Molecular Detection of *Rickettsia felis* and *Bartonella henselae* in Dog and Cat Fleas in Central Oromia, Ethiopia

Bersissa Kumsa, Philippe Parola, Didier Raoult, and Cristina Socolovschi*

Aix Marseille Université, Marseille, France; Department of Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia

Abstract. Fleas are important vectors of several *Rickettsia* and *Bartonella* spp. that cause emerging zoonotic diseases worldwide. In this study, 303 fleas collected from domestic dogs and cats in Ethiopia and identified morphologically as *Ctenocephalides felis felis*, *C. canis*, *Pulex irritans*, and *Echidnophaga gallinacea* were tested for *Rickettsia* and *Bartonella* DNA by using molecular methods. *Rickettsia felis* was detected in 21% of fleas, primarily *C. felis*, with a similar prevalence in fleas from dogs and cats. A larger proportion of flea-infested dogs (69%) than cats (37%) harbored at least one *C. felis* infected with *R. felis*. *Rickettsia typhi* was not detected. *Bartonella henselae* DNA was detected in 6% (2 of 34) of *C. felis* collected from cats. Our study highlights the likelihood of human exposure to *R. felis*, an emerging agent of spotted fever, and *B. henselae*, the agent of cat-scratch disease, in urban areas in Ethiopia.

INTRODUCTION

Fleas (order Siphonaptera) are holometabolous blood-feeding insects; the immature stages (egg, larva, and pupa) are found in burrows or nests, whereas adult fleas are usually found on animal hosts.[1] These insects are considered ectoparasites of dogs and cats[2] and also infest and readily feed on other species of domestic animals and humans.[3] *Ctenocephalides felis felis* (cat flea), *C. canis* (dog flea), *Pulex irritans* (human flea), *Echidnophaga gallinacea* (sticktight poultry flea), and *Xenopsylla cheopis* (rat flea) are the most commonly reported species on dogs and cats.[2,4] In infested dogs and cats, flea bites cause dermatologic problems such as severe pruritus, self-inflicted trauma, and flea allergy dermatitis.[5]

Fleas are important vectors and reservoirs of several *Rickettsia* and *Bartonella* spp. that cause emerging or re-emerging zoonotic infectious diseases in humans.[6,7] *Rickettsia* spp. (order Rickettsiales, family Rickettsiaceae) are small, gram-negative, obligate, intracellular fastidious bacteria.[8,9] Flea-transmitted *Rickettsia* spp. that are pathogenic to humans include *Rickettsia typhi*, which belongs to the typhus group and is the causative agent of murine typhus; and *R. felis*, which belongs to the spotted fever group. Laboratory studies have indicated that both *Rickettsia* species are maintained in fleas by vertical and horizontal transmission and can persist for life without causing damage.[11] *Rickettsia felis* is transmitted to humans by flea bites and contact with flea feces. Recently, *R. felis* was detected in patients with fever of unknown cause, with a prevalence of 4.4% in Senegal[11] and 3.7–7.2% in Kenya.[12] In addition, 3.4% of the afebrile patients in western Kenya were positive for *R. felis* by molecular methods.[13] Although *C. felis* is considered to be the only known biological vector of *R. felis*,[10] *R. felis* has recently been detected in different flea species in many countries in Europe,[14–16] Asia,[17] north and sub-Saharan Africa[18–20] and North and Latin America.[21,22] However, in Ethiopia, few reports are available,[23] although *R. felis* has recently been reported in a small number of *P. irritans* and *C. felis* specimens collected from human dwellings in southwestern regions of this country.[19]

Bartonella spp. are hemotropic, facultative, gram-negative, intracellular bacteria that are usually transmitted by arthropod vectors, such as fleas, lice, flies, and ticks. These bacteria are highly adapted to their mammalian hosts and infect erythrocytes and endothelial cells. To date, the genus *Bartonella* comprises more than 20 species and subspecies, including *B.* henselae, *B. quintana*, *B. alsatica*, *B. clarridgeiae*, *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis*, *B. vinsonii* subsp. *berkhoffii*, *B. washoensis*, *B. rochalima*, and *B. koehlerae*, which are pathogenic to humans and detected in fleas.[24] Cats are considered to be the main reservoir of *B. henselae* and can transmit the pathogen to humans via scratches or bites, although naturally infected cats are asymptomatic. Although several countries worldwide have reported *B. henselae* in cats and in fleas,[25,26] the presence of *Bartonella* spp. in fleas was not reported in Ethiopia until recently. However, an 11% (5 of 46) prevalence of antibodies against *Bartonella* spp. was reported in cats from Addis Ababa,[27] and *Bartonella* spp. related to *B. elizabethae* were reported in small mammals in northern Ethiopia.[28]

