAS Institute Inc, Cary, NC.

References


New Veterinary Biologic Products

<table>
<thead>
<tr>
<th>Product name</th>
<th>Species and indications for use</th>
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<td>Swine Influenza Vaccine, pH1N1, Killed Virus (Pfizer Animal Health, Lincoln, Neb, US Vet Lic No. 189)</td>
<td>For vaccination of healthy swine, including pregnant sows and gilts, 3 weeks of age or older against SIV subtype H1N1. The product is a freeze-dried preparation containing the pandemic A/California/04/2009 isolate. Reasonable expectation of efficacy was demonstrated in pigs that received 2 doses of the vaccine and seroconverted</td>
<td>IM</td>
<td>USDA licensed 12/11/09</td>
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