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Molecular survey of *Bartonella henselae* and *Bartonella clarridgeiae* in pet cats across Japan by species-specific nested-PCR

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SUMMARY

Cats are known to be the main reservoir for *Bartonella henselae* and *Bartonella clarridgeiae*, which are the agents of 'cat-scratch disease' in humans. In the present study, we investigated the prevalence of the two *Bartonella* species on 1754 cat bloods collected from all prefectures in Japan during 2007–2008 by a nested-polymerase chain reaction (PCR) targeting the 16S–23S rRNA internal transcribed spacer region. Overall, *Bartonella* DNA was detected in 4·6% (80/1754) of the cats examined. The nested-PCR showed that 48·8% (39/80) of the positive cats were infected with *B. henselae* mono-infection, 33·8% (27/80) with *B. clarridgeiae* mono-infection and 17·5% (14/80) were infected with both species. The prevalence (5·9%; 65/1103) of *Bartonella* infection in the western part of Japan was significantly higher than that (2·3%; 15/651) of eastern Japan ($P < 0·001$). Statistical analysis of the cats examined suggested a significant association between *Bartonella* infection and FeLV infection (OR = 1·9; 95% CI = 1·1–3·4), but not with FIV infection (OR = 1·6; 95% CI = 1·0–2·6).

Key words: *Bartonella clarridgeiae*, *Bartonella henselae*, Japan, species-specific nested-PCR.

INTRODUCTION

Bartonella bacteria are haemotropic Gram-negative organisms that persistently infect a wide variety of mammalian erythrocytes [1] and several *Bartonella* species have been recognized to be pathogenic for humans [2]. *Bartonella henselae* is known to be the agent of 'cat-scratch disease' (CSD) and *B. clarridgeiae* has also been suspected as causing a few cases of CSD in humans [3]. In addition, *Bartonella koehlerae* has been associated with culture-negative endocarditis in human [4].

Cats are recognized to be the reservoir for *B. henselae*, *Bartonella clarridgeiae* and *B. Koehlerae*, which are transmitted by cat fleas between cats [5–7]. In contrast to humans, cats infected with the *Bartonella* species do not usually develop any symptoms but present relapsing bacteraemia for months or years [8–10]. Epidemiological studies have been conducted in cats

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in many countries and the prevalence of *Bartonella* varies from 24·0% (65/271) to 39·5% (81/205) in USA [11, 12], 8·1% (8/99) to 16·5% (72/436) in France [13, 14], 13·0% (13/100) in Germany [15], 61·3% (19/31) in the Philippines [16], 27·6% (76/275) in Thailand [17] and 19·1% (25/131) in Taiwan [18]. In these studies, *B. henselae* tends to be the more dominant species than *B. clarridgeiae* in many countries. In Japan, the prevalence of *Bartonella* infection in cats from 10 prefectures was reported as 7·2% (50/690) and *B. henselae* was the predominant species among the isolates identified in 2000 [19]. On the other hand, *B. koehlerae* has never been isolated from any cats not only in Japan, but also in other Asian countries to date. Therefore, surveillance of *B. henselae* and *B. clarridgeiae* infection in cats in Japan seems to be more crucial for preventing cat-associated bartonellosis rather than that of *B. koehlerae*.

Isolation of *Bartonella* bacteria is the most important method for detection of current status of bacteraemic cats. However, primary culture of *Bartonella* requires long incubation periods since the bacteria are fastidious and the growth is considerably slow [20]. Several conventional and real-time polymerase chain reactions (PCRs) have been applied as alternative diagnostic tools for the isolation of *Bartonella* bacteria from clinical specimens [21]. On the other hand, identification of *Bartonella* species by DNA sequencing with PCR amplicons takes a long time and at considerable cost [22,23]. Consequently, it is necessary to develop an easy and rapid identification method for *Bartonella* instead of DNA sequencing at the clinical site.