All reports of fleas on animals in Ethiopia resulted from studies of other ectoparasites, specifically ticks, mange mites, and lice,[1] and these studies focused on the epidemiology, species composition, and impact of ectoparasites on the skin and hides of food animals. As a result, studies of the role of fleas as vectors of pathogens of veterinary and medical importance in Ethiopia are lacking. Despite the nationwide distribution and great economic significance of fleas, information on the occurrence of *Bartonella* spp. in fleas collected from domestic animals in this country is not available. In addition, little is known about *Rickettsia* species in fleas collected from domestic animals in Ethiopia. Therefore, the current study was conducted to determine the presence of *Bartonella* and *Rickettsia* species in fleas collected from domestic dogs and cats in central Oromia, Ethiopia.

MATERIALS AND METHODS

Study areas and animals. In this study, domestic dogs and cats in the town of Bishoftu, Ethiopia (8°44′37.69″N, 38°59′19.28″E) were studied for flea infestation. Bishoftu, the capitol of the Ada'a District, is located 47 km east of Addis Ababa and has an elevation of 1,880 meters above sea level. The town has a population of 95,000 persons. The area

* Address correspondence to Cristina Socolovschi, Faculté de Médecine, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5 France. E-mail: cristina.socolovschi@univ-amu.fr

receives an average annual rainfall of 8.00 mm[3], has average maximum and minimum temperatures of 30.7°C and 8.5°C, respectively, and a mean relative humidity of 61.3%.[29]

Fleas were collected during September–November 2011. In the study area, the main rainy season is during mid-June–September, and the dry season is during November–May. All study animals were selected irrespective of sex, age, and breed by using a simple random sampling method. The sex (female, male) and age (young, adult) were recorded for each study animal. The animals were categorized into two age groups, young (≤ 1 year) and adult (> 1 year) according to Kumsa and Mekonnen.[1] In the town of Bishoftu, dogs generally stay in gardens and their owners provide food. Cats also usually stay in gardens and are provided with food, water, and sleeping areas by the owners. In the present study, stray dogs and cats were not sampled.

Study methods. A cross-sectional study type was used to determine the species of fleas infesting dogs and cats in Bishoftu by using a house-to-house survey. All collected fleas were tested by using molecular methods for *Bartonella* spp. and spotted fever and typhus group *Rickettsia* spp.

Flea collection and identification. The skin of each study dog and cat was first rubbed with a piece of cotton soaked in ether and then combed for 10–15 minutes by using a one-toothed standard flea comb (0.08 cm distance between teeth), which other previous investigators recommended for flea collection from dogs and cats.[1,30] After combing, the flea comb was held over a white plastic tray, and the fleas were collected from the tray by using forceps. *Echidnophaga gallinacea* fleas, usually found firmly attached to the skin around the eyelids, snout, ears, lips and heads of the infested animals, were manually collected y using forceps and gloved hands. The collected fleas (minimum = 1 and maximum = 23) from each infested dog and cat were placed into pre-labeled small plastic tubes. All collected fleas from each animal were placed in separate vials containing 70% ethanol and transported to the Laboratory of the World Health Organization Collaborative Center for Rickettsial Diseases and Arthropod-borne Bacterial Diseases in Marseille, France.

Morphologic identification of fleas and molecular studies were performed from the end of 2011 through 2012. The flea species and sex were determined by using a microscope according to standard morphologic identification keys as described.[1] The head shape, length of the first and second spines of the genal comb, number of setae-bearing notches of the dorsal aspect of the hind tibiae, and the number of setae on the first segment of the neck were used to differentiate *C. felis* from *C. canis* as described.[1] Photographs of body parts of each flea were made.

DNA extraction from fleas. Before DNA extraction, each flea specimen was rinsed twice in sterile water for 15 minutes and dried on sterile filter paper. Each specimen was dissected laterally into two halves. Genomic DNA was extracted from each specimen by using the QIAamp DNA Tissue Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The DNA from each flea was eluted in 100 µL of Tris-EDTA buffer and stored at −20°C under sterile conditions to preclude contamination until the sample was used for polymerase chain reaction. The second half of each flea was stored at −80°C as a backup sample.

