The prevalence of persistent bacteremic *Bartonella* spp. and hemoplasma infections was determined in healthy pet cats in Ontario. Blood samples from healthy cats sent to a diagnostic laboratory for routine health assessment over the course of 1 y were tested for *Bartonella* spp. using both polymerase chain reaction (PCR) and blood culture, and for the presence of hemoplasma by PCR. The overall prevalence of *Bartonella* spp. by PCR and by culture combined was 4.3% (28/646) [3.7% (24/646) *Bartonella henselae*, 0.6% (4/646) *Bartonella clarridgeiae*]. The novel *B. henselae* PCR developed for this study demonstrated nearly twice the sensitivity of bacterial isolation. The overall prevalence of hemoplasma was 4% (30/742) [3.3% (25/742) *Candidatus Mycoplasma haemominutum*, 0.7% (5/742) *Mycoplasma haemofelis*]. There was no significant difference between the prevalence of infection by season or by age (≤ 2 y, > 2 y). *Candidatus Mycoplasma turicensis* was identified, for the first time in Canada, in 1 cat. The prevalence of *Bartonella* (58%) and hemoplasma (47% *M. haemofelis*, 13% *M. haemominutum*) in blood from a small sampling (*n* = 45) of stray cats was considerably higher than that found in healthy pet cats.

The prevalence of *Rickettsia felis* in cat fleas was also assessed. A pool of fleas from each of 50 flea-infested cats was analyzed for the presence of *R. felis* by PCR. *Rickettsia felis* was confirmed, for the first time in Canada, in 9 of the 50 samples. Therefore, the prevalence of *Bartonella* and hemoplasma infection in healthy pet cats is relatively low. Further, the control of cat fleas is important because of the public health significance of *Bartonella* and *R. felis* infection.

**Abstract**

La prévalence d’une bactériémie persistante à *Bartonella* spp. et d’une infection à hémoplasma a été déterminée dans une population de chats en santé provenant de l’Ontario. Des échantillons sanguins de chat en santé envoyés à un laboratoire de diagnostic pour un bilan de santé durant une période d’une année ont été éprouvés pour *Bartonella* spp. par réaction d’amplification en chaîne par la polymérase (PCR) et par hémoculture, et pour la présence d’hémoplasma par PCR. La prévalence globale de *Bartonella* spp. par PCR et culture combinés était de 4,3 % (28/646) [3,7 % (24/646) *Bartonella henselae*, 0,6 % (4/646) *B. clarridgeiae*]. L’épreuve PCR développée pour la présente étude avait une sensibilité presque le double de l’isoliement bactérien. La prévalence globale d’hémoplasma était de 4 % (30/742) [3,3 % (25/742) *Candidatus Mycoplasma haemominutum*, 0,7 % (5/742) *Mycoplasma haemofelis*]. Il n’y avait pas de différence significative entre les prévalences d’infection selon la saison ou le groupe d’âge (≤ 2 y, > 2 y). *Candidatus Mycoplasma turicensis* a été identifié chez un chat pour la première fois au Canada. La prévalence de *Bartonella* (58 %) et d’hémoplasma (47 % *M. haemofelis*, 13 % *M. haemominutum*) dans le sang d’un échantillonnage restreint (*n* = 45) de chats errants était considérablement plus élevée que chez des chats en santé.

La prévalence de *Rickettsia felis* chez des puces de chats a également été évaluée. Un pool de puces provenant de chacun de 50 chats infestés par des puces a été analysé par PCR pour la présence de *R. felis*. La présence de *R. felis* a été confirmée pour la première fois au Canada dans 9 des 50 échantillons. Ainsi, la prévalence d’infection par *Bartonella* et hémoplasma chez des chats en santé est relativement faible. De plus, la maîtrise des puces de chat est importante compte tenu de l’impact sérieux en santé publique des infections par *Bartonella* et *R. felis*.