The objectives of the present study are to investigate the prevalence of CSD-associated *Bartonella* species by a nested-PCR capable of discriminating between *B. henselae* and *B. clarridgeiae* and to evaluate correlation between FIV or FeLV and *Bartonella* infection in pet cats from all prefectures in Japan.

MATERIALS AND METHODS

Cat blood and DNA extraction

Between 2007 and 2008, blood samples were collected from 1754 cats in animal clinics from all (47) prefectures of Japan. In total 14–41 samples were collected from each prefecture. Western and eastern parts of Japan were divided at a longitude of approximately 137° east. The samples were transferred into commercial blood collection tubes and stored at 4 °C or −20 °C, until the DNA extraction.

Before collecting the samples, the physical condition of cats was checked and the demographic information, including age and sex were obtained by clinical veterinarians. In addition, FeLV and FIV infections in the cats were examined using the Snap Combo FeLV Ag/FIV Ab test kit (Idexx Laboratories, ME, USA). The genomic DNA of *Bartonella* was extracted from 200 µl of each blood sample by using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) and kept at −20 °C until further examination.

Detection of *B. henselae* and *B. clarridgeiae* DNAs by nested-PCR

A nested-PCR was applied to detect *Bartonella* DNAs by modifying the PCR targeting a part of the 16S-23S rRNA internal transcribed spacer (ITS) region of *Bartonella* species. The genus-specific primers (URBarto1 and URBarto2) reported in the previous study [24] were used for the first PCR. The primer sets used for the second PCR were newly designed *in silico* to be specific to *B. henselae* (URBhen-f and URBhen-r) or *B. clarridgeiae* (URBcla-f and URBcla-r), respectively (Table 1). *B. henselae* strains of Houston-I^T (Accession No. L35101), CAL-1 (Accession No. AF369527) and San Ant 2 (Accession No. AF369529) and *B. clarridgeiae* strains of Houston-II^T (Accession No. AF312497) and Kyoto19-2 (Accession No. AB674239) were used for design of the species-specific primers. The DNA sequences obtained from GenBank database were analyzed by using GENETYX-win software, version 9 (Genetyx Corp., Tokyo, Japan).

First and second steps of the nested-PCRs were carried out in a 20 µl volume of mixture as follows: 1 µl of a 10 µM solution of each primer, 10 µl of 2 × Go Taq DNA polymerase master mix (Promega, Madison, WI, USA), 7 µl of nuclease-free distilled water and 1 µl of a sample DNA (1–10 ng/µl) for the first PCR or an amplicon of the first PCR for the second PCR. The first PCR was performed in the same way as the previous report [24]. Conditions for the second PCR were as follows: 3 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 30 s at 50 °C, 1 min at 72 °C and a final extension of 3 min at 68 °C. Amplicons of the PCRs were separated by electrophoresis on 3% agarose gels and visualized by staining with ethidium bromide. Any sample showing the product size of 700–722 bp by the first PCR was considered

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Table 1. *Primer information for the first and second PCRs used in this study*

PCR	Target species	Primer	Sequence (5′→3′)	Reference
First	*Bartonella* species	URBarto1	CTTCGTTTCTCTTTCTTCA	[24]
		URBarto2	CTTCTCTTCACAATTTCAAT	
Second	*B. henselae*	URBhen-f	TTGCTTCTAAAAAGCTTATCAA	This study
		URBhen-r	CAAAAGAGGGATTACAAAATC	
Second	*B. clarridgeiae*	URBcla-f	ATGCTAAAAGTTGCTATATTGG	This study
		URBcla-r	CCTCACACTAAAATATAAAAAAC	

to be positive for genus *Bartonella* and used for the following second PCR. *B. henselae* or *B. clarridgeiae*-specific primers for the second PCR were predicted to amplify products of 254 bp or 283 bp to 285 bp, respectively. Positive controls were the genomic DNAs from *B. henselae* Houston-I^T and *B. clarridgeiae* Houston-II^T and negative control was nuclease-free distilled water in the first PCR.