Molecular detection of *Rickettsia* and *Bartonella* species. The DNA extracted from the fleas was individually tested by using genus-specific quantitative PCR (qPCR) for spotted fever group *Rickettsia* by targeting the *glt*A gene (RKND03 system)[19,20] in accordance with the manufacturer's recommendations and protocols (Applied Biosystems, Foster City, CA). In addition, each DNA sample was tested for the *bio*B gene by using a second qPCR with primers and a probe specific for *R. felis*.[20,25,31] All DNA samples positive for either the *glt*A or *bio*B genes were further confirmed by a third qPCR specific for the *orf*B gene, which is highly sensitive and specific for *R. felis*, by using described primers and probes.[20,31] A sample was considered positive for *R. felis* when it was showed a positive result for at least two of the genes described above.

In addition, 21 randomly selected *R. felis* DNA-positive samples were subjected to a standard PCR analysis specific for the *omp*A gene with primers 190.70, 190.180, and 190.701 (Eurogentec, Seraing, Belgium), which amplify a 629–632-basepair fragment.[20,31] Sterile water was used as a negative control and DNA from *R. montanensis* was used as a positive control. Sequencing was performed as described.[32] Sequences were aligned by using ChromasPro version 2.31 (http://en.bio-soft.net/dna/chromas.html) and then analyzed and compared with the *Rickettsia* sequences available in GenBank by using the BLAST search tool.

All flea samples were also individually tested by genus-specific qPCR for typhus group *Rickettsia* spp. by using a PCR specific for the *Rpr274P*[20,32] gene according to the manufacturer's protocol using a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Sterile water was used as a negative control and DNA from an *R. typhi* cell culture was used as a positive control.

All samples were individually tested for the presence of the 16S–23S ribosomal RNA intergenic spacer gene by qPCR with a 21-basepair probe specific for the genus *Bartonella* as described.[8,25] Sterile water was used as a negative control and DNA from *B. elizabethae* was used as a positive control. All DNA samples positive for the intergenic spacer gene were further tested for *B. henselae* by qPCR specific for the *pap31* gene, which is specific for this species, as described.[8,25]

Ethics. Ethical approval for the collection of fleas from domestic animals was obtained from the animal research ethics board (Agreement # 14/160/550/2011) of the College of Veterinary Medicine and Agriculture of Addis Ababa University. All necessary oral permits were obtained for the described field studies, including permission from the administration and agricultural office of the district and from each animal owner. Flea collection from the animals was not harmful to the animals.

Data analysis. Microsoft (Redmond, WA) Excel was used for data management. Descriptive statistics, such as percentages and means, were used to summarize the proportions of flea infestations among the animals and infection of fleas with *R. felis*. Statistical analysis was performed by using EpiInfo 7 (Centers for Disease Control and Prevention, Atlanta, GA), Associations between the sex and age groups of the dogs and cats, species and sex of fleas, and prevalence of flea infestation and *R. felis* in fleas were determined by using the Mantel-Haenszel test and EpiInfo 7. A *P* value ≤0.05 was considered significant.

RESULTS

Identification of fleas. In this study, 64% (30 of 47) of dogs and 50% (8 of 16) of cats were infested with at least with one

TABLE 1

Species composition and overall percentage of flea infestations by sex and age of dogs and cats in Oromia, Ethiopia*

Host factors		Host sex		Host age		
Host	Flea spp.	Female	Male	Young	Adult	Total
Dog	*Ctenophalides felis*	11/19 (58) (90:77)	18/28 (64) (114:38)	12/17 (71) (79:24)	17/30 (57) (112:27)	29/47 (62) (191:51)
	C. canis	2/19 (10) (2:4)	3/28 (11) (4:6)	3/17 (18) (4:9)	2/30 (7) (2:1)	5/47 (11) (6:10)
	Pulex irritans	2/19 (10) (1:1)	6/28 (21) (2:5)	4/17 (23) (2:3)	4/30 (13) (1:3)	8/47 (17) (3:6)
	Echidnophaga gallinacea	0/19 (0)	2/28 (7) (2:0)	1/17 (6) (1:0)	1/30 (3) (1:0)	2/47 (4) (2:0)
Overall in dogs		11/19 (58) (80:18)	19/28 (68) (122:49)	13/17 (76) (86:36)	17/30 (57) (116:31)	30/47 (64) (202:67)
Cat	*C. felis*	6/10 (60) (19:12)	2/6 (33) (2:1)	6/7 (86) (17:9)	2/9 (22) (4:4)	8/16 (50) (21:13)