(Traduit par Docteur Serge Messier)

**Introduction**

Healthy cats may be subclinical carriers of blood-borne bacterial infections that are potentially serious zoonotic pathogens and which may cause severe diseases in other cats. The genus *Bartonella* is comprised of fastidious hemotropic bacteria that have been increasingly identified in a wide range of domestic and wild animals (1). Domestic cats are the primary reservoir of 3 species of *Bartonella*, *B. henselae*, *B. clarridgeiae*, and *B. kohlerae* (2–4), all of which may cause various clinical manifestations in humans (4,5). *Bartonella henselae* is the major causative agent of cat scratch disease (CSD) in immunocompetent people and of bacillary angiomatosis and hepatic peliosis in patients with a defective immune system, especially those infected with human immunodeficiency virus (HIV) (5,6). As a result of the development of sensitive molecular techniques for the diagnosis of *Bartonella* organisms in human patient tissue samples, and because

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Received September 6, 2007. Accepted January 8, 2008.
of the increasing numbers of patients with defective immune systems through HIV infection or the use of immunosuppressive drugs, *Bartonella* spp. have emerged as agents of zoonotic infections (7). This study is the first to use both blood culture and polymerase chain reaction (PCR) to investigate the prevalence of *Bartonella* bacteremia in both pet and stray domestic cats in Ontario.

Feline hemobartonellosis is a bacteremic infection in cats that may be associated with severe hemolytic anemia. The etiologic agents of this infection, *Mycoplasma haemofelis* and *Mycoplasma haemominutum*, may cause severe hemolytic anemia, and subtle anemia or subclinical infection, respectively, in cats (8). *Candidatus Mycoplasma turicensis* is a recently recognized hemotropic mycoplasma, first identified in 2005 in Switzerland in a cat with hemolytic anemia (9). This study is the first to investigate the prevalence of the hemoplasmas, including *Candidatus Mycoplasma turicensis*, in pet and stray cats in Ontario.

*Rickettsia felis* is a recently recognized agent of flea-borne spotted fever in humans (10–12). The cat flea is the major vector of this organism. In endemic areas of the United States, peri-urban opossums are the major reservoir host of *R. felis*. The reservoir potential of cats has yet to be determined, although experimental infection of cats with *R. felis* has been demonstrated (13,14). One objective of this study was to determine if *R. felis* is present in cat fleas in Canada.

### Materials and methods

#### Blood samples

Blood from healthy domestic cats in southern Ontario was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) anticoagulant, and submitted to a laboratory (Vita-Tech Laboratories, Markham, Ontario) as part of a routine “wellness” program for cats. Care was taken to exclude samples from animals that were either suspect or clinically ill. A convenience sampling method was used, whereby approximately 45 cats > 2 years old, and 14–45 cats, ≤ 2 years old, were collected each month. A total of 742 samples were collected over a period of 12 mo (April 2006 to March 2007). Blood samples were also collected from 45 stray cats, found within Wellington County, Ontario. Blood was stored at −70°C prior to testing.

#### Cat fleas

Fleas were collected in the summer of 2006 from cats submitted for care to 3 local Humane Societies located in Guelph (*n* = 4), Kitchener-Waterloo (*n* = 7), and Cambridge (*n* = 3). In addition, fleas were collected from cats within the Wellington County area (*n* = 36), and submitted to the Ontario Veterinary College for teaching and research purposes. One flea sample consisted of 1–5 fleas pooled from each flea-infested cat. Fleas were identified as *Ctenocephalides felis* based on current taxonomic keys ([http://www.icb.usp.br/~marcelcp/Ctenocephalidesfelis.htm](http://www.icb.usp.br/~marcelcp/Ctenocephalidesfelis.htm)) and stored in 70% ethanol prior to testing.