Statistical analysis

Chi-square test was applied to analyze the association between *Bartonella* infection and demographic factors. A $P < 0{\cdot}05$ was considered as statistically significant in this study. In relation to the association between *Bartonella* and FeLV or FIV infections, the odds ratio was calculated with a 95% confidence interval.

RESULTS

Prevalence of *Bartonella* DNA in pet cats

The first PCR detected *Bartonella* DNA in 4·6% (80/1754) of the cats examined. The following second PCR demonstrated that the DNAs of *B. henselae*, *B. clarridgeiae* and both species accounted for 48·8% (39/80), 33·8% (27/80) and 17·5% (14/80) of the DNA-positive cats, respectively.

The *Bartonella* DNA by regions was detected in 2·3% (15/651) of cats from 7 of 18 prefectures in the eastern part of Japan (Fig. 1). In contrast, the positivity rate was found to be 5·9% (65/1103) in cats from 20 of 29 prefectures in the western part of Japan. The prevalence of *Bartonella* DNA in the western part was significantly higher than that observed in the eastern part of Japan ($P < 0{\cdot}001$; Table 2).

The prevalence of *Bartonella* DNA by gender was 4·6% (43/931) in male cats and 4·4% (36/815) in female cats. Only one of eight cats was positive for *Bartonella*, where gender was unknown. Prevalence by age was 6·4% (14/218) in cats under 1 year of age and 4·3% (65/1523) in cats over 1 year of age. Only one of 13 cats was positive for *Bartonella* where age was unknown. No significant difference was observed in the prevalence when sex and age of the cats were examined and compared (Table 2).

Table 3 summarizes the prevalence of *Bartonella* DNA in relation to FeLV or FIV infections of cats. Prevalence of *Bartonella* DNA in cats that were positive or negative for FeLV infection were 7·7% (16/209) and 4·1% (64/1545), respectively; in cats with FIV infection positive or negative the prevalence was 6·4% (26/407) and 4·0% (54/1347), respectively. Statistical analysis demonstrated a significant association between *Bartonella* infection and FeLV infection (OR = 1·9; 95% CI = 1·1–3·4) but not with FIV infection (OR = 1·6; 95% CI = 1·0–2·6).

DISCUSSION

The nested-PCR used in this study could differentiate *B. henselae* and *B. clarridgeiae* infections in cat blood. Bacterial culture with blood samples and PCR and DNA sequencing with the DNA of the isolates are considered as gold-standard diagnosis methods for *Bartonella* infection in cats and the identification of *Bartonella* species. In addition, the indirect immunofluorescence assay with serum samples is also used for diagnosis of *Bartonella* infection. However, primary culture of *Bartonella* bacteria requires a long period of time, which can extend to 4 weeks because of the fastidious property of the organism [20]. Furthermore, serological tests are not adequate in understanding the present status (*vs.* historic legacy) of infection in cats [25]. The nested-PCR applied in this study can be a useful tool for estimating active infection in cats without culture of *Bartonella* bacteria. Furthermore, the nested-PCR can differentiate *B. henselae* and *B. clarridgeiae* infection in cats. Consequently, the nested-PCR may contribute to the