*Values are no. infested/no. tested (%) (no. females:no. males).

species of flea (Table 1). Of the 269 fleas (202 females and 67 males collected from dogs, 242 *C. felis* (191 males and 51 females), 16 *C. canis* (6 females and 10 males), 9 *P. irritans* (three females and six males) and 2 *E. gallinacea* (two females) specimens were distinguished by morphologic identification. In female dogs, an overall prevalence of 58% (11 of 19) and 98 fleas (80 females and 18 males) was recorded. The corresponding values for male dogs were 68% (19 of 28) and 171 fleas (122 females and 49 males), respectively (Table 1). An overall prevalence of 76% (13 of 17) and 57% (17 of 30) was observed in young and adult dogs, respectively (Table 1). A total of 122 fleas (86 females and 36 males) and 147 fleas (116 females and 31 males) were collected from young and adult dogs, respectively (Table 1). There was no statistically significant difference in the overall prevalence of flea infestations between dogs of different ages (13 of 17 versus 17 of 30; $P = 0.2$, by Mantel-Haenszel test) and sex (11 of 19 versus 19 of 28; $P = 0.5$, by Mantel-Haenszel test) (Table 1).

A total of 34 (21 females and 13 males) *C. felis* were identified on cats (Table 1). In this study, *C. felis* was the most prevalent flea species on both hosts; overall prevalence of 62% (29 of 47) on dogs (29 of 47 versus 8 of 47; $P = 0.00002$, by Mantel-Haenszel test) and 50% (8 of 16) on cats. *Pulex irritans*, with an overall prevalence of 17% (8 of 47), was the second most prevalent species, followed by *C. canis*; the least prevalent species on dogs was *E. gallinacea* (Table 1).

For dogs (191 of 242 versus 51 of 242; $P < 0.0001$, by Mantel-Haenszel test) and cats (21 of 34 versus 13 of 34; $P = 0.05$, by Mantel-Haenszel test), the number of female *C. felis* was significantly higher than the number of males (Table 1). Furthermore, the prevalence of *C. felis* monospecies in dogs (60%) (18 of 30 versus 5 of 30; $P = 0.0006$, by Mantel-Haenszel test) and cats (100%, 8 of 8) was significantly higher than all other types of mixed species infestations (Table 2). The prevalence of

C. felis and *E. gallinacea* (3%, 1 of 30) mixed species infestations recorded in dogs was lower than all other types of mixed species infestations (Table 2).

Molecular detection of *Rickettsia* spp. in fleas. Molecular analysis of 303 fleas collected from dogs and cats identified *R. felis* DNA in 63 fleas (21%) (Table 3). In our study, *R. felis* DNA was detected in 21% (56 of 269) of fleas from dogs and in 21% (7 of 34) of fleas from cats. The overall prevalence of *R. felis* was not significantly different between fleas obtained from dogs and cats. In dogs, 11% (1 of 9) of *P. irritans* and 6% (1/16) of *C. canis* specimens were positive for *R. felis* DNA. However, *R. felis* DNA was not detected in *E. gallinacea* from dogs (Table 3). The overall percentage of *R. felis* was higher in *C. felis* than in *C. canis* (29 of 47 versus 5 of 47; $P < 0.0001$). However, no statistically significant differences were observed in the overall prevalence of *R. felis* between female and male *C. felis* from dogs (46 of 191 versus 8 of 51; $P = 0.2$, by Mantel-Haenszel test) and cats (4 of 21 versus 3 of 13; $P = 0.8$, Mantel-Haenszel test) (Table 3).

The overall prevalence of *R. felis* was 21% (21 of 98) and 20% (35 of 171) among fleas collected from female and male dogs, respectively. The corresponding values for fleas collected from young and adult dogs were 19% (23 of 122) and 22% (33 of 147), respectively (Table 3). No statistically significant differences were observed in the overall prevalence of *R. felis* between the fleas collected from female versus male dogs and young versus adult dogs.

