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Bartonella Infection among Cats Adopted from a San Francisco Shelter, Revisited

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***Bartonella* infection among cats from shelters can pose a health risk to adopters. *Bartonella henselae* is the most common species, with *B. clarridgeiae* and *B. koehlerae* being less common. The lower rates of infection by the latter species may reflect their rarity or an inefficiency of culture techniques. To assess the incidence of infection, blood cultures, serology, and PCR testing were performed on 193 kittens (6 to 17 weeks old) and 158 young adult cats (5 to 12 months old) from a modern regional shelter. Classical *B. henselae* culture medium was compared to a medium supplemented with insect cell growth factors. *Bartonella* colonies were isolated from 115 (32.8%) animals, including 50 (25.9%) kittens and 65 (41.1%) young adults. Therefore, young adults were twice as likely to be culture positive as kittens. Enhanced culture methods did not improve either the isolation rate or species profile. *B. henselae* was isolated from 40 kittens and 55 young adults, while *B. clarridgeiae* was cultured from 10 animals in each group. *B. koehlerae* was detected in one young adult by PCR only. *B. henselae* genotype II was more commonly isolated from young adults, and genotype I was more frequently isolated from kittens. Kittens were 4.7 times more likely to have a very high bacterial load than young adults. A significantly higher incidence of bacteremia in the fall and winter than in the spring and summer was observed. *Bartonella* antibodies were detected in 10% (19/193) of kittens and 46.2% (73/158) of young adults, with culture-positive kittens being 9.4 times more likely to be seronegative than young adults.**

Cat scratch disease is caused by the vector-borne Gram-negative proteobacterium *Bartonella henselae* (1). Domestic cats, especially strays, have been shown to be the main reservoir of this bacterium, and the cat flea, *Ctenocephalides felis*, is its main vector (2, 3). *Bartonella* infection in cats raised in catteries or brought to shelters for adoption or rescue cats can be a major issue, as many of these cats may be bacteremic and heavily infested with fleas (4–7), thus presenting extensive opportunities for horizontal transmission of the bacterium. At least two genotypes of *B. henselae* have been identified and designated Houston-1 (type I) and Marseille (previously BATF) (type II) (1).

In their study of pound cats from the San Francisco Bay area, Koehler et al. (7) reported a bacteremia prevalence of 41% (9/22) and that younger cats were more likely to be bacteremic than older cats. After >20 years, we have a unique opportunity to reassess the prevalence and dynamics of infection by *B. henselae* and other *Bartonella* species in cats from kittenhood to adulthood from a large regional shelter that receives and adopts out >4,500 cats a year. The San Francisco Society for the Prevention of Cruelty to Animals (SPCA) receives cats and kittens from the field and from cat rescue organizations, but the vast majority (>90%) of cats comes from San Francisco Animal Control. Kittens as young as 4 to 5 weeks old may reach the shelter, but cats brought to the SPCA are usually 8 to 12 weeks old or older. It is regular practice at the San Francisco SPCA to regularly use flea control products when cats arrive at the shelter and during their stay (our unpublished data).

In the present study, we aimed to assess the prevalence of *Bartonella* infection in the cat population aged 12 months old or younger, with a special emphasis on cats <6 months old, as this age group has not been particularly emphasized in previous stud-

ies (6, 7). Evaluation of flea infestation of these cats was not conducted in the present study, as most of the cats had received flea control products at the time of arrival in the shelter. It was hypothesized that (i) the prevalence of *Bartonella* infection in San Francisco shelter kittens/cats would be lower than that in 1998 due to better flea control, (ii) most *Bartonella* infections would occur prior to entry into the shelter, and (iii) the use of *B. henselae* culture techniques with insect cell growth medium would increase the detection of *B. clarridgeiae* and other *Bartonella* species (proportion of positive cats) and the level of bacteremia (colony counts) in cats.