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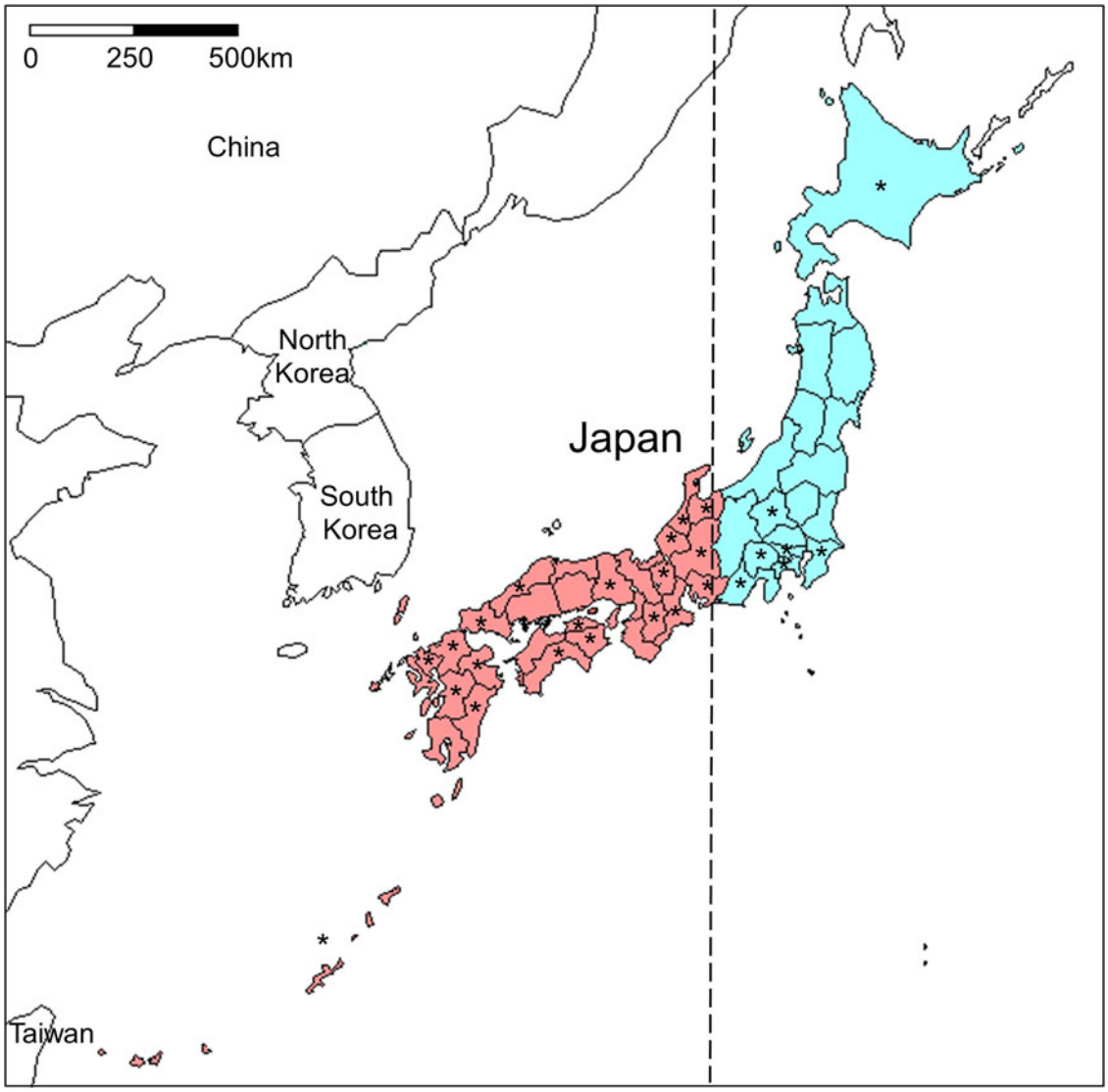

Fig. 1. Geographical distribution of the *Bartonella*-infected cats. Blue and red colors indicate the areas of eastern and western parts of Japan, respectively. The areas are segregated by a vertical dashed line located at a longitude of approximately 137° east. Asterisks represent the prefectures where *Bartonella*-infected cats were found.

rapid detection and identification of *Bartonella* in cats at the clinical site.

