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RESEARCH NOTE

BARTONELLA INFECTIONS IN FLEAS (SIPHONAPTERA: PULICIDAE) AND LACK OF BARTONELLAE IN TICKS (ACARI: IXODIDAE) FROM HUNGARY

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Abstract. Fleas (95 *Pulex irritans*, 50 *Ctenocephalides felis*, 45 *Ctenocephalides canis*) and ixodid ticks (223 *Ixodes ricinus*, 231 *Dermacentor reticulatus*, 204 *Haemaphysalis concinna*) were collected in Hungary and tested, in assays based on PCR, for *Bartonella* infection. Low percentages of *P. irritans* (4.2%) and *C. felis* (4.0%) were found to be infected. The *groEL* sequences of the four isolates from *P. irritans* were different from all the homologous sequences for bartonellae previously stored in GenBank but closest to those of *Bartonella* sp. SE-Bart-B (sharing 96% identities). The *groEL* sequences of the two isolates from *C. felis* were identical with those of the causative agents of cat scratch disease, *Bartonella henselae* and *Bartonella clarridgeiae*, respectively. The *pap31* sequences of *B. henselae* amplified from Hungarian fleas were identical with that of Marseille strain. No *Bartonella*-specific amplification products were detected in *C. canis*, *I. ricinus*, *D. reticulatus* and *H. concinna* pools.

Ticks transmit several bacterial pathogens including genospecies of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia* spp. of the spotted-fever group, *Coxiella burnetti* and *Francisella tularensis* in Europe (Parola and Raoult 2001, Parola et al. 2005). Fleas are known to be the vectors of *Rickettsia felis* on the continent (Parola et al. 2005). In the past years, several previously unrecognised bacterial pathogens belonging to the genus *Bartonella* were also described from ticks and fleas, and several new clinical syndromes caused by these bacteria were reported (Greub and Raoult 2002, Jacomo et al. 2002, Boulouis et al. 2005, Chomel et al. 2006). The tick and flea vectors of some of these bartonellae might be the sheep tick, *Ixodes ricinus* and the cat flea, *Ctenocephalides felis* on the continent (Schouls et al. 1999, Rolain et al. 2003, Sanogo et al. 2003, Shaw et al. 2004). No information is available on the infection rate of other tick and flea species. Although bartonellae are widely distributed throughout Europe, limited information is available for Hungary (Tokodi et al. 2001, Boulouis et al. 2005). Herein we report *Bartonella* infection in two flea species from Hungary.

Overall, 223 *I. ricinus*, 231 *Dermacentor reticulatus*, 204 *Haemaphysalis concinna* ticks, 95 human fleas (*Pulex irritans*) and 45 dog fleas (*Ctenocephalides canis*) were removed from carcasses of red foxes (*Vulpes vulpes*), and overall, 50 cat fleas (*C. felis*) were collected from domestic cats (*Felis catus* f. domestica). Fox and cat carcasses were sent to the Central Veterinary Institute, Budapest for rabies examination. The transportation and storage of the carcasses and the methods used to collect and identify fleas found on them have already been described (Szabó 1975, Sréter et al. 2003, Széll et al. 2006). The ticks and the fleas were stored as separate pools (each of five or fewer specimens) from each animal at –20ºC. The DNA was extracted from each pool (Kálmán et al. 2003), and fragments of gene coding 60 kDa heat shock protein (*groEL*) were amplified using the primer set of HSPps1, HSPps2 and HSPps4 primers (Zeaiter et al. 2002). From the *Bartonella henselae*-positive pool, fragments of gene coding hemin-binding protein (*pap31*) were also amplified using the primer sets of PAPn1, PAPn2 and PAPns2 (Zeaiter et al. 2002). Amplicons were further characterized by sequence analysis (Sréter et al. 2000). Sequences were identified by comparison with GenBank entries using the BLAST programme (http://www.ncbi.nlm.nih.gov/blastn).

No *Bartonella* specific amplification products were detected in *I. ricinus*, *D. reticulatus* and *H. concinna* pools. As *B. henselae* and *Bartonella quintana* have been reported from *Ixodes*, *Dermacentor* and *Haemaphysalis* spp. in some countries of Eurasia and North America (Table 1), it cannot be excluded that the lack of bartonellae in Hungarian and Swedish ticks (La Scola et al. 2004) might be attributed to the low prevalence of bartonellae in these ectoparasites in some regions of Europe. Moreover, the surprisingly high infection rate of ticks and the lack of sequencing results in some studies might represent laboratory contamination with DNA originally not present in these ectoparasites or might be attributed to the detection of aspecific products.