MATERIALS AND METHODS

Blood samples (0.5 to 1.5 ml) were collected in plastic EDTA tubes by venipuncture of the jugular or saphenous vein of cats estimated to be 12 months old or younger. Samples were collected between November 2011 and February 2013. The cats were divided into 10 age groups: 6 to 7 weeks (group A), 8 to 9 weeks (group B), 10 to 11 weeks (group C), 12 to 13 weeks (group D), 14 to 15 weeks (group E), 16 to 17 weeks (group F), 5 to

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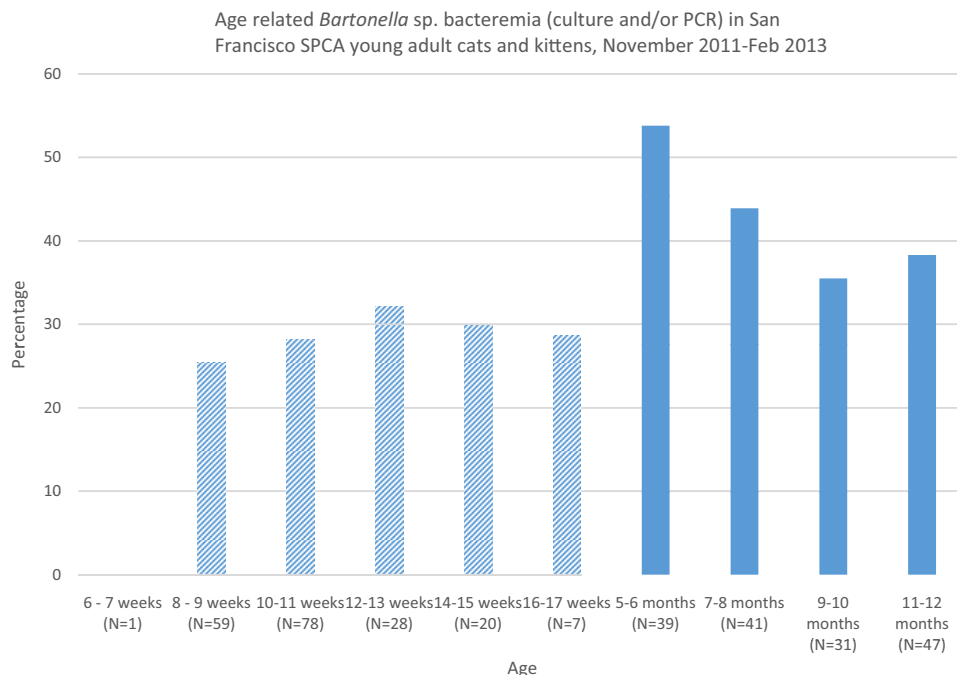


FIG 1 Age-related *Bartonella* sp. bacteremia (determined by culture and/or PCR) in young adult cats and kittens at the San Francisco SPCA from November 2011 to February 2013.

6 months (group I), 7 to 8 months (group II), 9 to 10 months (group III), and 11 to 12 months (group IV).

Collected blood samples were frozen at -70°C until testing. After thawing, whole blood was divided whenever possible into 4 aliquots of 250 μl , as follows.

Aliquot A was plated onto 5% rabbit blood agar plates after the addition of 62 μl of M199 growth mix consisting of M199 culture medium containing Earle's salts supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1 $\mu\text{g/ml}$ of amphotericin B and incubated at 35°C with 5% CO_2 for 4 weeks, as previously described (2, 8).

Aliquot B was plated onto 5% rabbit blood agar plates after the addition of 62 μl of 50% M199 growth mix and 50% Schneider's mix consisting of Schneider's medium supplemented with 10% fetal bovine serum, 5% sucrose, and 1 $\mu\text{g/ml}$ of amphotericin B (9) and incubated at 35°C with 5% CO_2 for 4 weeks.

Aliquot C was incubated for 4 days at 35°C without shaking in liquid medium consisting of 1 ml of 50% M199 growth mix and 50% Schneider's growth mix, using sterile 12-well microtiter plates. Next, 250 μl of the incubated liquid was plated onto 5% rabbit blood agar plates and incubated at 35°C with 5% CO_2 for 4 weeks.

Aliquot D was used for DNA extraction to detect *Bartonella* by PCR and for serology.

The isolated strains were confirmed to be *B. henselae* or *B. clarridgeiae* by PCR-restriction fragment length polymorphism (RLFP) analysis of the citrate synthase gene (*gltA*) using HhaI and TaqI endonucleases, as previously described (2). The number of colonies was estimated and calculated as CFU per milliliter. The 16S rRNA gene type (type I or type II) was determined by PCR, using previously designed primers (10).

