Fipronil and (S)-methoprene can lessen the risk of transmission of *Bartonella clarridgeiae* among cats with exposure to *Ctenocephalides felis*

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OBJECTIVE
To cohouse cats experimentally infected with *Bartonella clarridgeiae* (Bc) with naive cats in a flea-free environment or with *Ctenocephalides felis*, *Bartonella henselae* (Bh), *Mycoplasma haemofelis*, and *Candidatus Mycoplasma haemominutum* to determine which flea could be a vector and to assess whether transmission of the infectious agents could be blocked by fipronil and (S)-methoprene.

ANIMALS
Specific pathogen-free cats (n = 34).

METHODS
In experiment 1, Bc was inoculated in 1 cat that was housed with 9 naive cats without *C felis*. In experiment 2, the 2 cats inoculated with Bc were housed with 6 other cats (2 inoculated with Bh, 2 inoculated with *M haemofelis*, and 2 inoculated with *Candidatus M haemominutum*) in the center (enclosure 2) of 3 housing enclosures separated by mesh walls that allow passage of fleas but precludes fighting. *C felis* were placed only on cats in enclosure 2 (5 times). Cats in enclosures 1 (n = 8) and 2 (8) were untreated, and cats in enclosure 3 (8) were administered fipronil and (S)-methoprene. Blood was collected from all cats for PCR assays for the pathogens.

RESULTS
None of the cats housed with the cat inoculated with Bc became PCR positive in the absence of *C felis*. All cats in enclosure 2 became Bc DNA positive. While 2 of 8 cats in enclosure 1 became Bc PCR positive, none of the treated cats in enclosure 3 became infected.

CLINICAL RELEVANCE
The study demonstrated that *C felis* can be a vector for Bc. The results support the recommendation that flea control products can reduce the risk of transmission of flea-borne pathogens.

Keywords: *Bartonella clarridgeiae*, *Ctenocephalides felis*, fipronil and (S)-methoprene, transmission, vector

**B**artonella spp have been associated with cat scratch disease and a variety of other human illnesses and are increasingly being linked to clinical diseases in cats.1–3 Humans infected with *Bartonella* spp experience general systemic symptoms such as relapsing fever, poor appetite, and fatigue and can be afflicted by ocular disease, multifocal osteomyelitis, endocarditis, hepatic and splenic abscesses, encephalopathy, and pneumonia.4,5 *Bartonella henselae* (Bh) and *B clarridgeiae* (Bc) are the most common *Bartonella* spp of cats and are most strongly linked to fever, lymphadenopathy, and uveitis with other likely manifestations including endocarditis, myocarditis, meningoencephalitis, and granulomatous inflammation of various tissues.3,5–9 Cats are the reservoir for Bh and Bc, and *Bartonella* spp DNA has been amplified from cats and *Ctenocephalides felis* in studies around the world.10–18 *C felis* has been proven as a vector of Bh in experimental studies.5,19–22 However, whether *C felis* is a vector for Bc has not been studied extensively.
Mycoplasma haemofelis (Mhf), Candidatus Mycoplasma haemominutum (Mhm), and Candidatus M. turicensis are the most common haemoplasmas in cats around the world and are associated with fever and hemolytic anemia in cats. Clinical presentations in cats can be similar between haemoplasmosis and bartonellosis, but Bartonella spp are usually not associated with anemia. The DNA of the haemoplasmas has been amplified from C. felis from cats in the field with wide variations of estimated prevalence rates. Studies with the highest haemoplasma prevalence rates often used a primer set that also amplified a Spiroplasma of fleas that led to an overestimation. While C. felis allowed to feed on experimentally inoculated cats take up Mhf and Mhm in the blood meal, transmission to other cats exposed to infected fleas was uncommon and did not happen with cats fed with infected fleas.

In another study, Aedes aegypti fed on cats with experimental haemoplasma infections took up the agents in the blood meal but did not transfer the infections to new cats when reinfested, suggesting this mosquito is not a vector.

A number of groups interested in human health, including the CDC, the American Association of Feline Practitioners (AAFP) Zoonoses Guidelines Committee, the AAFP Panel Report on Bartonellosis, and the Companion Animal Parasite Council recommend the administration of flea control products to cats. In an experimental study, monthly administration of moxidectin-imidacloprid was shown to block the transmission of Bh among cats. It is currently unknown whether the administration of any flea control product can reduce the transmission rate of Bc, Mhf, or Mhm among cats. Fipronil and (S)-methoprene (Frontline Plus for Cats; Boehringer Ingelheim) is a well-established flea control product that also controls ticks and may potentially reduce transmission of aforementioned organisms.