Of the 19 pools of *P. irritans*, *Bartonella* DNA was detected in four pools coming from four different foxes. As there were four positive pools, there must have been at least four infected fleas among the 95 investigated, giving a minimum prevalence of 4.2% in *P. irritans*. All *Bartonella*-positive pools appeared to be identical in terms of their *groEL* sequences, which were deposited in GenBank under the accession no. DQ522300. The sequences were different from all the homologous sequences for bartonellae previously stored in GenBank but closest to those of *Bartonella* sp. SE-Bart-B detected in *Xenopsylla cheopis* fleas from Egypt (GenBank accession no. DQ166942; sharing 96% identities) and those of *Bartonella clarridgeiae* detected in *C. felis* fleas from some countries of Eurasia (GenBank accession no. AF014831; sharing 94% identities). Bartonellae have never been reported earlier in *P. irritans* from Europe, and almost no information is available on the infection rate of this flea species on other continents (Table 1). From Gabon and Peru, *B. quintana* and three novel *Bartonella* genotypes were recently detected in *Pulex* fleas (Parola et al. 2002, Rolain et al. 2005). The medical importance of the four new genotypes of bartonellae detected in human fleas from Hungary and Peru has yet to be clarified. Nevertheless, several previously unrecognised bacterial pathogens belonging to the genus *Bartonella* were recently described from ectoparasites including fleas (Greub and

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Table 1. Current knowledge on the prevalence and geographical distribution of *Bartonella* infections in fleas and ticks.*

Flea or tick vector	*Bartonella* spp. detected in the vector	Recorded prevalence (%) in vector		Known geographical distribution of bartonellae in vector
		Range	Mean	
Ctenocephalides felis	*B. henselae*, *B. clarridgeiae*, *B. quintana*, *B. koehlerae*	4–34	19	France, Hungary, Japan, New Zealand, Thailand, United Kingdom, United States (Alabama, Maryland, Texas)
Ctenocephalides canis	*B. henselae*	–	ND**	Japan
Pulex irritans/*simulans*	*B. quintana*, *Bartonella* spp.***	4–10	7	Gabon**, Hungary, Peru
Xenopsylla cheopis	*Bartonella* sp.***	–	20	Egypt
Leptopsylla segnis	*Bartonella* sp.***	–	3	Egypt
Nosopsylla fasciatus	*Bartonella* sp.***	–	4	Thailand
Oropsylla hirsuta	*Bartonella* spp.***	2–13	7	United States (Colorado)
Ixodes ricinus	*B. henselae*, NI****	0–77	16	France, Italy, The Netherlands
Ixodes persulcatus	*B. henselae*, *B. quintana*	–	38	Korea**, Russia (Siberia)
Ixodes turdus	NI****	–	11	Korea
Ixodes nipponensis	NI****	–	5	Korea
Ixodes scapularis	*B. henselae*	–	35	United States (New Jersey)
Ixodes pacificus	*B. henselae*	2–19	9	United States (California)
Dermacentor reticulatus	*B. henselae*, *B. quintana*	0–21	10	Russia (Siberia)
Dermacentor variabilis	NI****	–	14	United States (California)
Dermacentor occidentalis	NI****	–	3	United States (California)
Haemaphysalis longicornis	*Bartonella* sp.***	–	4	Korea
Haemaphysalis flava	NI****	–	3	Korea

*All data presented come from published reports (Chomel et al. 1996, Schouls et al. 1999, Chang et al. 2001, Ishida et al. 2001, Chang et al. 2002, Parola et al. 2002, 2003, Rolain et al. 2003, Sanogo et al. 2003, Stevenson et al. 2003, Adelson et al. 2004, Kelly et al. 2004, La Scola et al. 2004, Shaw et al. 2004, Halos et al. 2005, Kim et al. 2005, Rar et al. 2005, Rolain et al. 2005, Holden et al. 2006, Lappin et al. 2006, Loftis et al. 2006, this study). **Prevalence was not determined. ***As-yet-unnamed *Bartonella* genotypes with unknown pathogenicity. ****Bartonellae were not identified at species level.

Raoult 2002, Jacomo et al. 2002, Boulouis et al. 2005, Chomel et al. 2006). As *P. irritans* is a particularly 'anthropophilic' flea species, further studies are needed on the transmission potential of this flea species. Although bartonellae have been reported in *C. canis* in Japan (Ishida et al. 2001), no *Bartonella*-specific amplification products were detected in *C. canis* pools from Hungary. Of the 10 pools of *C. felis*, *Bartonella* DNA was detected in two pools coming from two cats. As there were two positive pools, there must have been at least two infected fleas among the 50 investigated, giving a minimum prevalence of 4.0% in *C. felis*. The *groEL* sequences of the two isolates were identical with those of the causative agents of cat scratch disease, *B. henselae* and *B. clarridgeiae*, respectively (GenBank accession nos. AF304019 and AF014831). The *pap31* sequences of *B. henselae* isolate amplified from Hungarian fleas were identical with that of Marseille strain (GenBank accession no. AF308169). Of *Bartonella* spp. with known pathogenicity, *B. clarridgeiae*, *B. henselae*, *B. quintana* and *Bartonella koehlerae* were detected in *C. felis* from some other countries of Eurasia, New Zealand and the United States (Chomel et al. 1996, Ishida et al. 2001, Parola et al. 2003, Rolain et al. 2003, Kelly et al. 2004, Shaw et al. 2004, Lappin et al. 2006). Although a few cases of cat scratch disease have been reported on the basis of serological results from Hungary (Tokodi et al. 2001), *B. clarridgeiae* and *B. henselae* have never been isolated earlier. The infection rate of Hungarian *C. felis* fleas with bartonellae was similar to that seen in New Zealand (Table 1).