#### DNA extraction and PCR amplification

The DNA for PCR amplification was extracted from a 200 µL aliquot of each EDTA-treated blood sample using a commercial kit (QIAamp DNA Blood Mini Kit; Qiagen, Mississauga, Ontario). Purified DNA was eluted in 150 µL elution buffer and stored at −20°C. Pooled flea samples were prepared for DNA extraction by first rinsing in sterile distilled water for 5 min at room temperature. Each sample was macerated completely in a 1.5 mL microcentrifuge tube using a sterile pipette tip. The DNA was extracted, according to the manufacturer’s instructions, using the QIAamp DNA Blood Mini Kit (Qiagen), eluted in 100 µL elution buffer and stored at −20°C. To monitor the extraction process for any foreign DNA contamination, a negative “DNA extraction” control, consisting of 200 µL sterile phosphate buffered saline (pH 7.2, PBS), was included in each batch of samples processed.

Various primer combinations, each targeting the 16S–23S rRNA intergenic region of the genus *Bartonella*, were assessed for their amplification efficiency of plasmid DNA containing the target gene of *B. henselae* (ATCC 4988). Oligonucleotide sequences of each primer are listed in Table I. The following primer combinations were examined: primer pair JF 311/JR 473, as described by Jensen et al (15); primer pair MF 321/MR 983, as described by Maggi and Breitschwerdt (16); and, a novel combination of the primers MF 321/JR 473. The PCR amplifications were performed in a 25 µL reaction mix containing a ready-to-use PCR buffer that included a proof-reading hot start DNA polymerase (Platinum PCR SuperMix High Fidelity; Invitrogen, Burlington, Ontario), 0.3 pmol/µL of each primer, and 2 µL DNA template (0.02 ng, plasmid DNA containing *B. henselae* target gene). Use of proof-reading DNA polymerase on *Bartonella* DNA when samples were extracted from blood, removed some nonspecific amplification observed with *Taq* polymerase (data not shown). Amplification conditions used in this assay consisted of denaturation at 94°C for 2 min, 45 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 58°C, 30 s of extension at 68°C, followed by an additional cycle of 7 min extension at 68°C. The predicted size of the amplicons resulting from this PCR reaction were calculated to be 152 bp, 136 bp, 233 bp, 242 bp, and 187 bp for *B. henselae*, *B. clarridgeiae*, *B. elizabethae*, *B. vinsonii* subsp. *berkhoffii*, and *B. bovis*, respectively. The PCR amplification products were visualized by ethidium bromide fluorescence after electrophoresis in a 3% agarose gel. The primer pair MF 321/JR 473 was used in subsequent PCR amplification of DNA isolated from feline blood and pooled flea samples.

A single-step PCR assay first described by Jensen et al (17) was used for the detection of the hemoplasmas. Oligonucleotide primer sequences are listed in Table I. Since *Candidatus M. turicensis* has the same amplicon size as *M. haemofelis* using this amplification protocol, *Candidatus M. turicensis*-specific primers (Table I) were used in a second PCR protocol on all *M. haemofelis* PCR-positive cases identified. This second PCR amplifies a 1317 bp region of the 16S rRNA gene of *Candidatus M. turicensis*. For both PCR reactions for hemoplasma detection, 2 µL of DNA template was added to make a total 25 µL reaction mixture containing 0.8 pmol/µL of each primer and a ready-to-use PCR mixture containing a proof-reading hot-start DNA polymerase (Platinum PCR SuperMix High Fidelity, Invitrogen). A touch-down PCR program was used: 95°C for 5 min, 35 cycles of 95°C for 30 s, 60°C to 50°C for 30 s/cycle (the annealing temperature is decreased by 1°C every cycle until 50°C, after which each cycle was for 30 s), 68°C for 2 min, and finally, 68°C for 10 min.
The PCR amplification products were visualized by ethidium bromide fluorescence following electrophoresis in a 2% agarose gel.

The DNA extracts from pooled fleas and blood from stray cats were amplified targeting 2 different genes of *R. felis* in 2 separate reactions. The first pair of primers amplified a 381 bp sequence of the citrate synthase (*gltA*) gene of the genus *Rickettsia* (Table I). The second pair of primers amplified a 840 bp sequence of an outer membrane protein B gene (*rOmpB*) of *R. felis* (Table I). For both reaction mixes, 5 μL of DNA template was added to make a total 25 μL volume of PCR reaction mix containing 1× PCR buffer (New England Biolabs, Pickering, Ontario), 0.2 mM dNTPs (Roche Diagnostics GmbH, Mannheim, Germany) 2.5 mM Mg²⁺, 1 pmol/μL of each primer and 1 U of Taq polymerase (New England Biolabs). The PCR was carried out with an initial denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. The amplification was completed with an additional elongation at 72°C for 7 min. The PCR products were electrophoresed through 2% agarose gel and visualized, after ethidium bromide labelling, under UV light.