Analysis of flea species positive for *R. felis* DNA on the infested animals showed that 69% (20 of 29) of dogs infested with *C. felis* harbored at least one flea that contained *R. felis* DNA, whereas 7% (2 of 29) of dogs infested with *C. felis* harbored fleas that contained *R. felis* DNA (Table 4). However, in the case of *C. canis*, *P. irritans*, and *E. gallinacea*, only a small percentage of infested dogs harbored fleas that contained *R. felis* DNA (Table 4). In contrast to dogs, only 37% (3 of 8) of cats infested with *C. felis* harbored at least one flea positive for *R. felis* DNA (Table 4).

The mean ± SD qPCR cycle threshold (C_t) values for positive samples were 21.1 ± 6.6 for the *orf*B gene, 28.4 ± 3.1 for the *bio*B, and 31.3 ± 2.3 for the *glt*A gene.

We amplified a 629–632-baspair fragment of the *ompA* gene from 18 samples that were representative of all of the species of fleas collected. A BLAST analysis of the *ompA* gene sequence from all flea species showed 99.6–100% nucleotide homology with the GenBank reference sequence of *R. felis* obtained from *C. felis* in Yucatan, Mexico (GenBank accession no. AJ563398), and one sample was 100% similar to *R. felis* originating from Guatemala and Costa Rica (GenBank accession no. JN990593).

TABLE 2

Percentage of mono- and multiple flea species infestations in positive dogs and cats in Oromia, Ethiopia*

Flea spp.	Dog	Cat	Overall
Ctenocephalides felis	18/30 (60)†	8/8 (100)	26/38 (68)
C. felis and *C. canis*	2/30 (7)	–	2/30 (7)
C. felis and *Pulex irritans*	5/30 (17)†	–	5/30 (17)
C. felis, *C. canis*, and *P. irritans*	3/30 (10)	–	3/30 (10)
C. felis and *Echidnophaga gallinacea*	1/30 (3)	–	1/30 (3)
E. gallinacean	1/30 (3)	–	1/30 (3)
Overall frequency	30/30 (100)	8/8 (100)	38/38 (100)

*Values are no. positive/no. tested %.
†$P = 0.0006$, by Mantel-Haenszel test.

TABLE 3
Prevalence of *Rickettsia felis* in different flea species collected from dogs and cats in Oromia, Ethiopia*

	Host factor		Host sex		Host age		
Host	Flea spp.	Flea sex	Female host	Male host	Young host	Adult host	Total hosts
Dog	*Ctenocephalides felis*	F	20/77 (26)	26/114 (23)	20/79 (25)	26/112 (23)	46/191 (24)
		M	1/13 (8)	7/38 (18)	2/24 (8)	6/27 (22)	8/51 (16)
		Total	21/90 (23)	33/152 (22)	22/103 (21)	32/139 (23)	54/242 (22)
	C. canis	F	0/2 (0)	0/4 (0)	0/4 (0)	0/2 (0)	0/6 (0)
		M	0/4 (0)	1/6 (17)	0/9 (0)	1/1 (100)	1/10 (10)
		Total	0/6 (0)	1/10 (10)	0/13 (0)	1/3 (33)	1/16 (6)
	Pulex irritans	F	0/1 (0)	1/2 (50)	1/2 (50)	0/1 (0)	1/3 (33)
		M	0/1 (0)	0/5 (0)	0/3 (0)	0/3 (0)	0/6 (0)
		Total	0/2 (0)	1/7 (14)	1/5 (20)	0/4 (0)	1/9 (11)
	Echidnophaga gallinacea	F	–	0/2 (0)	0/1 (0)	0/1 (0)	0/2 (0)
		M	–	–	–	–	–
		Total	–	0/2 (0)	0/1 (0)	0/1 (0)	0/2 (0)
Overall: dogs			21/98 (21)	35/171 (20)	23/122 (19)	33/147 (22)	56/269 (21)
Cat	*C. felis*	F	4/19 (21)	0/2 (0)	2/17 (12)	2/4 (50)	4/21 (19)
		M	3/12 (25)	0/1(0)	2/9 (22)	1/4 (25)	3/13 (23)
		Total	7/31 (23)	0/3 (0)	4/26 (15)	3/8 (37)	7/34 (21)
Overall: dogs and cats			28/129 (22)	35/174 (20)	27/148 (18)	36/155 (23)	63/303 (21)

*Values are no. positive/no. tested (%).

No typhus group *Rickettsia* species were detected in any of the 303 fleas collected from dogs and cats in Bishoftu in central Oromia.