Antibodies against *B. henselae* and *B. clarridgeiae* were detected by an immunofluorescence assay (IFA), as previously described (11). A fluorescence score of ≥ 2 at a 1:64 dilution was defined as a positive result.

Statistical analysis. *Bartonella* prevalence data resulting from diagnostic tests and the corresponding binomial proportion confidence intervals (CI) were calculated by using Wald and Fisher exact methods, where appropriate, in OpenEpi, version 3.03 (A. G. Dean, K. M. Sullivan, and M. M. Soe). All logistic regression models and the resulting prevalence

odds ratios (ORs) with 95% CIs were generated with SAS software, university edition (SAS Institute Inc., Cary, NC).

RESULTS

A total of 351 blood samples was available for testing and analysis, including 193 from kittens 6 to 17 weeks of age and 158 from young adult cats 5 to 12 months of age. Gender was not included in the database, as it is not a major risk factor for *Bartonella* infection (2). Fifty-eight kittens (30%) and 88 young adults (55%) were positive by either blood culture, blood PCR, or serology. Young adults were 2.93 times (95% CI = 1.89 to 4.54) more likely to be infected or to have been infected with *Bartonella* than kittens. Fifty-four kittens (28%) and 68 young adults (43%) were *Bartonella* culture and/or PCR positive (Fig. 1), indicating that young adults were 1.95 times more likely to be bacteremic than kittens (95% CI = 1.25 to 3.04). Among the 106 blood PCR-positive animals, 49 (25.4%) were kittens and 57 (36.1%) were young adults, a difference still statistically significant between the two groups (prevalence OR = 1.66; 95% CI = 1.05 to 2.62). Blood culture yielded growth of *Bartonella* colonies from 115 (32.8%) animals, including 50 (25.9%) kittens and 65 (41.1%) young adults. Young adults were twice as likely to be culture positive as kittens (OR = 2.0; 95% CI = 1.27 to 3.14), but the culture methods did not yield major differences in the numbers of positive animals. For the kittens, 47 isolates were obtained by both methods A and B, and only 43 were obtained by method C. For the young adult cats, 60, 59, and 58 isolates were obtained for each of the 3 methods, respectively. Overall, 87% (96/111) of the isolates grew on all three media. Four isolates (3 *B. henselae* genotype I [2 young adults and 1 kitten] and 1 *B. clarridgeiae* [1 kitten]) grew only on medium A, 3 isolates grew only on medium B (1 *B. henselae* genotype I [young adult], 1 *B. henselae* genotype II [kitten], and 1 *B. clarridgeiae* [young adult]), and 4 isolates grew only on

TABLE 1 Prevalences of *Bartonella henselae* genotypes in kittens and young adult cats at the San Francisco SPCA

Genotype	No. (%) of isolates ^a		
	Kittens (n = 40)	Young adults (n = 55)	Total (n = 95)
I only	21 (56.7)*	16 (43.2)	37 (39)
II only	17 (31.5)	37 (69.5)*	54 (56.8)
I/II	2 (5.0)	2 (5.0)	4 (4.2)

^a Asterisks indicate that the difference was highly statistically significant.

medium C (3 *B. henselae* genotype II [2 young adults and 1 kitten] and 1 *B. henselae* genotype I [1 young adult]). Six isolates grew only on media A and B (3 *B. henselae* genotype II [1 young adult and 2 kittens], 2 *B. henselae* genotype I [1 young adult and 1 kitten], and 1 *B. clarridgeiae* [1 young adult]), and 1 *B. clarridgeiae* isolate from a 12-month-old cat grew on media A and C.

Blood-extracted DNA was either only culture positive or only PCR positive for only a limited number of animals. Sixteen animals were culture positive but PCR negative, including 5 kittens (2 infected with *B. henselae* genotype I, 2 infected with *B. clarridgeiae*, and 1 infected with *B. henselae* genotype II) and 11 young adults (5 infected with *B. henselae* genotype II, 4 with *B. clarridgeiae*, and 1 with *B. henselae* genotype I). These 16 culture-positive and PCR-negative animals had low colony counts, ranging between 4 and 24 CFU/ml for the 5 kittens and between 4 CFU/ml and <400 CFU/ml for the 11 young adults. Seven animals were culture negative but PCR positive, including four kittens (three infected with *B. clarridgeiae* and one with *B. henselae*), and three young adults (two infected with *B. clarridgeiae* and a 9-month-old cat with *B. koehlerae*).