All PCR assays were carried out in a Model T Gradient thermocycler (Biometra, Göttingen, Germany). To avoid PCR amplification product carryover, DNA extraction, PCR reaction set-up, and gel electrophoresis were each carried out in a different room. Appropriate negative reagent controls and positive DNA plasmid controls, containing the target gene of each relevant organism, were included in each PCR assay.

### DNA sequencing

To confirm the identity of PCR-amplified products, selected amplicons were sequenced (Guelph Molecular Supercentre, Laboratory Services Division, University of Guelph, Guelph, Ontario) using an ABI Prism 3100 Genetic Analyzer, and the Big Dye Terminator Reaction Kit (Version 3) (Applied Biosystems) with either universal or specific primers. Sequencing was performed directly on amplicons resulting from PCR amplification using primers specific for *B. clarridgeiae*, *R. felis*, or *Candidatus M. turicensis*. The PCR products resulting from DNA amplification specific for *B. henselae* were cloned into pGEM-T Easy vector (Promega; Fisher Scientific, Nepean, Ontario) and sequenced. The obtained sequences were compared to known sequences held in the GenBank database using the nucleotide-nucleotide BLAST function.

### Blood culture for *Bartonella* spp.

Initially, 2 culture methods were used for isolation of *Bartonella*. The first was direct culture of 200 μL of each blood sample on Brain Heart Infusion Agar (BHIA) (Difco, Detroit, Michigan, USA) with 5% chcolatized sheep blood (“chocolate agar”). Agar plates were incubated at 35°C in a 5% CO₂ water-saturated atmosphere for 6 wk. The DNA was extracted from suspect colonies (small, slow growing) and sequences specific to *Bartonella* spp. were targeted as described previously. The other method used was a novel liquid medium, *Bartonella-Alphaproteobacteria* growth medium (BAPGM) (18). A 200 μL aliquot of blood was inoculated into a tube containing BAPGM. Each tube was cotton plugged and incubated for 12 d at 35°C in a 5% CO₂ water-saturated atmosphere. After incubation, 200 μL of the blood-inoculated medium was used for DNA extraction and amplification. In addition, 200 μL of the blood-inoculated liquid medium that had been incubated for 12 d was plated on BHIA with 5% chcolatized sheep blood. The plates were then incubated for another 12 d at 35°C, as described. The DNA was isolated from

<table>
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<tr>
<th>Table I. Sequence of oligonucleotide primers used for PCR assays</th>
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<tr>
<td><strong>Primer</strong> (target gene)</td>
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<tr>
<td><strong>JF 311</strong> (16S–23S rRNA)</td>
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<td><strong>JR 473</strong> (16S–23S rRNA)</td>
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<td><strong>MF 321</strong> (16S–23S rRNA)</td>
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<td><strong>MR 983</strong> (16S–23S rRNA)</td>
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<td><strong>HF</strong> (16S rRNA)</td>
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<td><strong>HR</strong> (16S rRNA)</td>
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<td><strong>MTR</strong> (16S rRNA)</td>
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<td><strong>CSF</strong> (<em>gltA</em>)</td>
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<td><strong>CSR</strong> (<em>gltA</em>)</td>
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<td><strong>OMF</strong> (<em>rOmpB</em>)</td>
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suspect colonies (small, slow growing) and tested in the PCR assay for confirmation as Bartonella spp.