The primary objective of experiment 1 was to cohouse cats experimentally infected with Bc with naive cats while maintaining the cats in a flea-free environment to determine if Bc is transmitted among cats living passively. The objectives of experiment 2 were to house cats with Bartonella spp and haemoplasma infections together with or without fleas to gather further information on which agents were transmitted by fleas and to determine whether flea-borne infection could be reduced in cats treated with fipronil and (S)-methoprene.

The hypotheses were that Bartonella spp, but not the haemoplasmas, would be transmitted by C. felis and that flea-borne Bartonella spp transmission would be prevented by the monthly administration of fipronil and (S)-methoprene.

Methods

Cats

Sixteen-week-old, vaccinated, FeLV- and FIV-negative, laboratory-reared cats (n = 34) from a colony at a research facility were used and managed under approved institutional animal care and use protocols (No. 158.006). The cats were shown to be negative for Bartonella spp antibodies in serum as well as Bartonella spp, Mhf, Mhm, and Candidatus M. turicensis DNA in blood by PCR before use in this study.

Organisms

The strain of Bh used in this study was used in the previous imidacloprid-moxidectin study. The strains of Bc, Mhf, and Mhm that were used were originally isolated from the blood of feral cats in Florida. For this study, blood in EDTA and freezing solution that had been stored at −80 °C was thawed, and 1 mL was inoculated into specific pathogen-free cats (1 cat for each organism) to document viability, and infection was confirmed by PCR assay results.

Experimental design

Experiment 1

The cat that was inoculated on day 0 with the field strain of Bc became strongly PCR positive by day 19 after IV inoculation. This cat was then cohoused with 9 other Bc naive cats in the absence of C. felis for >150 days. The number of cats studied was selected arbitrarily based on availability. Blood for Bartonella spp PCR assay was collected every 7 to 21 days from these 10 cats.

Experiment 2

On day −21, 8 randomly selected cats were chosen for experimental infection; 2 mL of blood in EDTA from the carrier cats (PCR confirmed; see Organisms) of each of the 4 organisms was given by IV inoculation to 2 naive cats. Day −21 was chosen because by day 0 peak bacteremia should be occurring, which may potentiate flea-borne transmission. The 8 experimentally infected cats were housed together as 1 group in the center (enclosure 2 (E2)) of 3 enclosures, and the other 16 cats were divided into two additional groups containing 8 cats each (Figure 1). The cat numbers and design were based on our previous study using Bh. The sections were separated from each other by mesh (1-cm apertures) so that C. felis could move among the cats, but the cats could not bite or scratch the members of other groups. Approximately 25% of the floor space in each section was covered in carpet to promote the survival of C. felis within the room and to allow larvae to migrate between enclosures. At the ends of each section, enrichment perches were placed next to the mesh walls to encourage the travel of C. felis among the cats of each section.

A total of 100 adult C. felis (50 male and 50 female) were placed on each E2 cat starting 21 days after inoculation with the infectious agents (day 0). The infestations were repeated on days 14, 28, 42, and 56. To lessen the potential that aerosol transmission of fipronil and (S)-methoprene (applied topically at label dose) could affect the results of the study, the treated cats housed in enclosure 3 (E3) were treated in another room and held 3 days before being returned to E3.
On day 168, all cats were treated with the product and moved to a flea-free room.

Monitoring
The cats were examined daily for attitude and appetite throughout the study. Mucous membrane color assessment and body temperature measurement were to be made on any cat exhibiting depression or inappetence, and if abnormalities were detected, a CBC and serum biochemical panel would have been performed. Blood was drawn (3 mL) for the performance of PCR assays for *Bartonella* spp and haemoplasmas weekly for the duration of the study. On completion of the study, all cats were shown to be negative for *Bartonella* spp on 3 sequential PCR assays performed every 2 weeks and were adopted to private homes or transferred to other studies.

Statistical analyses
Since hemoplasma and *Bartonella* PCR results can vary in infected cats over time, any cat with at least 2 positive PCR assay results in a row for an organism was considered infected. The proportion of infected cats for each agent in each enclosure was compared using a 2-tailed Fisher exact test. Significance was defined as \( P < .05 \).