The *Bartonella* species isolated were *B. henselae* (95 isolates; 40 isolates from kittens and 55 isolates from young adults) and *B. clarridgeiae* (20 isolates; 10 isolates from kittens and 10 isolates from young adults). *B. clarridgeiae* accounted for 20% of the kitten isolates and 15.4% of the young adult isolates, a difference that was not statistically significant. No coinfection with *B. henselae* and *B. clarridgeiae* was detected in these cats, and no *B. koehlerae* strains were isolated. Overall, *B. clarridgeiae* was isolated ($n = 20$) or detected ($n = 5$) in 25 animals, but 6 (30%) of the 20 isolates were not detected by PCR, and 5 (25%) were detected only by PCR.

Among the *B. henselae* isolates, *B. henselae* genotype II was more commonly isolated from young adults than kittens, and conversely, *B. henselae* genotype I was more frequently isolated from kittens (Table 1). Kittens were 2.22 times more likely to be infected with *B. henselae* genotype I than young adults (95% CI = 1.0 to 4.92; $P = 0.0499$), and young adults were 2.57 times more likely to be infected with *B. henselae* genotype II than kittens (95% CI = 1.20 to 5.51). In kittens, prevalences of infection were almost

equal for each genotype, including the two coinfecting kittens (genotype I, 23 kittens; genotype II, 19 kittens). In young adult cats, genotype II was isolated from more than twice as many cats (39 young adults) as genotype I (18 young adults). Furthermore, kittens were 4.7 times (95% CI = 2.13 to 10.38) more likely to have very high numbers of CFU per milliliter (>3,000 CFU/ml) than young adults.

Seasonal trends could not be easily evaluated, as blood samples were collected mainly in the summer and fall for kittens and in the fall and winter for young adult cats. Although kittens were more frequently bacteremic in the summer and fall than in the spring (Table 2), the difference was not statistically significant for each season. However, when combining data for fall and winter versus spring and summer, both kittens and young adults were more likely to be bacteremic in the fall and winter (OR = 1.93; 95% CI = 1.20 to 3.09). When adjusting for age, the association of bacteremia and season was no longer statistically significant, suggesting age as a confounder. For young adults, bacteremia prevalence declined significantly (OR = 0.30; 95% CI = 0.12 to 0.79) for the winter season, from 53.3% (45/84) in 2012 to 26% (7/27) in 2013.

The presence of *Bartonella* antibodies against either *B. henselae* or *B. clarridgeiae* was detected in 92 (26.2%) of the 351 animals tested. Antibodies were present in almost 10% (19/193) of the kittens and 46.2% (73/158) of the young adults. Therefore, young adults were almost 8 times more likely to be seropositive than kittens (OR = 7.87; 95% CI = 4.46 to 13.87). Forty-three (46.7%) of these 92 seropositive animals were positive for both antigens, including 2 kittens and 41 young adults. Thirty-two (34.8%) animals, including 12 kittens and 20 young adults, were seropositive for *B. clarridgeiae* only, and 17 (18.5%), including 5 kittens and 12 young adults, were seropositive for *B. henselae* only. Seropositivity was more frequent in young adults than in kittens for the animals that were culture positive and/or PCR positive. However, culture-positive kittens were 9.4 times (95% CI = 3.98 to 22.02) more likely to be seronegative than young adults. Among the 32 animals seropositive for *B. clarridgeiae* only, 12 (40%; 9 young adults and 3 kittens) were culture or PCR positive for the same species, 9 (30%; 7 young adults and 2 kittens) were culture and PCR negative, and another 9 (30%; 4 young adults and 5 kittens) were culture or PCR positive for *B. henselae*. Among the 17 animals that were seropositive for *B. henselae* only, 11 (64.7%; 7 young adults and 4 kittens) were culture or PCR positive for the same species, 6 (35.3%; 5 young adults and 1 kitten) were culture or PCR negative, and none were culture or PCR positive for *B. clarridgeiae*. Among the animals that were culture and/or PCR positive, 77.9% of the young adults were also seropositive. Of the culture- and/or PCR-positive kittens, only 27.8% were seropositive. Therefore, young adult cats

