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Relationship between the Presence of *Bartonella* Species and Bacterial Loads in Cats and Cat Fleas (*Ctenocephalides felis*) under Natural Conditions

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Cats are considered the main reservoir of three zoonotic *Bartonella* species: *Bartonella henselae*, *Bartonella clarridgeiae*, and *Bartonella koehlerae*. Cat fleas (*Ctenocephalides felis*) have been experimentally demonstrated to be a competent vector of *B. henselae* and have been proposed as the potential vector of the two other *Bartonella* species. Previous studies have reported a lack of association between the *Bartonella* species infection status (infected or uninfected) and/or bacteremia levels of cats and the infection status of the fleas they host. Nevertheless, to date, no study has compared the quantitative distributions of these bacteria in both cats and their fleas under natural conditions. Thus, the present study explored these relationships by identifying and quantifying the different *Bartonella* species in both cats and their fleas. Therefore, EDTA-blood samples and fleas collected from stray cats were screened for *Bartonella* bacteria. Bacterial loads were quantified by high-resolution melt real-time quantitative PCR assays. The results indicated a moderate correlation between the *Bartonella* bacterial loads in the cats and their fleas when both were infected with the same *Bartonella* species. Moreover, a positive effect of the host infection status on the *Bartonella* bacterial loads of the fleas was observed. Conversely, the cat bacterial loads were not affected by the infection status of their fleas. Our results suggest that the *Bartonella* bacterial loads of fleas are positively affected by the presence of the bacteria in their feline host, probably by multiple acquisitions/accumulation and/or multiplication events.

Bartonellae are vector-borne hemotropic bacteria of numerous mammalian hosts, in which they typically establish persistent and subclinical infections (1). Several *Bartonella* species are considered pathogens of many incidental hosts, including humans and domesticated animals (2). Cats (*Felis catus*) are the reservoirs of three zoonotic *Bartonella* species: *Bartonella henselae*, *Bartonella clarridgeiae*, and *Bartonella koehlerae* (2). Of these, *B. henselae* is considered the main causative agent of cat scratch disease (CSD) in humans (3), while *B. clarridgeiae* has been implicated as a cause of a CSD-like disease in several cases (4–6), and *B. koehlerae* was reported to cause endocarditis in humans and dogs (7, 8). Commonly, infected cats are subclinical persistent carriers of these *Bartonella* species (9–11). Moreover, other *Bartonella* species, including *Bartonella quintana* (12, 13), *Bartonella bovis* (14), and *Bartonella vinsonii* subsp. *berkhoffii* (15), were occasionally isolated from cats. Cat fleas (*Ctenocephalides felis*) are considered the major vector of feline bartonellae. They have been experimentally proved to be a competent vector of *B. henselae* (11) and have been proposed as the potential vector of *B. clarridgeiae* and *B. koehlerae*, since DNA sequences from these *Bartonella* species have been commonly detected in *C. felis* collected from cats worldwide (16).

The distribution of *Bartonella* species in cat populations varies notably across geographic regions. Infection rates of 0 to 62% in cats have been reported worldwide (17–19) but can reach even higher percentages in isolated cat populations (11). Although the natural transmission of *Bartonella* species among cats requires the presence of fleas (11, 20) and no vertical transmission of bartonellae has been proven in either cats (maternal transmission; 21, 22) or cat fleas (23), previous studies reported a lack of association between *Bartonella* infection in cats and their fleas (11, 18). The presence and/or level of infection (bacterial loads) of the *Bartonella* species in the cats tested did not mirror the infection status of the fleas they hosted (11, 18). It has also been observed that infected cats can host negative fleas and vice versa or can be infected with different *Bartonella* species (18). The latter studies have based the infection status and quantification of bacterial loads on bacterial isolation (11, 18). However, it has been shown that isolation-based methods underestimate the rates of *Bartonella* species infection in cats in comparison to molecular-analysis-based assays (24). Thus, investigation of the distribution of *Bartonella* species and their infection loads in both hosts and vectors through a molecular-analysis-based quantitative approach was warranted. This study aimed to further investigate the relationships between *Bartonella* bacterial loads in cats and their fleas under natural conditions by quantifying their infection loads by high-resolution melt (HRM) real-time quantitative PCR (qPCR) assays and evaluating ecological factors that could influence the infection status and/or the level of *Bartonella* bacteremia.

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MATERIALS AND METHODS

Animal sample collection. Thirty-six stray cats (21 females and 15 males) were caught in the eastern suburbs of Rishon-LeZion, Israel, during August to November 2012 as part of a neutering campaign by the Municipal Veterinary Services of the city. Nineteen of the 36 cats, were kittens (<1 year old), and 17 were adults (1 to 4 years old). The animals were anesthetized with a combination of ketamine at 10 mg/kg and xylazine at 1 mg/kg injected intramuscularly. Blood samples were drawn from the cephalic or jugular vein of each cat into EDTA tubes after disinfection of the skin with 70% ethanol. All of the fleas found on each cat (one to six per cat), were collected and kept in 70% ethanol until further analyzed. After collection, EDTA-blood and flea samples were kept in a cool box (4°C), transported to the laboratory, and kept frozen at −20°C and room temperature, respectively. Prior to molecular analysis, the fleas were taxonomically identified and sexed by morphological characteristics by microscopy. The study was approved by the Hebrew University Institutional Animal Care and Use Committee (MD-12-13461-2).

Screening for feline retroviruses FeLV and FIV. Cat EDTA-blood samples were screened for feline immunodeficiency virus (FIV) antibodies and feline leukemia virus (FeLV) antigen with the commercial combined SNAP Combo Plus test (IDEXX Inc., Westbrook, ME).

DNA extraction. (i) Blood samples. Genomic DNA was extracted from 50 μl of EDTA-blood from each cat with a DNA extraction kit (BiOstic Bacteremia DNA isolation kit; MO BIO Laboratories, Inc., Carlsbad, CA). DNA was obtained in 50 μl of elution buffer. For quality assurance, a sample with all of the reagents except blood was processed in parallel with the blood samples and used as a negative control.

(ii) Flea samples. DNA was extracted individually from 90 fleas (1 to 3 per cat; 35 female fleas, 19 male fleas, and 36 fleas whose gender was undetermined) as follows. Each flea was washed once in 1 ml of 70% ethanol for 5 min and three times in 1 ml of sterile phosphate-buffered saline (PBS) for 5 min. Thereafter, the flea was homogenized in 50 μl of sterile PBS with a sterile pestle until a clear solution was obtained. Finally, the DNA was extracted with a DNA extraction kit (Illustra Tissue & Cells GenomicPrep Mini Spin kit; GE Healthcare, Buckinghamshire, United Kingdom) with an incubation step in lysis buffer and proteinase K for 2.5 h. DNA was obtained in 100 μl of elution buffer. For quality assurance, a sample with all of the reagents but no flea components was processed in parallel with the samples and used as a negative control.