Comparison of the sensitivity of blood culture and PCR for Bartonella detection

The sensitivity of PCR and of blood culture on solid or in liquid media for detection of Bartonella spp. in blood was compared in the following way. Colonies of an isolate of B. henselae made from one of the cats in the study were suspended in 700 μL sterile PBS. After homogenization of the suspension, 10-fold serial dilutions were made in PBS until 10^{-9}; 200 μL of each dilution was used for DNA extraction and PCR amplification using primers specific for Bartonella spp. In addition, 200 μL of each dilution was plated on chocolate agar. The number of colony-forming units for each dilution was determined following 12 d incubation at 35°C. Furthermore, 200 μL of each dilution was inoculated directly into BAPGM and incubated for 12 d at 35°C. The DNA was extracted from the BAPGM and tested using the Bartonella spp. specific PCR. Aliquots of the inoculated BAPGM media were subcultured onto chocolate agar for a further 12 d; this study was done in triplicate.

Statistical analysis

The association of categorical variables of age categories and seasons of the year with each infection were tested by a chi-squared or Fisher’s exact test. The level of significance was considered at P < 0.05. All of the statistical analyses were carried out using statistical software (MINITAB, version 15.1; Minitab, State College, Pennsylvania, USA).

Results

PCR assay for Bartonella detection

Various primer combinations, each targeting the 16S–23S rRNA intergenic region of the genus Bartonella, were assessed for their efficiency of amplification of plasmid DNA containing the target gene of B. henselae. Based on band intensity, the novel combination of the forward primer MF 321 and the reverse primer JR 473 (Table I) (9,15–17,19) gave better amplification than the original combination of MF 321 and MR 983 or JF 302 and JR 473 suggested by Maggi and Breitschwerdt (16) and by Jensen et al (15), respectively (Figure 1), and was therefore used throughout the study. The PCR was optimized for DNA template concentration and annealing temperature (data not shown).

Results of PCR on blood from healthy cats

A total of 646 and 742 blood samples, from July 2006 to March 2007, were tested by PCR for the presence of Bartonella and hemoplasma, respectively. The overall results of specific PCR on blood samples are shown in Table II, while the monthly results for PCR are given in Table III. The prevalence of infection with Bartonella and hemoplasma in different seasons is illustrated in Figure 2. There was no association between the presence of Bartonella spp. and of hemoplasma in healthy cats (Fisher exact probability test: P = 0.42).

No association was found between age category, ≤ 2 and > 2 years old, for either infection (P = 0.064 for Fisher’s exact test for Bartonella infection and P-value = 0.457 for Fisher’s exact test for hemoplasma infection). No association was found between each infection and the season of sampling (P = 0.36 for chi-squared test for Bartonella infection and P = 0.33 for chi-squared test for hemoplasma infection).

After applying Candidatus M. turicensis-specific PCR to all M. haemofelis PCR-positive samples, 1 cat from the domestic pet cat population yielded a PCR product consistent with the expected Candidatus M. turicensis amplicon of approximately 1300 bp. The sequenced PCR product was 99% identical to, and had the highest homology with, the 16S rRNA gene sequence of Candidatus M. turicensis in GenBank (Accession number DQ464422.1).

Comparison of the sensitivity of culture and PCR

Comparison of the sensitivity of detection of Bartonella, which had been serially diluted in saline, showed that PCR was up to 10 times more sensitive than culture on chocolate agar, and that PCR
detected between 1 and 10 colony-forming units. The broth culture medium BAPGM, which was subcultured on chocolate agar, was found to be between 100 and 1000 times less sensitive than direct culture on chocolate agar. Culture in BAPGM broth, followed by DNA extraction and PCR analysis was found to be about 100 times less sensitive than direct culture on chocolate agar. Because of the toxicity of the BAGPM medium identified in the controlled sensitivity experiments, its use was discontinued after the first 200 blood samples were cultured.

### Results of blood culture on chocolate agar

Initially 938 blood samples were cultured for *Bartonella* detection from April 2006 to March 2007. Of these samples, 42 blood samples were discarded because of contamination. There was no significant association between *Bartonella* isolation and season (chi-squared test 1.15, *P* = 0.76), nor between *Bartonella* isolation and age (Fisher’s exact probability test, *P* = 0.78). With the exception of 3 samples, all blood samples positive by culture were also PCR positive. Direct PCR on blood was found to be 1.7 times more sensitive than blood culture for *B. henselae* (Table III). *Bartonella clarridgeiae* was not isolated from any of the samples tested.