TABLE 2 Seasonal prevalence of *Bartonella* bacteremia (culture and/or PCR positive) in kittens and young adult cats at the San Francisco SPCA

Culture/PCR result	No. (%) of isolates from:							
	Kittens				Young adult cats			
	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
Positive	0	3 (17.6)	32 (27.8)	19 (31.1)	52 (46.8)	0	0	16 (36.4)
Negative	0	14 (82.3)	83 (72.2)	42 (68.9)	59 (53.2)	3 (100)	0	28 (63.6)
Total	0	17	115	61	111	3	0	44

were 9.2 times (95% CI = 4.02 to 21.0) more likely to be seropositive than kittens.

DISCUSSION

The present study revealed that a high percentage (bacteremia rate of 32.8%) of young adult cats and kittens brought to the San Francisco SPCA were already infected with *Bartonella* species, mainly *B. henselae* and *B. clarridgeiae*, as only one young adult cat was PCR positive for *B. koehlerae*, confirming that this *Bartonella* species is quite uncommon (<1%) in young adult (≤ 12 months of age) domestic cats from Northern California and has a prevalence much lower than the 8% (2/25) reported previously (7). Furthermore, the actual prevalence was not much different from the prevalence of 41% that had been observed at this shelter and in stray cats from the San Francisco Bay area >20 years ago (7).

B. henselae genotype II was also the most common genotype isolated from these animals (60%), as previously reported (2). However, a major difference was observed for the prevalences of isolation of these genotypes between the two groups, as kittens were twice as likely to be culture or PCR positive for *B. henselae* genotype I as young adults. This observation raises an interesting question concerning the zoonotic aspect of *Bartonella* infection. It has been suggested that *B. henselae* genotype I is more likely to be zoonotic than genotype II (12–14). Human cases of cat scratch disease are more likely to be acquired from kittens (2, 15–17). This increased risk could be associated with a higher level of bacteremia in kittens than in young adults but also with frequent infection with *B. henselae* strains belonging to genotype I. As the present study is certainly one of the first ones to investigate natural infection of very young cats by *B. henselae*, our results will need to be confirmed by similar studies of various cat populations. On the contrary, *B. clarridgeiae* infections were quite similar in kittens and young adults and were less prevalent than *B. henselae* infections, as previously reported (6, 18). It will be of interest to investigate why kittens were almost equally infected with both genotypes, whereas older cats were twice as likely to be infected with genotype II. Such an observation suggests that in kittens, the risks of being infected by either genotype are similar, whereas in young adults, there might be an immunological selection pressure, such as a strong anti-type I immune response, as twice as many young adult cats were infected by genotype II as genotype I, including seronegative young adult cats.

This study demonstrated that kittens as young as 8 weeks old can have *Bartonella* bacteremia under natural environmental conditions. Infection at this very young age is unlikely to be due to transplacental transmission, which apparently does not occur with *B. henselae* infection (19, 20). Infection at a very young age is more likely due to natural exposure. In one study, 12-week-old specific-pathogen-free (SPF) kittens were bacteremic within 2 weeks of experimental *B. henselae* infection (21). Neonatal kittens (3 to 5 days old) were also susceptible to *Bartonella* infection following intradermal or oral inoculation in the absence of maternal immunity (22). Maternal antibodies to *B. henselae* persist for only the first 6 weeks of life (19). Therefore, very young kittens can be infected if they are born from noninfected queens (20) or after their maternal immunity is lost. In the present study, most of the kittens were IgG seronegative at the time of testing even if they were bacteremic, which is suggestive of recently acquired infection. However, cats were not tested for IgM antibodies, which may have been a better indicator of early infection.