Molecular quantification of *Bartonella* species bacterial loads in cats and fleas by HRM qPCR for *Bartonella* DNA. An intercalating-dye-based qPCR assay was developed to quantify the *Bartonella* bacterial loads in the cat and flea samples. Accordingly, a 190-bp fragment of the internal transcribed spacer (ITS) was targeted with primers 321s (AGATGATGATCCCAAGCCTTCTGG) and H493as (TGAACCTCCGACCTCACGCTTATC) (25). All reactions were performed in the Life Technologies StepOnePlus real-time PCR system (Thermo Fisher Scientific, Waltham, MA) in 96-well plates. The amplification protocol used was 4 min at 95°C, followed by 50 cycles of 5 s at 95°C, 30 s at 60°C (data collection on HRM reporter), and 2 s at 72°C. The HRM stage was performed at the end of the cycling stage as follows: 15 s at 95°C, followed by a temperature increase from 70 to 95°C (data collection set in 0.3%, HRM reporter). Genomic DNA samples obtained from *Bartonella*-free cat blood and *Bartonella*-free fleas were used as negative controls, and ultrapure water was used as a nontemplate control (NTC). The expected HRM profiles of *Bartonella* species ranged from 80 to 85°C (24); thus, melt profiles out of this range were considered nonspecific products.

The qPCR tests were carried out with a 20-μl final volume containing 0.5 μl of a 10 μM solution of each primer, 0.6 μl of a 50 μM Syto9 solution (Invitrogen, Carlsbad, CA), 10 μl of Maxima Hot-Start PCR Master Mix (2X) (Thermo Scientific, Surrey, United Kingdom), 3.9 μl of ultrapure water (Sigma-Aldrich, St. Louis, MO), 0.5 μl of a 25 μM solution of $MgCl_2$, and 4 μl of each genomic DNA. Cat blood DNA was diluted 1:2 in ultrapure water for the analyses. Each qPCR run included duplicates of each (blood or flea) DNA sample, *B. henselae* reference points (described below), negative-control samples, and the NTC. Any amplification with a quantification cycle (C_q) value of <40 and with an HRM pattern within the expected range was sequenced and confirmed as a positive result.

All samples were retested with an additional *Bartonella* real-time qPCR assay in order to evaluate the performance of the ITS assay (see the supplemental material). Accordingly, a 380-bp fragment of the citrate synthase gene (*gltA*) was targeted with primers 443F (GCTATGTCTGCATTCTATCA) (26) and 781R (CCACCATGAGCTGGTCCCC) (based on Bhcs.781F from Norman et al. [27]). All reactions were run under amplification conditions equal to those used for the ITS HRM qPCR assay. The PCR tests were carried out with a 20-μl final volume containing 0.5 μl of a 10 μM solution of each primer, 0.6 μl of a 50 μM Syto9 solution (Invitrogen, Carlsbad, CA), 10 μl of Maxima Hot-Start PCR Master Mix (2X) (Thermo Scientific, Surrey, United Kingdom), 4.4 μl of ultrapure water (Sigma-Aldrich, St. Louis, MO), and 4 μl of each genomic DNA.

Sequencing. All positive PCR products obtained in the ITS and *gltA* assays were purified with a PCR purification kit (Exo-SAP; New England BioLabs, Inc., Ipswich, MA) and sequenced by BigDye Terminator cycle sequencing chemistry with the Applied Biosystems ABI 3700 DNA Analyzer and the ABI Data Collection and Sequence Analysis software (ABI, Carlsbad, CA). Further analyses of sequences were done with MEGA alignment software, version 5.05 (The Biodesign Institute, Tempe, AZ).

***B. henselae* reference points for the standard curve.** A *B. henselae* isolate (cultured from blood of a stray cat from Israel) was used as the reference for quantification of *Bartonella* infection loads. Accordingly, a highly concentrated inoculum of fresh *B. henselae* colonies was homogenized in 200 μl of sterile PBS, and 10-fold dilutions ranging from 10^{-1} to 10^{-8} were prepared in sterile PBS (final volume of 400 μl). DNA was then extracted from 50 μl of each of the 10-fold bacterial dilutions and used as the reference points for the standard curve of the qPCR assay. DNA was extracted with the Illustra Tissue & Cells GenomicPrep Mini Spin DNA extraction kit as described above. The concentration of the original bacterial solution in CFU per microliter was determined by seeding 100 μl of each of the bacterial dilutions (10^{-6} to 10^{-8}) in chocolate agar plates (in duplicate). The plates were incubated at 37°C in a 5% CO_2 atmosphere for 7 days.

Standard curve for the ITS HRM qPCR assay. The standard curve for the ITS HRM-qPCR assay was obtained by running triplicates of the *B. henselae* reference points and plotting their C_q values against the log of the number of *Bartonella* CFU/μl. The linear equation of the curve and the correlation coefficients (r^2 values) were obtained with Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA). The amplification efficiency (E) of the qPCR was calculated with the formula $E = (10^{-1/\text{slope}} - 1) \times 100$. Threshold values were adjusted manually according to the geometric phase of the amplification plot of the reference points. Baselines were fixed manually (start 6, end 12 cycles) for all runs.

Real-time PCR for *C. felis* DNA. The variability in flea size between the samples, which could potentially affect the DNA extraction yield, was corrected by targeting an internal reference gene of *C. felis* in a real-time PCR assay (see data analysis section below). Accordingly, a 120-bp fragment of the cytochrome oxidase subunit 2 gene (*cox-2*) of *C. felis* was targeted with primers cox-2F (CTGCTACCGATGTTCTTCATTCA) and cox-2R (TGTCCAAAATATAATCCTGGTCGAT) (this study). The qPCRs were carried out with a 20-μl final volume containing 0.15 μl of a 10 μM solution of each primer, 0.6 μl of a 50 μM Syto9 solution (Invitrogen, Carlsbad, CA), 5.1 μl of ultrapure water (Sigma-Aldrich, St. Louis, MO), 10 μl of Maxima Hot-Start PCR Master Mix (2X) (Thermo Scientific, Surrey, United Kingdom), and 4 μl of each flea DNA sample. The amplification protocol was the same as for the ITS HRM qPCR assay. The specificity of this assay for *C. felis* only was corroborated with DNA extracted from *Ctenocephalides canis*, *Xenopsylla cheopis*, *Pulex irritans*, and *Bartonella* species. All flea samples were run in duplicate. Threshold values

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were adjusted manually and baselines were fixed automatically according the StepOnePlus software version 2.2.2.

Real-time PCR for *F. catus* DNA. An internal reference cat gene was used for a cat blood meal size estimation of all flea samples (see data analysis section below). Thus, a 286-bp amplicon of the cytochrome *b* fragment gene (*cytB*) from *F. catus* was screened with primers F3 (ATCT CAGCCTTAGCAGGAGTACAC) and R2 (TGGATCGGAGAATTGCGT ATGCGA) (28). The PCR tests were carried out with a 20-μl final volume containing 0.3 μl of a 10 μM solution of each primer, 0.6 μl of a 50 μM Syto9 solution (Invitrogen, Carlsbad, CA), 4.8 μl of ultrapure water (Sigma-Aldrich, St. Louis, MO), 10 μl of Maxima Hot-Start PCR Master Mix (2X) (Thermo Scientific, Surrey, United Kingdom), and 4 μl of each flea DNA sample. The amplification protocol was identical to that of the other PCR assays (described above). The specificity of this assay for cat blood DNA only was corroborated with DNA extracted from dog blood, cow blood, *Bartonella* species, and *C. felis* fleas collected from dogs. All flea samples were run in duplicate. Threshold values were adjusted manually and baselines were fixed automatically according the StepOnePlus software version 2.2.2.