Molecular detection of *Bartonella* spp. in fleas. Two of 303 fleas tested were positive for *Bartonella* spp. by *Bartonella* qPCR and *B. henselae*-specific qPCR, and had mean ± SD C_t values of 31.0 ± 3.2 and 21.2 ± 1.1, respectively. *Bartonella henselae* DNA was detected in 6% (2 of 34) *C. felis* collected from cats. One *C. felis* was coinfected with *R. felis*.

DISCUSSION

We detected *R. felis* in *C. canis* collected from dogs. The results of our study also provide additional molecular and geographic evidence for *R. felis* in *C. felis* and *P. irritans* specimens collected from dogs and cats in central Oromia. Our molecular findings were validated by the facts that all negative control samples had negative results and all positive control samples had positive findings in all quantitative and conventional PCRs.

The detection of an overall percentage of 21% of *R. felis* DNA in fleas collected from dogs and cats is comparable to the prevalence of *R. felis* reported by investigators in several countries.[14,18,21,33] However, overall prevalence values that are lower[17,25] and higher[12,22] than our results for *R. felis* have

TABLE 4
Proportion of infested dogs and cats harboring fleas infected with *Rickettsia felis* and *Bartonella henselae* in Oromia, Ethiopia*

		R. felis		B. henselae
Flea spp.	Infection status	Dog	Cat	Cat
Ctenocephalides felis	At least 1 infected	20/29 (69)	3/8 (37)	1/8 (12)
	All infected	2/29 (7)	0/8 (0)	1/8 (12)
	None infected	7/29 (24)	5/8 (62)	6/8 (75)
C. canis	At least 1 infected	0/5 (0)	–	–
	All infected	1/5 (20)	–	–
	None infected	4/5 (80)	–	–
Pulex irritans	At least 1 infected	0/8 (0)	–	–
	All infected	1/8 (12)	–	–
	None infected	7/8 (87)	–	–
Echidnophaga gallinacea	None infected	2/2 (100)	–	–

been reported, a variation that is most likely attributable to differences in geographic and animal factors. The observation of a higher overall percentage of *R. felis* in *C. felis* than in other flea species and detection of *R. felis* in *C. canis* and *P. irritans* collected from dogs in our study is consistent with those of other reports.[18,19] The absence of a significant difference in the overall prevalence of *R. felis* between fleas from female and male dogs and fleas from young and adult dogs in this study suggests that the sex and age of the host does not affect the prevalence of *R. felis* in fleas.

Average C_t values for the *orf*B, *bio*B, and *glt*A genes in detection of *R. felis* DNA in fleas in the present study are consistent with reported values.[31] This variation in C_t values most likely reflects differences in sensitivity and specificity among different assays and specific gene targets for the DNA of this bacterium. In addition, fifty randomly selected second half-cut female flea backup samples were negative for *R. felis* DNA, confirming our initial negative observations for the corresponding first half-cut female flea samples.

The finding of a higher proportion of flea-infested dogs (69%) than infested cats (37%) carrying *C. felis* that contain *R. felis* DNA is consistent with previous findings.[22] The significantly higher count, prevalence, and number of species of fleas on dogs than on cats in this and previous studies[1] underscores the need for additional studies to clearly determine the relative importance of dogs and cats in the transmission of *R. felis*. In addition, we detected *R. felis* in one of two *C. felis* collected from a goat in Ethiopia, but *R. felis* DNA was not detected in one *C. felis* flea taken from a cow.

Furthermore, we report detection of *B. henselae* in *C. felis* collected from cats in Ethiopia. *Bartonella henselae* DNA was detected in only in 6% (2 of 34) of *C. felis* fleas collected from cats in our study. This finding suggests that cats are important sources of this bacterium and is consistent with reports of *B. henselae* in fleas from several countries worldwide.[18,25,26,33] *Bartonella henselae* is the major cause of cat-scratch disease, for which cats are implicated as the main animal reservoir.[6,7,24,25,34] The finding of a single *C. felis* coinfected with *B. henselae* and *R. felis* in this study implies the possibility of dual infections in exposed humans. Benign lymphadenopathy, low-grade fever, malaise and aching, headache, anorexia, and

splenomegaly are the major clinical signs in patients affected with cat-scratch disease.[6,7,24]

The high overall prevalence of flea infestation in dogs (64%) and cats (50%) in the present study supports the observation of a prevalence of 99.5% in dogs and 91.5% in cats in Hawassa in southern Ethiopia.[1] These observations suggest the presence of favorable climatic conditions important for flea survival, reproduction, and development on dogs and cats in the study area. These findings also indicate that in Ethiopia, dogs and cats are not generally treated for any ectoparasites, including fleas.