Results

**Experiment 1**
The cat that was inoculated IV with Bc-containing blood was PCR positive in 9 of the 10 assays performed over the 150-day observation period. None of the 9 cats cohoused with this cat became positive, there was no evidence of fighting documented, and none of the 10 cats were known to be clinically ill.

**Experiment 2**
By day 0 of the study (21 days after inoculation), both cats primarly inoculated with Bc-containing blood in E2 were PCR positive and all other cats were negative. Neither of the 2 cats inoculated with Bh was PCR positive on day 0 or any other day after inoculation, and it was concluded that the infection with this organism failed.

The DNA of Mhm was amplified from both of the primarily inoculated cats on day 0, before flea infestation, as well as 2 additional cats in E2 and 1 cat in E3 (days 0 and 7 only). The DNA of Mhf was amplified from 1 of the 2 primarily inoculated cats but none of the other cats on day 0.

After flea infestations were started, Bc DNA was amplified multiple times from all the cats in E2, 2 of 8 cats in E1, but none of the fipronil-treated cats in E3. When the percent positive rates were compared

Table 1—Distribution of *Bartonella clarridgeiae* (Bc) PCR assay results in infected cats.

<table>
<thead>
<tr>
<th>Enclosure</th>
<th>First positive Bc PCR</th>
<th>No. positive Bc PCR (total = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enclosure 2 (primary agent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFN2 (Bc)</td>
<td>Day 0</td>
<td>18 (72%)</td>
</tr>
<tr>
<td>OFA3 (Bc)*</td>
<td>Day 0</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>OFQ1 (Bh)</td>
<td>Day 21</td>
<td>21 (84%)</td>
</tr>
<tr>
<td>OFQ3 (Bh)</td>
<td>Day 35</td>
<td>20 (80%)</td>
</tr>
<tr>
<td>OFM2 (Mhm)*</td>
<td>Day 56</td>
<td>17 (68%)</td>
</tr>
<tr>
<td>OFR6 (Mhm)*</td>
<td>Day 77</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>OFR5 (Mhf)*</td>
<td>Day 45</td>
<td>12 (48%)</td>
</tr>
<tr>
<td>OFX2 (Mhf)*</td>
<td>Day 42</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>Enclosure 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UFU1 (NA)</td>
<td>Day 119</td>
<td>8 (32%)</td>
</tr>
<tr>
<td>OFU4 (NA)</td>
<td>Day 63</td>
<td>15 (60%)</td>
</tr>
</tbody>
</table>

Primary agents were inoculated on day −21. Fleas were added to the cats in chamber 2 on days 0, 14, 28, 42, and 56. Each cat had a total of 25 Bc PCRs run over the course of the study. None of the product-treated cats in chamber 3 became infected by Colorado State University Bc-1.

Bh = *Bartonella henselae*. Mhf = *Mycoplasma haemofelis*. Mhm = *Candidatus* Mycoplasma haemominutum. NA = Not applicable.

*Cats positive for DNA of a haemoplasma over the course of the study.*
between treated cats (0 of 8) and untreated cats (8 of 14 cats [the 2 primarily inoculated cats were excluded]), the results were significantly different (P = .017). The distribution of positive Bc PCR assay results is shown (Table 1).

After starting flea infestations, haemoplasma DNA was only occasionally amplified in cats of E1 and E3 and was never amplified on 2 bleed dates, concurrently, so infection as defined was not documented. In E2, 3 cats ultimately were infected by Mhf and 5 were infected by Mhm with the proportions of positive tests listed as shown (Table 2).

There was no evidence of fighting or bite wounds among the cats cohoused in the different enclosures over the course of experiment 2. None of the cats in experiment 2 developed measurable clinical signs of disease, even when infection with Mhf or coinfections with haemoplasmas and Bc occurred.

**Discussion**

Experiment 1 results suggest that Colorado State University (CSU) Bc-1 is not easily transmitted among cats living passively as reported by Bh. However, it is possible the cats were exposed but mounted effective immune responses that blocked the cats from becoming PCR positive during the times of testing. Experiment 2 results suggest that CSU Bc-1 is transmitted by fleas as reported for Bh. The results support public health guidelines suggesting that flea control should be maintained to limit the zoonotic risk of *Bartonella* spp. Amplification of *CSU Mhm-2* DNA from the 2 cats in enclosure 2 that were neither inoculated primarily nor were infected before exposure to *C felis* (OFU5; OFX2) suggests the organism was transmitted directly to these cats. The cat that developed consistent Mhm infection starting on day 156 could have been infected by direct contact or by flea exposure. One study in Southern Germany found that mult-cat households had a higher prevalence of haemoplasma infection, suggestive of direct transmission. Another study suggested that haemoplasmas could be transmitted directly from the saliva of infected cats. These results should be confirmed in studies using larger numbers of cats.