Data analysis. The *Bartonella* ITS C_q values of positive samples were used to quantify the *Bartonella* bacterial load (number of CFU per flea or milliliter of blood) in each sample. First, the variability between runs was corrected by calculating the difference between the C_q of the reference point of each run and the C_q of the standard curve point. The latter was subtracted from the mean C_q of each positive sample. Additionally, the *Bartonella* ITS C_q values from fleas were corrected according to flea size, with the internal C_q values of the internal reference gene for *C. felis* (*cox-2*) as described elsewhere (23, 29). Finally, the *Bartonella* bacterial load (number of CFU per μl) in each sample was calculated according to the equation of the linear regression of the standard curve. For a blood meal size analysis, the difference between the mean C_q value of the *cytB* cat gene in each flea and the *cox-2* flea gene C_q value was calculated (ΔC_q) and then divided by the ratio of the *Bartonella* bacterial load of the host's blood and the median bacterial load of the cat population tested in this study. The result of the latter equation was used as a relative measurement of the blood meal size of each flea.

Statistical analyses. The *Bartonella* bacterial loads obtained by ITS quantification were compared with those obtained by *gltA* quantification by Pearson's correlation test. The bacterial loads in the cat blood samples were compared according to the cat gender, categorical age (kitten versus adult), *Bartonella* species detected, FeLV infection status, and the *Bartonella* infection status of the fleas by the nonparametric Mann-Whitney U and Kruskal-Wallis tests for two or more than two independent groups, respectively. The relationship between the categorical variables of *Bartonella* and FeLV infection statuses was evaluated by Fisher's exact test. *Bartonella* bacterial loads in fleas were quantified according to flea sex by paired analysis with the Wilcoxon signed-rank test. The effects of the cat host infection status and the *Bartonella* species detected in the fleas were evaluated by the Kruskal-Wallis and Mann-Whitney U tests. Spearman's rho correlation coefficient test was used to evaluate the *Bartonella* loads in cat blood and the average bacterial load of the fleas infected with the same *Bartonella* species and to evaluate if there is a correlation between the bacterial load in the fleas and their relative blood meal size (both analyses were performed only for fleas infected with the same *Bartonella* species as their hosts). All statistical analyses were performed in IBM SPSS Statistics software version 20 (IBM Corp., Armonk, NY). Median values and interquartile ranges (IQRs) of the groups were calculated. Statistical significance was defined as $P < 0.05$. Significance levels were adjusted for multiple tests by Bonferroni correction.

RESULTS

ITS HRM qPCR assay performance. The concentration of the original *B. henselae* bacterial solution was 9.7×10^6 CFU/μl. The standard curve for the ITS HRM qPCR assay showed an efficiency (E) of 90.2%, a y intercept of 34.204, a slope of -3.583, and a linear correlation (R^2) of 0.9941. The assay showed a detection limit of 0.32 CFU/4 μl of extracted DNA. However, the assay maintained linearity until a concentration of 1.5 CFU/4 μl of extracted DNA; thus, the latter was considered the quantifiable detection limit (QDL), corresponding to a C_q of 33.6. Consequently, the *Bartonella* bacterial loads in positive samples (confirmed by sequencing) with C_q values higher than 33.6 (QDL) were defined as 1.5 CFU/4 μl. The *Bartonella* bacterial loads estimated by the ITS qPCR assay correlated significantly with those obtained by the confirmatory *gltA* qPCR assay (Pearson's correlation, $r = 0.907$, $P < 0.001$; see Fig. S1 in the supplemental material).

Detection of *Bartonella* DNA in cats and fleas. The detection of *Bartonella* DNA in cats and their fleas is shown in Table 1. Sixty-four percent (23/36) of the cats were positive for *Bartonella* DNA. Of these, *B. clarridgeiae* DNA was identified in 36.1% (13/36), *B. henselae* DNA was identified in 22.2% (8/36), and *B. koehlerae* DNA was identified in 5.6% (2/36). The *Bartonella* species was confirmed in 91.3% (21/23) of the cases by the *gltA* assay (see Table S1 in the supplemental material). No additional coinfecting *Bartonella* species was detected in the positive cat blood samples, either when a different locus was targeted or by detection of a double HRM pattern. Seventy-eight percent (18/23) of the positive cats harbored at least one flea infected with the same *Bartonella* species, 47.8% (11/23) of the bacteremic cats harbored at least one flea infected with a different *Bartonella* species, and 26.1% (6/23) of the bacteremic cats hosted at least one negative flea. Moreover, 84.6% (11/13) of the nonbacteremic cats harbored at least one *Bartonella*-positive flea (Table 1).

Bartonella DNA was detected in 75.6% (68/90) of the *C. felis* fleas. DNA of four different *Bartonella* species was identified in these fleas. Single infection with *B. clarridgeiae* was detected in 38.9% (35/90), *B. henselae* in 26.7% (24/90), *Bartonella elizabethae*-like bacteria in 6.7% (6/90), and *B. koehlerae* in 1.1% (1/90) of the fleas. In 79.4% (54/68) of the ITS-positive samples, the *gltA* fragment was also amplified (see Table S1 in the supplemental material). In 96% (52/54) of these cases, the *Bartonella* species was confirmed. Fleas infected with *B. elizabethae*-like organisms harbored ITS sequences 100% identical to that of *B. elizabethae* (GenBank accession number L35103.1) and *gltA* sequences 98% identical to that of *Bartonella tribocorum* (GenBank accession number HG969192.1). It is noteworthy that both ITS and *gltA* sequences were found to be 100% identical to *Bartonella* sp. strain Tel Aviv Rr, which was previously isolated from commensal rats in Israel (30). In two cases, the ITS and *gltA* sequences from the same flea corresponded to different feline *Bartonella* species and were defined as coinfections (Table 1; see Table S1 in the supplemental material). Moreover, one sample was found to be positive for *B. clarridgeiae* DNA only by *gltA* real-time PCR assay (see Table S1). Of the fleas collected from bacteremic cats, 66.7% (40/60) were positive for the same *Bartonella* species as their host, while 20.0% (12/60) harbored a different *Bartonella* species and 13.3% (8/60) were negative for *Bartonella* DNA.

FeLV and FIV infection status of the cats. Eighty-one percent (29/36) of the cats were positive for the FeLV antigen, while only 5.6% (2 cats) carried antibodies against FIV. None of the cats was positive for both FeLV antigen and FIV antibodies.