The absence of statistically significant variations in the overall prevalence of flea infestation among dogs of different age and sex groups in our study is consistent with those of earlier observations.[5,35,36] Previous investigators argued that differences in the prevalence of flea infestation is associated with host habitat and environmental factors rather than host-dependent factors.[36] Female *C. felis* were more abundant than males in dogs and cats, a result consistent with previous findings.[1,3,5,35,37–39] Factors such as the longer time required by female fleas to imbibe large quantities of blood for reproduction, higher female survival rates, and the greater ability of females to evade capture during host grooming have been suggested as possible explanations.[1,3,5,35–39]

The higher overall percentage, overall flea count. and four flea species on dogs compared with cats (*C. felis* only) in the present study is consistent with findings of a previous report[1] and the following reports: three species on dogs and one species on cats in Libya,[3] three species on dogs and one species on cats in Albania,[35] and three species on dogs and two species on cats in Hungary.[5] These finding suggest that dogs are the preferred hosts of fleas, which is most likely caused by stronger grooming behavior of cats in removing fleas from their bodies.[36,37] In general, dogs have thicker, longer, and denser fur than cats, which favors infestations.

The predominance of *C. felis* over all other flea species in both host species is in consistent with results of an earlier study in Ethiopia[1] and with other studies worldwide.[5,37–39] *Ctenocephalides felis* is generally considered the predominant species found on dogs and cats, and has replaces *C. canis* as the most common flea species on domestic dogs in many countries.[37] This flea species is better adapted than *C. canis* to a wider range of environmental factors.[38,39] Furthermore, previous studies have shown that *C. felis* is the most common flea in urban areas, whereas *C. canis* is the most common in rural areas.[30] The finding that *C. canis* was the second most abundant species, followed by *P. irritans* and *E. gallinacea*, on dogs in our study is consistent with results of reports from Hungary,[5] Albania,[35] Spain,[36] and Iran.[39] Consistent with previous reports,[1,3,36] *E. gallinacea* is a species frequently found on birds and only occasionally reported on dogs and cats in various regions because of transient infestations from contact with birds. In our study, the absence of *X. cheopis*, the tropical rat flea, is explained by the fact that this flea species is rarely found on carnivores except during seasons of higher rat density and occurrence of disease epizootics. In addition, highland agroecology is more favorable for *X. cheopis* than areas such as Bishoftu, which is located in midland altitudes. Possible explanations for the absence of *C. felis strongylus* in our study are that this flea species is adapted to domestic ruminants, as observed in Libya[3] or is restricted to rural areas and stray dogs and cats in Ethiopia.

In conclusion, our study shows that *C. felis* is the most common ectoparasite of dogs and cats in urban central Oromia, Ethiopia. This study provides molecular evidence of *R. felis* and *B. henselae* in the fleas collected from dogs and cats in this region. To our knowledge, this is the first study to report the presence of *B. henselae* in fleas collected from cats in Ethiopia. The high prevalence of flea infestations in dogs and cats and the finding that a high percentage of fleas are infected with these important zoonotic bacteria in this study suggest that *R. felis* and *B. henselae* may be more important in Ethiopia than previously believed. Our study demonstrates that dogs, cats and their fleas may play important roles in increasing the risk of human exposure to these pathogenic bacteria in this country. Therefore, *R. felis* and *B. henselae* should be considered in the differential diagnosis of any fever of unknown cause among patients in this country. Future studies are required to address the isolation and culture of the bacteria, serologic and molecular prevalence, clinical signs, treatment, and potential risk factors in humans in Ethiopia. Additional epidemiologic studies in different animal and arthropod species and the public health significance of these zoonotic bacteria in different agroecologic zones are urgently needed in this country.

Authors' addresses: Bersissa Kumsa, Didier Raoult, and Cristina Socolovschi, URMITE, UMR, CNRS 6236, IRD 198, Faculté de Médecine, Aix Marseille Université, Marseille, France, E-mails: bersissak@yahoo.com, didier.raoult@gmail.com, and cristina.socolovschi@univ-amu.fr. Philippe Parola, Service des Maladies Infectieuses et Tropicales, Hôpital Nord, Marseille, France, E-mail: philippe.parola@gmail.com.

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