The higher prevalence of bacteremia in kittens in the summer and fall could correspond to a higher load of fleas during these seasons. Unfortunately, only a small number of kittens was tested in the spring, which may have biased the data toward a lack of statistical significance. Similarly, the fact that we did not test young adults during the same time period did not allow for comparisons between the two age groups. The difference in the prevalence of bacteremia in young adults between winter 2012 and winter 2013 could be related to a much drier winter leading to a lower prevalence of flea infestation in these young adult cats, as California has been suffering a severe drought in the last few years, and the variation in the amount of precipitation from year to year is high. In 2013, a record low 142 mm (5.59 in.) of rainfall was recorded for downtown San Francisco, where records have been kept since 1849 (NowData, National Oceanic and Atmospheric Administration [NOAA] Online Weather Data [<https://www.ncdc.noaa.gov/cdo-web/>]; accessed 16 March 2015). In January and February 2012, the amounts of rainfall in San Francisco were 72 mm (2.82 in.) and 15 mm (0.59 in.), respectively, whereas the amounts were 14 mm (0.56 in.) and 22 mm (0.86 in.), respectively, the following year, almost a 60% decrease (<http://www.usclimatedata.com/climate/san-francisco/california/united-states/usca0987/2013/4> [accessed 16 March 2015]). However, the number of young adults tested in 2013 was much smaller than the number tested in 2012, which could be a source of selection bias.

No significant differences were observed for the yield of isolation of *Bartonella* strains based on the culture medium used. The two media composed of Schneider's growth medium with or without preincubation in liquid medium did not improve the yield of bacterial isolation by the classical approach of plating cat blood onto fresh 5% rabbit blood agar (2). Such an observation for feline *Bartonella* isolates is quite surprising compared to the successful isolation or detection of *Bartonella* in humans and dogs using a preenrichment liquid medium, bartonella-alphaproteobacterium growth medium (BAPGM), as previously reported (23, 24).

In our laboratory, direct blood culture has always been a very sensitive method for isolation of *B. henselae* from cats and is more sensitive than direct PCR of blood samples (B. B. Chomel and R. W. Kasten, unpublished data). Such an observation was confirmed in the present study, as all 11 young adult cats and 5 kittens that were culture positive and PCR negative had low CFU/milliliter counts. However, for *B. clarridgeiae*, the combination of blood culture and direct PCR substantially increased the number of positive samples detected, as 6 samples were culture positive only and 5 samples were PCR positive only. On the contrary, the two media composed of Schneider's growth medium with or without preincubation in liquid did not improve the isolation of *B. clarridgeiae* over the use of classical medium and direct PCR of blood.

Young adult cats were more likely to have been exposed to *Bartonella* than kittens, as they were almost 8 times more likely to be seropositive. Interestingly, kittens were more likely to be seropositive for one antigen only (either *B. henselae* or *B. clarridgeiae*), which could suggest a primary infection. Furthermore, the present data support the fact that there is limited cross-reactivity between *B. henselae* and *B. clarridgeiae*, as all four kittens that were culture positive and seropositive for *B. henselae* were culture positive only for *B. henselae*. However, five kittens that were *B. henselae* type I culture positive with very high bacterial loads (>3,000

CFU/ml) were seropositive for *B. clarridgeiae* only. Such a result could be related to a previous infection with *B. clarridgeiae* and a very recent infection with *B. henselae*, as a lack of cross-protection between infections with *B. henselae* type I and *B. clarridgeiae* was previously reported (25). It is also possible that there is cross-reactivity between the two antigens. However, such cross-reactivity was not observed for kittens infected by *B. henselae* type II, which is more suggestive of sequential infections. The 8 young adult cats that were seropositive for *B. henselae* were all bacteremic for only *B. henselae* type II (7 young adults) or *B. henselae* type I (1 young adult). However, for the 12 young adults that were seropositive for *B. clarridgeiae*, only 7 were also *B. clarridgeiae* culture positive, and 5 were *B. henselae* culture positive (4 positive for *B. henselae* genotype II and 1 positive for *B. henselae* genotype I), including 3 young adults with very high *B. henselae* type II bacterial loads, which is suggestive of previous infection with *B. clarridgeiae* and recent *B. henselae* infection. Finally, our data strongly support that serodiagnosis of acute *Bartonella* infection is very insensitive and that detection of *Bartonella* infection in kittens should rely primarily on blood culture.

The present study demonstrates that *Bartonella* infection is commonly occurring in young kittens and cats prior to their arrival at the San Francisco SPCA, making the management of such infections difficult and underlining the importance of regular flea control within the cat population in shelters as well as in adopting homes. Determination of the zoonotic potential of the isolates will be of importance, especially as kittens were more likely to be infected with *B. henselae* genotype I than older cats.

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