In the present study, *Bartonella* DNA was detected from 4·6% (80/1754) of the cats collected from all the prefectures in Japan using PCR. Maruyama *et al.* [19] showed that the prevalence of *Bartonella* by culture was 7·2% (50/690) in pet cats derived from 10 prefectures in Japan. It has been reported that the detection of *Bartonella* DNA by PCR is more sensitive than culture when performed on clinical specimens [26]. However, the positivity rate of the previous study by culture was higher than that of the present study by PCR, suggesting that the difference may be due to epidemiological biases, such as the number of prefectures examined and the study date reflecting a change in hygienic status of rearing environment. We investigated the *Bartonella* prevalence from cat samples collected from all prefectures in Japan, whereas only 10 out of 47 prefectures had been examined in the previous study [19]. Thus, the data of the present study may reflect the accurate status of *Bartonella* infection in domestic cats throughout Japan.

Our data showed that *Bartonella* prevalence in cats in the western part of Japan was significantly higher than that in the eastern part. According to previous studies in Asian countries excepting Japan, the prevalence of *Bartonella* infection in cats ranged from 19·1% in Taiwan [18] to 64·3% in Indonesia [27].

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Table 2. Bartonella *DNA prevalence in cats in Japan by respective demographic factors*

Demographic factor		No. examined	No. positive for *Bartonella* DNA (%)	*P* value
Area of Japan	East	651	15 (2·3)	<0·001
	West	1103	65 (5·9)	
Sex	Male	931	43 (4·6)	0·840
	Female	815	36 (4·4)	
	Unknown	8	1 (12·5)	–
Age	1 year >	218	14 (6·4)	0·153
	1 year ⩽	1523	65 (4·3)	
	No record	13	1 (7·7)	–

Bartonella prevalence in Asian cats varies by country, suggesting that the prevalence in tropical and sub-tropical countries tend to be higher than those of the countries with temperate climates, such as Japan. It has also been reported that cats raised in countries with high temperatures and humid climates tend to exhibit a higher seroprevalence of *B. henselae* than those cats, which dwell in cold and dry climates [28]. High temperature and humid climate is thought to be suitable for growth of ectoparasites such as cat fleas, which is the vector for *B. henselae* and *B. clarridgeiae* [5, 26]. Maruyama *et al.* [29] reported that a significantly higher seroprevalence of *B. henselae* was observed in cats with flea infestations than that in flea-free cats. *Bartonella* prevalence in cats in Kagoshima and Okinawa Prefectures located in the most south-western part of Japan was significantly higher than the other areas in Japan and flea infestation rates in the cats have also been reported to be 46·0% in Kagoshima Prefecture and 58·0% in Okinawa Prefecture. Of which, Okinawa Prefecture belong geographically to the sub-tropical region [19]. In the present study, although we could not investigate flea infestation in cats examined, the higher prevalence of this infection in the western part of Japan may possibly be attributed to the status of flea infestation in the cat population.

The percentages of PCR positive cats for *B. henselae*, *B. clarridgeiae* and both species were found to be 48·8% (39/80), 33·8% (27/80) and 17·5% (14/80), respectively. In contrast, it has been reported that *B. henselae* is much more prevalent (88·0%: 44/50) in cats followed by *B. clarridgeiae* (10·0%: 5/50), while co-infection with both species was detected only on one cat (2·0%: 1/50) [19]. The previous report suggested that [3, 30] isolation of *B. clarridgeiae* from cat blood appears to be more difficult than that of *B. henselae*. Then, the data of the present study based on PCR detection may reflect the accurate infection status of the two *Bartonella* species in pet cats in Japan.

Though no significant association was observed between prevalence and sex or age of the cats examined, cats <1 year-old tended to show higher prevalence than those aged over 1 year-old. Previous reports in the USA and the Netherlands also indicated that cats under 1 year of age showed a higher prevalence of *B. henselae* by culture and serology than the older cats [11, 31]. These facts suggest that juvenile cats rather than the adults may be more important reservoirs of CSD for humans. In fact, an association between owning a kitten was demonstrated in CSD patients in Connecticut, USA when compared with healthy persons [32].