Quantification of *Bartonella* bacterial loads in cats and fleas. The *Bartonella* bacterial loads determined in the positive cats ranged from 7.5×10^2 to 5.7×10^5 CFU/ml of blood, with a median bacterial load of 2.7×10^3 CFU/ml of blood (IQR, $1.1 \times$

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TABLE 1 Detection of *Bartonella* DNA in cats and their fleas, arranged according to the *Bartonella* species in the host cat[a]

		Bartonella species identified in:			
No.	Host ID	Host cat	Flea 1	Flea 2	Flea 3
1	3	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*
2	29	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*
3	30	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*
4	32	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*
5	15	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	*B. elizabethae*-like organism
6	38	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*, *B. henselae*[b]
7	7	*B. clarridgeiae*	*B. henselae*	*B. henselae*	Negative
8	26	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	Negative
9	20	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*	
10	18	*B. clarridgeiae*	*B. clarridgeiae*	*B. henselae*	
11	36	*B. clarridgeiae*	*B. henselae*	*B. henselae*	
12	1	*B. clarridgeiae*	Negative	Negative	
13	21	*B. clarridgeiae*	*B. clarridgeiae*		
14	8	*B. henselae*	*B. henselae*	*B. henselae*	*B. henselae*
15	10	*B. henselae*	*B. henselae*	*B. henselae*	*B. henselae*
16	11	*B. henselae*	*B. henselae*	*B. henselae*	*B. henselae*
17	14	*B. henselae*	*B. henselae*	*B. henselae*	*B. henselae*, *B. koehlerae*[b]
18	35	*B. henselae*	*B. henselae*	*B. henselae*	*B. elizabethae*-like organism
19	37	*B. henselae*	*B. henselae*	*B. elizabethae*-like organism	Negative
20	9	*B. henselae*	*B. henselae*	*B. clarridgeiae*	Negative
21	19	*B. henselae*	*B. elizabethae*-like organism	Negative	Negative
22	12	*B. koehlerae*	*B. clarridgeiae*	*B. clarridgeiae*	
23	16	*B. koehlerae*	*B. koehlerae*		
24	22	Negative	*B. clarridgeiae*	*B. clarridgeiae*	*B. clarridgeiae*
25	5	Negative	*B. clarridgeiae*	*B. clarridgeiae*	Negative
26	2	Negative	*B. clarridgeiae*	Negative	Negative
27	33	Negative	*B. henselae*	Negative	Negative
28	28	Negative	*B. elizabethae*-like organism	Negative	Negative
29	27	Negative	Negative	Negative	Negative
30	4	Negative	*B. clarridgeiae*	*B. clarridgeiae*	
31	24	Negative	*B. henselae*	*B. clarridgeiae*	
32	6	Negative	*B. elizabethae*-like organism	Negative	
33	13	Negative	*B. clarridgeiae*	Negative	
34	23	Negative	*B. henselae*		
35	34	Negative	*B. henselae*		
36	17	Negative	Negative	Negative	

[a] Sequence identification was performed according to the GenBank database (identities of 97 to 100%).
[b] Coinfection case identified while sequencing different *Bartonella* loci (ITS and *gltA*).

10^4). The bacterial loads were not significantly different between female and male positive cats (Mann-Whitney U test, $P = 0.727$) or between kittens and adult cats that were positive (Mann-Whitney U test, $P = 0.309$). No differences in bacterial loads were observed between the cats either according to the *Bartonella* species identified (Kruskal-Wallis test, $P = 0.133$) or according to the FeLV infection status (infected versus uninfected; Mann-Whitney U test, $P = 0.754$). Moreover, no relationship was found between *Bartonella* infection status and FeLV infection status (Fisher's exact test, $P = 0.501$).

The bacterial loads in single *Bartonella*-infected fleas ranged from 3.8×10^1 to 2.4×10^6 CFU/flea. The median bacterial loads of female and male fleas were 1.2×10^3 (IQR, 2.0×10^5) and 1.2×10^2 (IQR, 6.1×10^3) CFU/flea, respectively. No statistically significant difference was found between female and male flea pairs collected from the same host and infected with the same *Bartonella* species (paired analysis, Wilcoxon signed-rank test, $P = 0.237$). The *Bartonella* bacterial loads varied significantly according to the *Bartonella* species identified in the fleas (Kruskal-Wallis test, $P = 0.009$). The greater bacterial loads were determined in fleas infected with the three feline *Bartonellae* species (*B. henselae*, *B. clarridgeiae*, and *B. koehlerae*), while fleas infected with the *B. elizabethae*-like bacteria had bacterial loads below the QDL (37.5 CFU/flea). No significant difference was observed between the bacterial loads of fleas infected with the most prevalent feline *Bartonella* species, *B. clarridgeiae* and *B. henselae* (Mann-Whitney U test, $P = 0.415$). Moreover, positive fleas with the same *Bartonella* species as their hosts showed no correlation between their relative blood meal size and their *Bartonella* bacterial load (Spearman's rho, $r_s = -0.322$, $P > 0.05$).

The infection status of the cat host and/or the flea ectoparasites had contrasting effects on the *Bartonella* bacterial loads in the cats and/or the fleas. The *Bartonella* bacterial loads in fleas differed significantly according to the infection status of their host (Kruskal-Wallis test, $P < 0.0005$; Fig. 1). Fleas infected with the same *Bartonella* species as their host cat harbored greater bacterial loads than fleas collected from nonbacteremic cats (Mann-Whitney U test, $P < 0.0005$) or fleas infected with a *Bartonella* species

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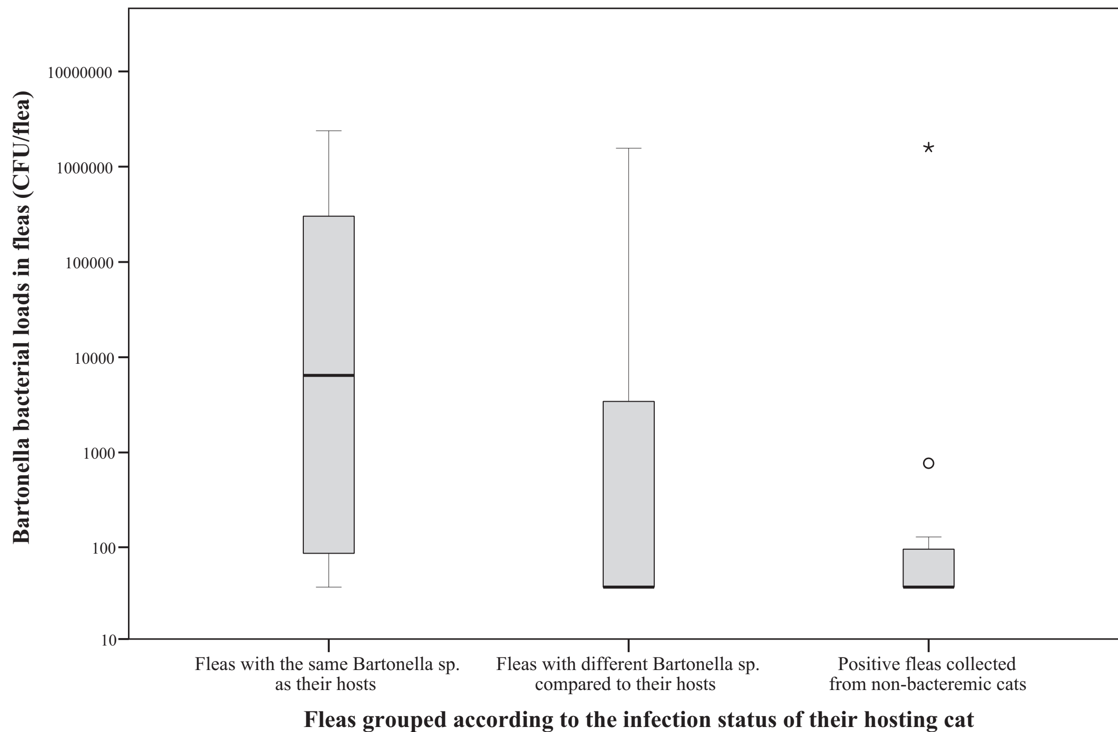