In the majority of studies of feline haemoplasmas, once infections are induced, the organisms are present in blood in high numbers, with consistently positive PCR assay results. If haemoplasma infection is defined as the presence of haemoplasma DNA in greater than or equal to 2 consecutive samples, then none of the cats in E1 became infected and only 1 cat in enclosure 3 became infected (day 0 and day 7 PCR positive only). The results suggest that neither CSU Mhm-2 nor CSU Mhf-2 was effectively transmitted by *C felis* that traveled from E2 to E1 or E3. This result is consistent with what we previously described with a study using caged fleas. In addition, the results of a study on transmission of Mhm and Mhf suggested that ingestion of *C felis* infected by *Mycoplasma* or

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**Table 2**—Distribution of haemoplasma PCR assay results in infected cats.

<table>
<thead>
<tr>
<th>Enclosure 2 (primary agent)</th>
<th>First-day Mhf PCR positive</th>
<th>No. positive Mhf PCR (n = 25)</th>
<th>First-day Mhm PCR positive</th>
<th>No. positive Mhm PCR (total = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFM2 (Mhm)</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>OFR6 (Mhm)</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>23 (92%)</td>
</tr>
<tr>
<td>OFU5 (Mhf)</td>
<td>0</td>
<td>13 (52%)</td>
<td>0</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>OFX2 (Mhf)</td>
<td>14</td>
<td>17 (68%)</td>
<td>0</td>
<td>23 (92%)</td>
</tr>
<tr>
<td>OFQ1 (Bh)</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>OFQ3 (Bh)</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>OFA3 (Bc)</td>
<td>70%</td>
<td>13 (52%)</td>
<td>156</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>OFN2 (Bc)</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Proportions</td>
<td>3/8 cats (37.5%)</td>
<td>5/8 cats (63.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Primary agents inoculated on day - 21. Fleas were added to the cats in enclosure 2 on days 0, 14, 28, 42, and 56. Each cat had a total of 25 *Candidatus* Mycoplasma haeminominutum (Mhm) PCRs run over the course of the study. One cat in enclosure 3 was infected with Mhm based on positive PCR assay results on days 0 and 7 only. All of these cats were positive for *Bartonella clarridgeiae* (Bc) during the course of the study.

*Bh* = *Bartonella henselae*. *Mhf* = *Mycoplasma haemofelis*. *Mhm* = *Mycoplasma haeminominutum*. *NA* = Not applicable.
their by-products was not an important route of transmission. However, this conclusion should be made cautiously as the numbers of C felis–infested cats in E1 and E3 were likely to be lower than those in E2 (experimentally infested).

None of the cats developed measurable clinical signs of disease after infection with CSU Mhf-2 or Mhm-2, even when coinfected with a Bartonella spp on multiple days. For CSU Mhm-2, this finding was expected as most Mhm isolates have been non-pathogenic when inoculated into specific pathogen-free cats. However, failure to document clinical illness after primary or secondary infection with CSU Mhf-2 suggests that nonpathogenic strains of Mhf also exist or that passage or storage of the agent can lessen virulence over time.

Our study has some limitations, including the use of a small number of cats, precluding adequate statistical analysis such as in regard to comparing Bh infection to other studies and assessing clinical signs after infection of Bartonella spp and haemoplasmas. In addition, we chose to not do flea counts on the cats due to the potential for zoonotic transfer to employees and since we had already validated the model previously. While none of the cats developed clinical signs, only 1 strain per organism was used, which would suggest these isolates are non-pathogenic. However, it is not known if this would be the same in a field study rather than compared to employees.”

In conclusion, this study suggests that Bc is not likely to be transmitted among cats by passive contact, Bc is transmitted by C felis, and the lower proportion of infected cats in the treated group when compared to the treated group was statistically significant. The present strain of Bc was not pathogenic in the cats in this model.

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None reported.

Disclosures

Dr. Douglas S. Carithers and Dr. Frederik Beugnet are professionally affiliated with Boehringer Ingelheim as current employees.

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References


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