### Results of PCR on blood from stray cats

The results of PCR for *Bartonella* spp., hemoplasma, and *R. felis* shown in Table II indicate the high prevalence of *B. henselae* (58%) and the absence of *B. clarridgeiae* in these cats. The high prevalence of *M. haemofelis* (47%) and its greater prevalence than *M. haemoninutum* (13%) is also shown (Table II).

### Results of PCR on fleas

Results of specific PCR on pooled flea samples are shown in Table II. Using primers targeting the *gltA* gene of *R. felis*, 5 of 50 samples were identified as positive and confirmed to be positive by sequencing of the resulting amplicons. In contrast, 9 of 50 samples were identified as being positive using primers targeting the *rOmpB* of *R. felis*. Sequencing of the amplicons showed 99% homology to the *rOmpB* sequence of *R. felis* in GenBank (Accession Number CP000053.1). Three samples that yielded PCR products
of the size expected for *B. henselae* were cloned into pGEM-T Easy vector, and sequenced. Resulting sequences shared 99% homology to a 16S–23S rRNA ITS sequence of *B. henselae* Houston-1 strain in GenBank (Accession Number BX897699.1). To confirm the identity of PCR products identified as *B. clarridgeiae*, 2 samples were found to be 98% identical to the 16S–23S rRNA ITS sequence of *B. clarridgeiae* Houston-2 strain in GenBank (Accession Number AF312497).

### Discussion

This is the first systematic study of the prevalence of *Bartonella* spp. and hemoplasma infection in healthy cats in Ontario, and the first to report the presence of *R. felis* in cat fleas in Canada. The study also documents the uncommon presence of *Candidatus* *M. turicensis*. There was a marked contrast between the relatively low prevalence of these bacteremic infections in healthy pet cats versus their high prevalence in stray cats, and a strikingly higher frequency of *M. haemofelis* in stray cats than in pet cats. In contrast to the blood from stray cats, the dominant hemoplasma species in fleas was *M. haemominutum*, as it was in pet cats, but the prevalence of *B. henselae* in fleas was similarly very high.

The PCR assay suggested by Jensen et al (15) for diagnosis of bartonellosis has been widely used in various epidemiological studies (20–23). The primers used may nonspecifically amplify the ubiquitous organisms of *Mesorhizobium* spp., so that Maggi and Brettschwerdt suggested that other primers be used (16). However these primers amplify a region of *B. henselae* in which there are variable repeats, resulting in amplicons of unpredictable sizes (24). The use of a novel combination of these primers (MF321/JR473) was chosen for study in this investigation. These primers amplify the most common veterinary-relevant *Bartonella* spp. but do not target the variable repeat region of *B. henselae*, thus yielding PCR products of predictable and consistent size. In addition, PCR using this novel primer pair may be more sensitive for the detection of *Bartonella* spp. in blood than other PCR assays reported.

For clinical samples, PCR detection of *Bartonella* spp. was almost twice as sensitive as culture of blood samples on chocolate agar, which was less than the sensitivity assessed using serially diluted cultures. Since 3 culture-positive samples were missed by PCR, the new PCR approach used here is not perfect, so that the best method for diagnosis of *Bartonella* bacteremia may be a combination of PCR and culture (25). In some other studies, blood culture has been shown to be more sensitive than PCR (25). Culture of blood would intuitively be an optimal approach to diagnosis, because of the relatively large volumes of blood that can be assessed compared with PCR. We observed that PCR was, surprisingly, more sensitive than blood culture. The novel liquid culture medium (BAGPM) described by Maggi et al (18) was, however, far less sensitive than culture of blood in chocolate agar or direct PCR. Recently described changes in BAGPM may have improved the sensitivity of this medium (25). We isolated no *B. clarridgeiae* which underlines its difficulty of isolation (26).