FIG 1 Effect of the *Bartonella* infection status of host cats on the *Bartonella* bacterial loads of their fleas. Box plots illustrate the distribution of the samples according to the infection status of the host cat. Boxes represent IQRs, and horizontal black thick lines represent median values. Vertical lines (whiskers) represent the distribution of maximum and minimum values. The symbols ° and * represent outliers. The values on the *y* axis are on a log scale. Kruskal-Wallis test, $P < 0.0005$.

different from that of their cat hosts (Mann-Whitney U test, $P = 0.012$). On the contrary, the cat bacterial loads did not vary significantly according to their fleas' infection status (Fig. 2). The *Bartonella* bacterial loads of cats that harbored at least one flea infected with the same *Bartonella* species did not show any significant difference from the *Bartonella* bacterial loads of cats that did not harbor any flea with the same infection status (Mann-Whitney U test, $P = 0.259$). Nevertheless, in cases where the flea and the cat hosted the same *Bartonella* species, a moderate positive correlation was observed between the average *Bartonella* loads of the positive fleas and the bacterial loads of their host cats (Spearman's rho, $r_s = 0.553$, $P = 0.017$; Fig. 3). This correlation was also confirmed with the *gltA* qPCR data (Spearman's rho, $r_s = 0.742$, $P = 0.002$; see Fig. S2 in the supplemental material).

DISCUSSION

In this study, *Bartonella* bacterial loads were measured and compared between cats and their fleas in a confined stray cat community. Our results showed a quantitative relationship between the presence of *Bartonella* bacteria in host cats and their fleas, corresponding to the dominant *Bartonella* species involved. Accordingly, a moderate positive correlation was found between the infection loads of the host cats and their fleas infected with the same *Bartonella* species. Moreover, fleas infected with the same *Bartonella* species as their host cats harbored larger numbers of bacteria than did fleas that carried *Bartonella* species other than those of their hosts or fleas collected from nonbacteremic cats. On the contrary, no significant differences were noted between the bacterial loads of cats that carried positive fleas for the same *Bartonella* species and those that carried fleas infected with other *Bartonella* species. Thus, the presence of a particular *Bartonella* species in the cat host seemed to have a positive effect on the bacterial loads of its fleas, but not the opposite. This relationship could be explained by continuous acquisitions/accumulations of bartonellae and/or multiplication events that might have occurred in the fleas, as opposed to the more complex infection dynamics in the host cats. It has been reported that *B. henselae* can persist and multiply in the gut of the flea (i.e., multiplication event) (23, 31), and since a flea can consume multiple meals from its host cat (32), new acquisitions of *Bartonella* organisms from the host may increase the number of bacteria in the flea (i.e., accumulation event). In addition, no correlation was observed between the relative amount of blood consumed by the flea and its *Bartonella* bacterial loads, suggesting that the *Bartonella* loads estimated are not necessarily a result of a recent blood meal. On the other hand, cyclic bacteremia (9, 22, 33) due to apparent cyclic release of bartonellae from a primary niche (or from secondary niches yet to be elucidated) into the bloodstream may explain the apparent independence of cat bacterial loads from the infection status of the fleas they host (infected or not infected with the same *Bartonella* species as the cat). Thus, it seems reasonable that, at a particular moment, the bacterial load of a cat is less affected by the presence of the *Bartonella* bacteria in its fleas, while bacterial loads in the fleas seem to be associated with new bacterial acquisition or input from the infected host.

The detection of a *Bartonella* species in a particular cat and its fleas has shown some inconsistencies in previous studies (11, 18, 34, 35). It has been observed that *Bartonella*-positive cats can harbor negative fleas or fleas infected with other *Bartonella* species and that nonbacteremic cats can harbor *Bartonella*-positive fleas.

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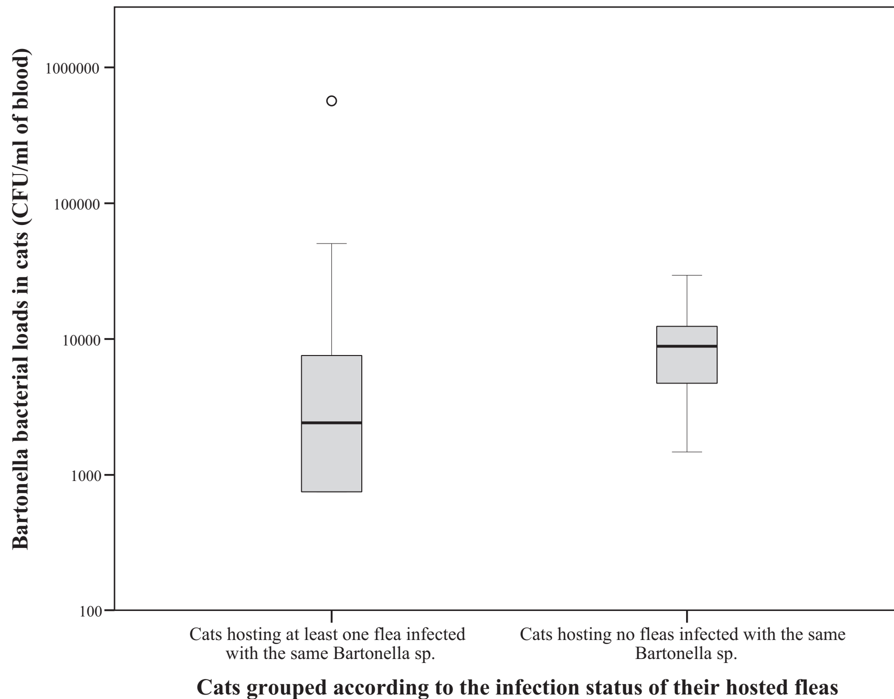

FIG 2 Effect of the *Bartonella* infection status of fleas on the *Bartonella* loads of their host cats. Box plots illustrate the distribution of the samples according to the infection status of the fleas. Boxes represent IQRs, and horizontal black thick lines representing median values. Vertical lines (whiskers) represent the distribution of maximum and minimum values. The symbol ° represents an outlier. The values on the *y* axis are on a log scale. Mann-Whitney U test, $P = 0.259$.