In the present study, *Bartonella* infection was significantly associated with FeLV infection but not with FIV infection in the cats examined. The present data may support the hypothesis that susceptibility of *Bartonella* infection is possibly increased in cats with FeLV infection, regardless of whether infection is latent or progressive [33]. In contrast, it has also been suggested that *Bartonella* infection in cats was not affected by FIV [29, 34] nor by FeLV infections [35]. Therefore, the association between feline *Bartonella* bacteria and the immunosuppressive

Table 3. *Correlation between FeLV or FIV infections and* Bartonella *infection in cats*

Feline immunosuppressive viral diseases		No. examined	No. positive for *Bartonella* (%)	OR (95% CI)
FeLV infection	Positive	209	16 (7·7)	1·9 (1·1–3·4)
	Negative	1545	64 (4·1)	
FIV infection	Positive	407	26 (6·4)	1·6 (1·0–2·6)
	Negative	1347	54 (4·0)	

OR, odds ratio; CI, confidence interval.

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viruses remains controversial. Additional epidemiological studies should be conducted to assess the cause-and-effect relationship in other countries.

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REFERENCES

1. **Dehio C.** *Bartonella*-host-cell interactions and vascular tumour formation. *Nature Reviews Microbiology* 2005; **3**: 621–631.
2. **Chomel BB, Kasten RW.** Bartonellosis, an increasingly recognized zoonosis. *Journal of Applied Microbiology* 2010; **109**: 743–750.
3. **Chomel BB, *et al.*** *Bartonella* spp. in pets and effect on human health. *Emerging Infectious Diseases* 2006; **12**: 389–394.
4. **Avidor B, *et al.*** *Bartonella koehlerae*, a new cat-associated agent of culture-negative human endocarditis. *Journal of Clinical Microbiology* 2004; **42**: 3462–3468.
5. **Chomel BB, *et al.*** Experimantal transmission of *Bartonella henselae* by the cat flea. *Journal of Clinical Microbiology* 1996; **34**: 1952–1956.
6. **Tsai YL, *et al.*** *Bartonella* species and their ectoparasites: selective host adaptation or strain selection between the vector and the mammalian host? *Comparative Immunology Microbiology and Infectious Diseases* 2011; **34**: 299–314.
7. **Droz S, *et al.*** *Bartonella koehlerae* sp. nov., isolated from cats. *Journal of Clinical Microbiology* 1999; **37**: 1117–1122.
8. **Guptill L, *et al.*** Experimental infection of young specific pathogen-free cats with *Bartonella henselae*. *Journal of Infectious Diseases* 1997; **176**: 206–216.
9. **Kordick DL, Breitschwerdt EB.** Relapsing bacteremia after blood transmission of *Bartonella henselae* to cats. *American Journal of Veterinary Research* 1997; **58**: 492–497.
10. **Kabeya H, *et al.*** Genomic variations among *Bartonella henselae* isolates derived from naturally infected cats. *Veterinary Microbiology* 2002; **89**: 211–221.
11. **Chomel BB, *et al.*** *Bartonella henselae* prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. *Journal of Clinical Microbiology* 1995; **33**: 2445–2450.
12. **Guptill L, *et al.*** Prevalence, risk factors, and genetic diversity of *Bartonella henselae* infections in pet cats in four regions of the United States. *Journal of Clinical Microbiology* 2004; **42**: 652–659.
13. **Gurfield AN, *et al.*** Epidemiology of *Bartonella* infection in domestic cats in France. *Veterinary Microbiology* 2001; **80**: 185–198.
14. **Rolain JM, *et al.*** Prevalence of *Bartonella clarridgeiae* and *Bartonella henselae* in domestic cats from France and detection of the organisms in erythrocytes by immunofluorescence. *Clinical and Diagnostic Laboratory Immunology* 2004; **11**: 423–425.
15. **Sander A, *et al.*** Detection and identification of two *Bartonella henselae* variants in domestic cats in Germany. *Journal of Clinical Microbiology* 1997; **35**: 584–587.
16. **Chomel BB, *et al.*** *Bartonella henselae* and *Bartonella clarridgeiae* infection in domestic cats from The Philippines. *American Journal of Tropical Medicine and Hygiene* 1999; **60**: 593–597.
17. **Maruyama S, *et al.*** Prevalence of *Bartonella* species and 16s rRNA gene types of *Bartonella henselae* from domestic cats in Thailand. *American Journal of Tropical Medicine and Hygiene* 2001; **65**: 783–787.
18. **Chang CC, *et al.*** Cat-scratch disease in veterinary-associated populations and in its cat reservoir in Taiwan. *Veterinary Research* 2006; **37**: 565–577.
19. **Maruyama S, *et al.*** Prevalence of *Bartonella henselae*, *Bartonella clarridgeiae* and the 16S rRNA gene types of *Bartonella henselae* among pet cats in Japan. *Journal of Veterinary Medical Science* 2000; **62**: 273–279.
20. **Brenner SA, *et al.*** Isolation of *Bartonella* (*Rochalimaea*) *henselae*: effects of methods of blood collection and handling. *Journal of Clinical Microbiology* 1997; **35**: 544–547.
21. **Fenollar F, Raoult D.** Molecular genetic methods for the diagnosis of fastidious microorganisms. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 2004; **112**: 785–807.
22. **Angelakis E, *et al.*** Real-time PCR strategy and detection of bacterial agents of lymphadenitis. *European Journal of Clinical Microbiology and Infectious Diseases* 2009; **28**: 1363–1368.
23. **Gutierrez R, *et al.*** Bartonellae in domestic and stray cats from Israel: comparison of bacterial cultures and high-resolution melt real-time PCR as diagnostic methods. *Vector-Borne and Zoonotic Diseases* 2013; **13**: 857–864.
24. **Rolain JM, *et al.*** Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. *Emerging Infectious Diseases* 2003; **9**: 338–342.
25. **Bergmans AM, *et al.*** Pitfalls and fallacies of cat scratch disease serology: evaluation of *Bartonella henselae*-based indirect fluorescence assay and enzyme-linked immunoassay. *Journal of Clinical Microbiology* 1997; **35**: 1931–1937.
26. **Ciervo A, Ciceroni L.** Rapid detection and differentiation of *Bartonella* spp. by a single-run real-time PCR. *Molecular and Cellular Probes* 2004; **18**: 307–312.
27. **Marston EL, *et al.*** Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in an urban Indonesian cat population. *Clinical and Diagnostic Laboratory Immunology* 1999; **6**: 41–44.