The prevalence of bacteremia in healthy cats obtained in the present study was 3.8% and 1.6%, based on the PCR and culture methods, respectively, for a combined prevalence of 4.3%. It appears that *Bartonella* bacteremia is uncommon in healthy pet cats in Ontario, which may reflect the type of cats sampled as well as the geographic location. Since these were animals in which blood profiles were being analyzed for evidence of “wellness,” it is likely that these cats were well cared for and would be unlikely to have fleas. It was, however, impossible to obtain such details. In the United States, Guppitt et al (26) found 6%, 12%, 28%, and 33% prevalence rates for *Bartonella* bacteremia in pet cats in Chicago, Washington DC, California, and Florida, respectively. Since Ontario is most similar climatically to Chicago, our findings are consistent with those of Guppitt et al (26).

As in the present study, Guppitt et al (26) observed no association between *Bartonella* bacteremia and season of sampling. The prevalence of *Bartonella* bacteremia is significantly higher in regions with higher average temperatures and humidity, favoring flea infestation, than in regions with temperate climatic conditions (26). Flea infestation, age < 13 mo, and adoption from a shelter or as a stray cat appear to be significant risk factors for *Bartonella* bacteremia (27,28). In the current study, although the prevalence of *Bartonella* bacteremia was higher in the age group of ≤ 2 years old, the difference was not significant. Lappin et al (23) conducted a PCR-based study of *Bartonella* species in 92 flea-infested cats in Alabama, Maryland, and Texas, and found *B. henselae* in 32 (34.7%), *B. clarridgeiae* in 9 (20.6%), and both species in 7 (7.6%) cats, suggesting an association between fleas and bacteremia. Many studies have determined the prevalence of *Bartonella* bacteremia in different countries. In the Netherlands, 22% of 113 cats were positive by blood culture (27). In France, 16.5% of domestic cats in Paris were found to be bacteremic (28). In the United Kingdom, 34 (9.4%) out of 360 cats sampled were bacteremic (29). It is difficult to make direct comparisons with other studies since variables such as climate, flea infestation, age, and source all appear to influence the prevalence of bartonellosis.

The prevalence of *Bartonella* bacteremia in stray cats in Ontario was remarkably high compared with pet cats. This finding is generally supported by the results of other studies (26) that suggest that being a stray cat is a risk factor for bartonellosis (27,28,30). Although a higher prevalence of *Bartonella* bacteremia has been reported from tropical countries (31,32), the high prevalence of *Bartonella* infection in the current study is striking, considering the differences in climatic conditions in Ontario compared to tropical countries or the southern United States. The prevalence of *Bartonella* infection in stray cats probably reflects higher exposure to cat fleas, which are considered the major route of *Bartonella* transmission between cats (26,28), and
is supported by the very high prevalence of *Bartonella* spp. in cat fleas in Ontario (Table II).

Numerous studies have determined the prevalence of hemoplasma infection in healthy and diseased cats in different countries. In a PCR-based study, Jensen et al (17) found an overall prevalence of 19.5% hemoplasma in a population of healthy and anemic cats in the United States. Although similar numbers of anemic and nonanemic cats were infected with *M. haemominutum*, significantly higher numbers of anemic cats were infected with *M. haemofelis*. In a PCR-based study of healthy and diseased cats from Saskatchewan and Alberta (33), most cats with regenerative anemia were infected with *M. haemofelis* rather than *M. haemominutum*. In the United Kingdom, Tasker et al (34) found the overall prevalence of hemoplasma infection in healthy and sick cats to be 18.5% but, in contrast with the results of Jensen et al (17), most sick-anemic cats were infected with *M. haemominutum* rather than *M. haemofelis*. In a study of 92 healthy cats in the United States (23), 7.6% and 21.7% were infected with *M. haemofelis* and *M. haemominutum*, respectively. The prevalence of hemoplasma infection in healthy cats in the current study was low, consistent with our earlier speculation that these cats would be unlikely to be exposed to risk factors for hemoplasma infection, and *M. haemominutum* was more common in healthy cats than *M. haemofelis*, a finding consistent with that of most previous studies. By contrast, the prevalence of hemoplasma infection in the stray cats was considerably higher. This may reflect the likelihood that being a stray implies a greater probability of flea-infestation and of fighting, but no details were available to support this speculation. Besides the high prevalence, a second striking feature in stray cats was the relatively high prevalence of *M. haemofelis* compared to *M. haemominutum*. One explanation might be the ability of *M. haemofelis* to produce prolonged bacteremia than *M. haemominutum* and *Candidatus* *M. turicensis* (35).