Our results also demonstrated these phenomena (Table 1). However, it seems that these events are less likely to occur than in cases in which the cat and its fleas host the same *Bartonella* species. In bacteremic cats, a larger percentage (66.7%) of the fleas collected contained the same *Bartonella* species as their hosts than that of fleas that harbored a different *Bartonella* species (20.0%) or did not harbor bartonellae (13.3%). The nonmatched cases could be explained by a previous encounter of those fleas with another positive host or by a hidden coinfection that was below the assay's detection limit in either the cat or the flea. Coinfection was detected in only two fleas, which were harboring two *Bartonella* species, including the *Bartonella* species that was present in their hosts. Nevertheless, we are aware that the methodology applied in this study is biased toward the detection of the dominant *Bartonella* species; thus, other coinfections could have been overlooked. The negative fleas hosted by bacteremic cats could be the result of a recent feline acquisition of noninfected fleas or newly emerged fleas that had not become infected at the time of molecular screening or had not reached detectable levels of bartonellae. On the other hand, almost all of the nonbacteremic cats (11/13) were carrying *Bartonella*-positive fleas. Interestingly, these fleas presented the lower bacterial loads in this study, further strengthening our above-mentioned presumptions about the *Bartonella* acquisition/accumulation phenomenon, which could not take place in these fleas. The apparent dissimilar scenarios of the *Bartonella* infection of hosts compared to their vectors can be further explained by unexplored events such as the recent clearance of a previous *Bartonella* species from the cats and/or fleas, nonbacteremic periods of infected cats due to the cyclic pattern of *Bartonella* bacteremia, and potential competition between different *Bartonella* species in the cats and/or fleas.

Four distinct *Bartonella* species were detected in the fleas sampled in this study, including the three acknowledged feline-associated *Bartonella* species (*B. henselae*, *B. clarridgeiae*, and *B. koehlerae*) and one rodent-associated *Bartonella* species (a *B. elizabethae*-like isolate), while only the three feline-associated *Bartonella* species were detected in the cats. The detection of a rodent-associated *Bartonella* strain closely related to *Bartonella* sp. strain Tel Aviv Rr, *B. elizabethae*, and *B. tribocorum* in the *C. felis* fleas studied supports the notion that these arthropods are in close contact with other mammal hosts and not restricted to one host species only, even though the mobility of the fleas between hosts has been estimated to be low (36). Previous experimental studies have proven that *C. felis* can acquire and maintain persistent infection with non-feline-associated bartonellae, including *B. tribocorum* (23, 37), and evidence of *C. felis* (collected from dogs) carrying *B. elizabethae* DNA has been recently reported (38). Interestingly, the transmission of this rodent-associated *Bartonella* strain from fleas to cats and the potential establishment of infection in the cats seem to be limited in the population studied, as no cat was found to be infected with this strain. In a recent experimental-infection study, Chomel et al. (33) have shown that the ability to produce bacteremia in cats varies among non-feline-associated *Bartonella* species. In a previous study performed by our group, a different rodent *Bartonella* genotype was detected in a cat (24), suggesting that cats may be infected with other rodent-associated strains. Thus, the biological role of *C. felis* fleas in the transmission of nonfeline bartonellae to cats and other mammalian hosts (including humans) needs to be further investigated.

Cat populations can represent substantial reservoirs of bartonellae under natural conditions (39). In a cat population with

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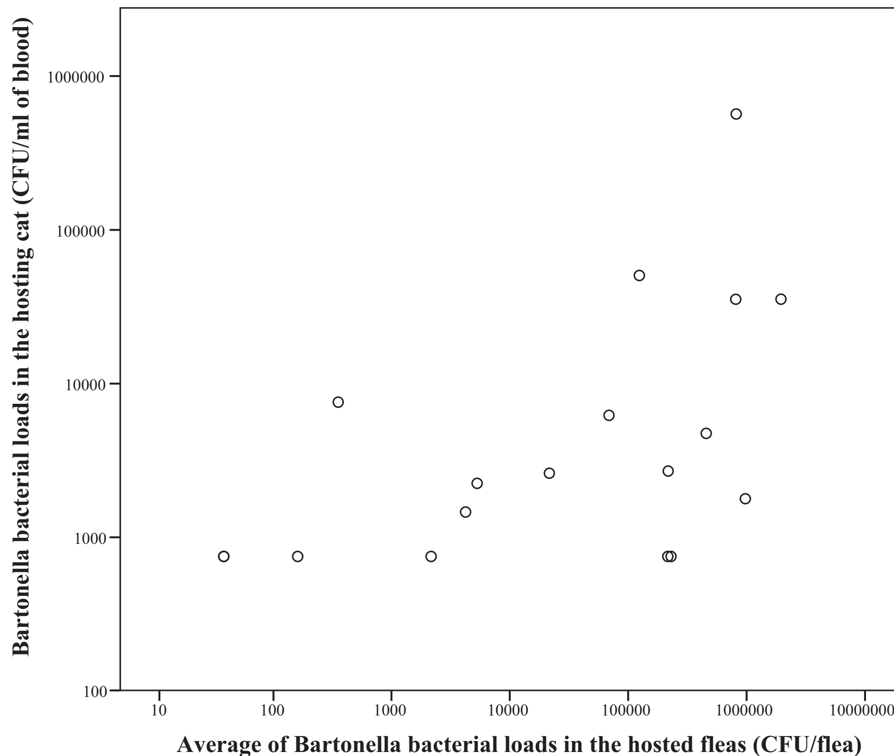

FIG 3 Correlation of *Bartonella* bacterial loads of host cats and their fleas infected with the same *Bartonella* species. Each flea bacterial load point represents the average of the fleas collected from a host cat in which the same *Bartonella* species was detected. The values on the axes are on log scales. Spearman's rho correlation coefficient, $r_s = 0.553$, $P = 0.017$.

close interactions, *Bartonella* transmission can reach high percentages, as observed in an isolated cattery with infection rates that varied from 67 to 100% over a year (11). Accordingly, the cat population studied appeared to be a focus of *Bartonella* infection in Israel, since the infection rate of the cats sampled (64%) was more than twice the general *Bartonella* prevalence in Israeli stray cats determined in a previous study (30.7%) (24). Moreover, the distribution of the *Bartonella* species differed from the general Israeli prevalence, since the percentage of infected cats with *B. clarridgeiae* (36.1%) surpassed that of cats infected with *B. henselae* (22.2%). Similarly, *B. koehlerae* was the least prevalent species detected (5.6% in both studies). The distribution of the *Bartonella* species in fleas mirrored this prevalence order. Interestingly, in other regions, such as the Palestinian Authority and France, *Bartonella* species in fleas showed a similar distribution, in which *B. clarridgeiae* was the most prevalent, more than *B. henselae* and *B. koehlerae* (40, 41). Furthermore, no differences were observed in the bacterial loads of cats according to gender (female versus male) or categorical age (kittens versus adults), reflecting a very homogeneous distribution of *Bartonella* infection within the cat population. In addition, most of the cats were infected by the immunosuppressing retrovirus FeLV (81%) or FIV (5.6%). The high percentage of retroviral infection of the cats in this study suggests that their immunological status was compromised. This might explain the higher *Bartonella* prevalence than in the Israeli stray cat population (42). However, no difference or association was observed between the FeLV infection status and the *Bartonella* loads and *Bartonella* infection status of the cats, respectively. These findings might have resulted from the low number of FeLV-negative cats included in this study. Thus, the cat population from the eastern suburbs of Rishon-LeZion, Israel, enabled us to add valuable information relating to the distribution of bartonellae in cats and fleas under natural conditions.