28. **Jameson P, *et al.*** Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. *Journal of Infectious Diseases* 1995; **172**: 1145–1149.
29. **Maruyama S, *et al.*** Seroprevalence of *Bartonella henselae*, *Toxoplasma gondii*, FIV and FeLV infections in domestic cats in Japan. *Microbiology and Immunology* 2003; **47**: 147–153.
30. **Breitschwerdt EB, *et al.*** *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbiology Reviews* 2000; **13**: 428–438.
31. **Bergmans AM, *et al.*** Prevalence of *Bartonella* species in domestic cats in the Netherlands. *Journal of Clinical Microbiology* 1997; **35**: 2256–2261.
32. **Zangwill KM, *et al.*** Cat scratch disease in Connecticut. Epidemiology, risk factors, and evaluation of a new diagnostic test. *New England Journal of Medicine* 1993; **329**: 8–13.
33. **Buchmann AU, *et al.*** Does a feline leukemia virus infection pave the way for *Bartonella henselae* infection in cats? *Journal of Clinical Microbiology* 2010; **48**: 3295–3300.
34. **Ueno H, *et al.*** Does coinfection of *Bartonella henselae* and FIV induce clinical disorders in cats? *Microbiology and Immunology* 1996; **40**: 617–620.
35. **Glaus T, *et al.*** Seroprevalence of *Bartonella henselae* infection and correlation with disease status in cats in Switzerland. *Journal of Clinical Microbiology* 1997; **35**: 2883–2885.