*Candidatus* Mycoplasma turicensis was recently discovered in a Swiss cat with severe hemolytic anemia (9). In the current study, one presumptively *M. haemofelis* PCR-positive samples was found to be *Candidatus* *M. turicensis*, the first time this has been reported in North America. In a study of 713 healthy and ill cats of Switzerland, Willi et al (35) later reported a 1.1% infection rate for *Candidatus* *M. turicensis*, all from ill cats. An infection rate for *Candidatus* *M. turicensis* of 2.3%, 10% and 26% was observed in a sample of healthy and ill cats in the United Kingdom, Australia, and ill cats of South Africa (36). The current study suggests that the infection is uncommon in cats in Ontario.

*Rickettsia felis* was identified here in cat fleas for the first time in Canada. Others have found somewhat similar or higher infection rates elsewhere (10,37,38). The PCR based on the *ompB* gene was twice as sensitive as that based on the *gltA* gene. Despite the high prevalence of *Bartonella* and hemoplasma infection, no stray cat blood was PCR positive for *R. felis*. Natural infection of cats with this organism has not been reported. Besides opossums, which are the reservoir of this organism in North America, it is believed that fleas are the main reservoir for *R. felis*, since vertical transmission of this organism has been reported (14). Experimental infection studies suggest that cats may naturally be infected by *R. felis*, but that the period of bacteremia is short (13). The current study supports the conclusion that *R. felis* bacteremia in cats is rare. In people, although this organism usually causes subclinical or mild “flu-like” symptoms, serious or even fatal infections especially in elderly and young people have been reported (11,12). The presence of this organism in cat fleas in Ontario underlines the need for flea control in domestic cats and for physicians to consider this infection in people presented with febrile illness and flea bites.

*Bartonella* DNA was amplified from 49 of the 50 flea pools, including 43 infected with *B. henselae*, 4 with *B. claridgeiae*, and 2 that were dually infected. This is higher than observed elsewhere (23,37,39). The relatively high prevalence of infection of cat fleas in the current study might relate to differences in sensitivity of the PCR assays used in these studies. The infection rates for hemoplasma in pooled fleas (33 of 50, 10% and 56% for *M. haemofelis* and *M. haemominutum*) are higher than the rates obtained in similar studies by Shaw et al (37) (49%, all *M. haemominutum*) and Lappin et al (23) (*M. haemofelis* 3.3%, and *M. haemominutum* 23.9%), but the higher prevalence of *M. haemominutum* is similar. Since fleas are hematophagous, the detection of pathogen DNA in fleas may reflect the existence of these infections in their hosts as well, but the prevalence of infection in the source cats was not determined. However the conclusion is that cats infested with *C. felis* must be exposed to a combination of flea-associated bacterial organisms, and that these may be present at high prevalence in fleas.

## Acknowledgments

The authors thank the Ontario Veterinary College’s Pet Trust for financial support. We are also grateful to Drs. A. Abrams-Ogg, H. Cai, and C. Ribble for discussions during the course of this work, and to Drs. K.R. Macaluso and B. Willi for provision of control DNA from *R. felis* and *M. turicensis*. Dr. H. Cai kindly provided control DNA from *M. haemofelis* and *M. haemominutum*. Dr. D. Bienzle provided blood from the stray cats. The authors also thank the Humane Societies of Cambridge, Kitchener, and Guelph for assistance with obtaining cat fleas.

## References