In conclusion, a quantitative relationship between the bacterial loads in cats and their fleas was found, especially when both harbored the same *Bartonella* species. The positive host effect on the bacterial loads of the fleas, but not the opposite, supports the notion that cats act as the source of bartonellae while fleas act as accumulators/proliferators and vehicles for dispersal. Moreover, the detection of rodent-associated bartonellae in the fleas evidences the movement of fleas between different host species. The present study suggests that the exploration and analysis of the distribution of *Bartonella* species within hosts and vectors need to be accomplished by quantitative and qualitative approaches. Accordingly, disregarding one approach might result in erroneous epidemiological conclusions.

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REFERENCES

1. **Dehio C.** 2005. *Bartonella*-host-cell interactions and vascular tumour formation. Nat Rev Microbiol **3:**621–631. http://dx.doi.org/10.1038/nrmicro1209.

Downloaded from https://journals.asm.org/journal/aem on 17 June 2024 by 44.192.48.196.

2. **Chomel BB, Kasten RW.** 2010. Bartonellosis, an increasingly recognized zoonosis. J Appl Microbiol **109:**743–750. http://dx.doi.org/10.1111/j.1365-2672.2010.04679.x.
3. **Mainardi JL, Figliolini C, Goldstein FW, Blanche P, Baret-Rigoulet M, Galezowski N, Fournier PE, Raoult D.** 1998. Cat scratch disease due to *Bartonella henselae* serotype Marseille (Swiss cat) in a seronegative patient. J Clin Microbiol **36:**2800.
4. **Kordick DL, Hilyard EJ, Hadfield TL, Wilson KH, Steigerwalt AG, Brenner DJ, Breitschwerdt EB.** 1997. *Bartonella clarridgeiae*, a newly recognized zoonotic pathogen causing inoculation papules, fever, and lymphadenopathy (cat scratch disease). J Clin Microbiol **35:**1813–1818.
5. **Margileth AM, Baehren DF.** 1998. Chest-wall abscess due to cat-scratch disease (CSD) in an adult with antibodies to *Bartonella clarridgeiae*: case report and review of the thoracopulmonary manifestations of CSD. Clin Infect Dis **27:**353–357. http://dx.doi.org/10.1086/514671.
6. **Sander A, Zagrosek A, Bredt W, Schiltz E, Piemont Y, Lanz C, Dehio C.** 2000. Characterization of *Bartonella clarridgeiae* flagellin (FlaA) and detection of antiflagellin antibodies in patients with lymphadenopathy. J Clin Microbiol **38:**2943–2948.
7. **Avidor B, Graidy M, Efrat G, Leibowitz C, Shapira G, Schattner A, Zimhony O, Giladi M.** 2004. *Bartonella koehlerae*, a new cat-associated agent of culture-negative human endocarditis. J Clin Microbiol **42:**3462–3468. http://dx.doi.org/10.1128/JCM.42.8.3462-3468.2004.
8. **Ohad DG, Morick D, Avidor B, Harrus S.** 2010. Molecular detection of *Bartonella henselae* and *Bartonella koehlerae* from aortic valves of Boxer dogs with infective endocarditis. Vet Microbiol **141:**182–185. http://dx.doi.org/10.1016/j.vetmic.2009.08.005.
9. **Kabeya H, Maruyama S, Irei M, Takahashi R, Yamashita M, Mikami T.** 2002. Genomic variations among *Bartonella henselae* isolates derived from naturally infected cats. Vet Microbiol **89:**211–221. http://dx.doi.org/10.1016/S0378-1135(02)00175-X.
10. **Yamamoto K, Chomel BB, Kasten RW, Hew CM, Weber DK, Lee WI, Droz S, Koehler JE.** 2002. Experimental infection of domestic cats with *Bartonella koehlerae* and comparison of protein and DNA profiles with those of other *Bartonella* species infecting felines. J Clin Microbiol **40:**466–474. http://dx.doi.org/10.1128/JCM.40.2.466-474.2002.
11. **Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K, Roberts-Wilson J, Gurfield AN, Abbott RC, Pedersen NC, Koehler JE.** 1996. Experimental transmission of *Bartonella henselae* by the cat flea. J Clin Microbiol **34:**1952–1956.
12. **La VD, Tran-Hung L, Aboudharam G, Raoult D, Drancourt M.** 2005. *Bartonella quintana* in domestic cat. Emerg Infect Dis **11:**1287–1289. http://dx.doi.org/10.3201/eid1108.050101.
13. **Breitschwerdt EB, Maggi RG, Sigmon B, Nicholson WL.** 2007. Isolation of *Bartonella quintana* from a woman and a cat following putative bite transmission. J Clin Microbiol **45:**270–272. http://dx.doi.org/10.1128/JCM.01451-06.
14. **Chomel BB, Boulouis HJ, Breitschwerdt EB.** 2004. Cat scratch disease and other zoonotic *Bartonella* infections. J Am Vet Med Assoc **224:**1270–1279. http://dx.doi.org/10.2460/javma.2004.224.1270.
15. **Varanat M, Travis A, Lee W, Maggi RG, Bissett SA, Linder KE, Breitschwerdt EB.** 2009. Recurrent osteomyelitis in a cat due to infection with *Bartonella vinsonii* subsp. *berkhoffii* genotype II. J Vet Intern Med **23:**1273–1277. http://dx.doi.org/10.1111/j.1939-1676.2009.0372.x.
16. **Tsai YL, Chang CC, Chuang ST, Chomel BB.** 2011. *Bartonella* species and their ectoparasites: selective host adaptation or strain selection between the vector and the mammalian host? Comp Immunol Microbiol Infect Dis **34:**299–314. http://dx.doi.org/10.1016/j.cimid.2011.04.005.
17. **Bergh K, Bevanger L, Hanssen I, Loseth K.** 2002. Low prevalence of *Bartonella henselae* infections in Norwegian domestic and feral cats. APMIS **110:**309–314. http://dx.doi.org/10.1034/j.1600-0463.2002.100405.x.
18. **La Scola B, Davoust B, Boni M, Raoult D.** 2002. Lack of correlation between *Bartonella* DNA detection within fleas, serological results, and results of blood culture in a *Bartonella*-infected stray cat population. Clin Microbiol Infect **8:**345–351. http://dx.doi.org/10.1046/j.1469-0691.2002.00434.x.
19. **Chomel BB, Carlos ET, Kasten RW, Yamamoto K, Chang CC, Carlos RS, Abenes MV, Pajares CM.** 1999. *Bartonella henselae* and *Bartonella clarridgeiae* infection in domestic cats from The Philippines. Am J Trop Med Hyg **60:**593–597.
20. **Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB.** 2005. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Vet Res **36:**383–410. http://dx.doi.org/10.1051/vetres:2005009.
21. **Guptill L, Slater LN, Wu CC, Lin TL, Glickman LT, Welch DF, Tobolski J, HogenEsch H.** 1998. Evidence of reproductive failure and lack of perinatal transmission of *Bartonella henselae* in experimentally infected cats. Vet Immunol Immunopathol **65:**177–189. http://dx.doi.org/10.1016/S0165-2427(98)00153-6.
22. **Abbott RC, Chomel BB, Kasten RW, Floyd-Hawkins KA, Kikuchi Y, Koehler JE, Pedersen NC.** 1997. Experimental and natural infection with *Bartonella henselae* in domestic cats. Comp Immunol Microbiol Infect Dis **20:**41–51. http://dx.doi.org/10.1016/S0147-9571(96)00025-2.
23. **Bouhsira E, Ferrandez Y, Liu MF, Franc M, Boulouis HJ, Biville F.** 2013. *Ctenocephalides felis* an in vitro potential vector for five *Bartonella* species. Comp Immunol Microbiol Infect Dis **36:**105–111. http://dx.doi.org/10.1016/j.cimid.2012.10.004.
24. **Gutiérrez R, Morick D, Gross I, Winkler R, Abdeen Z, Harrus S.** 2013. Bartonellae in domestic and stray cats from Israel: comparison of bacterial cultures and high-resolution melt real-time PCR as diagnostic methods. Vector Borne Zoonotic Dis **13:**857–864. http://dx.doi.org/10.1089/vbz.2013.1308.
25. **Maggi RG, Breitschwerdt EB.** 2005. Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. J Clin Microbiol **43:**1171–1176. http://dx.doi.org/10.1128/JCM.43.3.1171-1176.2005.
26. **Birtles RJ, Raoult D.** 1996. Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. Int J Syst Bacteriol **46:**891–897. http://dx.doi.org/10.1099/00207713-46-4-891.
27. **Norman AF, Regnery R, Jameson P, Greene C, Krause DC.** 1995. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol **33:**1797–1803.
28. **Natonek-Wisniewska M.** 2009. Species Identification of feline DNA based on analysis of cytochrome *b*. Ann Anim Sci **9:**379–383. http://www.izoo.krakow.pl/czasopisma/annals/2009/4/6.pdf.
29. **Robinson MT, Morgan ER, Woods D, Shaw SE.** 2010. Real-time and multiplex real-time polymerase chain reactions for the detection of Bartonella henselae within cat flea, *Ctenocephalides felis*, samples. Med Vet Entomol **24:**449–455. http://dx.doi.org/10.1111/j.1365-2915.2010.00901.x.
30. **Harrus S, Bar-Gal GK, Golan A, Elazari-Volcani R, Kosoy MY, Morick D, Avidor B, Baneth G.** 2009. Isolation and genetic characterization of a *Bartonella* strain closely related to *Bartonella tribocorum* and *Bartonella elizabethae* in Israeli commensal rats. Am J Trop Med Hyg **81:**55–58.
31. **Higgins JA, Radulovic S, Jaworski DC, Azad AF.** 1996. Acquisition of the cat scratch disease agent *Bartonella henselae* by cat fleas (Siphonaptera: Pulicidae). J Med Entomol **33:**490–495. http://dx.doi.org/10.1093/jmedent/33.3.490.
32. **Rust MK, Dryden MW.** 1997. The biology, ecology, and management of the cat flea. Annu Rev Entomol **42:**451–473. http://dx.doi.org/10.1146/annurev.ento.42.1.451.
33. **Chomel BB, Kasten RW, Stuckey MJ, Breitschwerdt EB, Maggi RG, Henn JB, Koehler JE, Chang CC.** 2014. Experimental infection of cats with *Afipia felis* and various *Bartonella* species or subspecies. Vet Microbiol **172:**505–510. http://dx.doi.org/10.1016/j.vetmic.2014.05.033.
34. **Bergmans AM, de Jong CM, van Amerongen G, Schot CS, Schouls LM.** 1997. Prevalence of *Bartonella* species in domestic cats in The Netherlands. J Clin Microbiol **35:**2256–2261.
35. **Lappin MR, Hawley J.** 2009. Presence of *Bartonella* species and *Rickettsia* species DNA in the blood, oral cavity, skin and claw beds of cats in the United States. Vet Dermatol **20:**509–514. http://dx.doi.org/10.1111/j.1365-3164.2009.00800.x.
36. **Rust MK.** 1994. Interhost movement of adult cat fleas (Siphonaptera: Pulicidae). J Med Entomol **31:**486–489. http://dx.doi.org/10.1093/jmedent/31.3.486.
37. **Kernif T, Leulmi H, Socolovschi C, Berenger JM, Lepidi H, Bitam I, Rolain JM, Raoult D, Parola P.** 2014. Acquisition and excretion of *Bartonella quintana* by the cat flea, *Ctenocephalides felis felis*. Mol Ecol **23:**1204–1212. http://dx.doi.org/10.1111/mec.12663.
38. **Sofer S, Gutiérrez R, Morick D, Mumcuoglu KY, Harrus S.** 11 April 2015. Molecular detection of zoonotic bartonellae (*B. henselae*, *B. elizabethae* and *B. rochalimae*) in fleas collected from dogs in Israel. Med Vet Entomol. http://dx.doi.org/10.1111/mve.12116.
39. **Breitschwerdt EB, Kordick DL.** 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for

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human infection. Clin Microbiol Rev **13:**428–438. http://dx.doi.org/10.1128/CMR.13.3.428-438.2000.

40. **Nasereddin A, Risheq A, Harrus S, Azmi K, Ereqat S, Baneth G, Salant H, Mumcuoglu KY, Abdeen Z.** 2014. *Bartonella* species in fleas from Palestinian territories: prevalence and genetic diversity. J Vector Ecol **39:** 261–270. http://dx.doi.org/10.1111/jvec.12100.
41. **Rolain JM, Franc M, Davoust B, Raoult D.** 2003. Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. Emerg Infect Dis **9:**338–342. http://dx.doi.org/10.3201/eid0903.020278.
42. **Harrus S, Klement E, Aroch I, Stein T, Bark H, Lavy E, Mazaki-Tovi M, Baneth G.** 2002. Retrospective study of 46 cases of feline haemobartonellosis in Israel and their relationships with FeLV and FIV infections. Vet Rec **151:**82–85. http://dx.doi.org/10.1136/vr.151.3.82.

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