The role of ixodid ticks and the majority of flea species in the direct transmission of bartonellae is not fully confirmed (Chomel et al. 1996, Eskow et al. 2001, Chomel and Boulouis 2005); nevertheless, the present and other data (Table 1) raise the possibility that several flea and tick species might play a role in the epidemiology of bartonelloses in Eurasia and Americas. Therefore, studies on the distribution of bartonellae in fleas and ticks and on the transmission potential of these parasites are encouraged. Recently reported seroprevalences in man (1–62%) indicate that *Bartonella* infections are frequent in human populations in some regions of Eurasia and North America (Breitschwerdt and Kordick 2000, Chomel et al. 2006), and these bacteria are responsible for diverse and in many cases serious clinical manifestations in man (Breitschwerdt and Kordick 2000, Jacomo et al. 2002, Chomel et al. 2006). Considering the relatively high prevalence of various bartonellae in ticks and fleas (Table 1) and the high infection rate of these ectoparasites with several other emerging bacterial pathogens in Hungary and other European countries, clinicians need to be more aware of, and more familiar with, the clinical manifestations of flea- and tick-borne infections including bartonelloses, the best methods for their laboratory confirmation, and the appropriate therapy (Rolain et al. 2004, Boulouis et al. 2005). As the pathogenicity of bartonellae was recently demonstrated in dogs, and the seroprevalences vary between 1% and 92% in various mammals (Breitschwerdt and Kordick 2000, Chomel et al. 2006), further studies are needed on the veterinary significance of these bacteria.

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References

ADELSON M.E., RAO R.V., TILTON R.C., CABETS L., ESKOW E., OCCI J.L., MORDECHAI E. 2004: Prevalence of *Borrelia burgdorferi*, *Bartonella* spp., *Babesia microti*, and *Anaplasma phagocytophila* in *Ixodes scapularis* ticks collected in Northern New Jersey. J. Clin. Microbiol. 42: 2799–2801.

BREITSCHWERDT E.B., KORDICK D.L. 2000: *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin. Microbiol. Rev. 13: 428–438.

BOULOUIS H.J., CHANG C.C., HENN J.B., KASTEN R.W., CHOMEL B.B. 2005: Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Vet. Res. 36: 383–410.

CHANG C.C., CHOMEL B.B., KASTEN R.W., ROMANO V., TIETZE N. 2001: Molecular evidence of *Bartonella* spp. in questing adult *Ixodes pacificus* ticks. J. Clin. Microbiol. 39: 1221–1226.

CHANG C.C., HAYASHIDANI H., PUSTERLA N., KASTEN R.W., MADIGAN J.E., CHOMEL B.B. 2002: Investigation of *Bartonella* infection in ixodid ticks from California. Comp. Immunol. Microbiol. Infect. Dis. 25: 229–236.

CHOMEL B.B., BOULOUIS H.J. 2005: [Zoonotic diseases caused by bacteria of the genus *Bartonella*: New reservoirs? New vectors?] Bull. Acad. Natl. Med. 189: 477–480. (In French.)

CHOMEL B.B., KASTEN R.W., FLOYD-HAWKINS K., CHI B., YAMAMOTO K., ROBERTS-WILSON J., GHURFIELD A.N., ABBOTT R.C., PEDERSEN N.C., KOEHLER J.E. 1996: Experimental infection with *Bartonella henselae* in domestic cats. J. Clin. Microbiol. 34: 1952–1956.

CHOMEL B.B., BOULOUIS H.J., MARUYAMA S., BREITSCHWERDT E.B. 2006: *Bartonella* in pets: impact on human health. Emerg. Infect. Dis. 12: 389–394.

ESKOW E., RAO K., MORDECHAI E. 2001: Concurrent infection of the central nervous system by *Borrelia burgdorferi* and *Bartonella henselae*: evidence of a novel tick-borne disease complex. Arch. Neurol. 58: 1357–1363.

GREUB G., RAOULT D. 2002: *Bartonella*: new explanations for old diseases. J. Med. Microbiol. 51: 915–923.

HALOS L., JAMAL T., MAILLARD R., BEUGNET F., LE MENACH A., BOULOUIS H.J., VAZSSIER-TAUSSAT M. 2005: Evidence of *Bartonella* sp. in questing adult and nymphal *Ixodes ricinus* ticks from France and co-infection with *Borrelia burgdorferi* sensu lato and *Babesia* sp. Vet. Res. 36: 79–87.

HOLDEN K., BOOTHBY J.T., KASTEN R.W., CHOMEL B.B. 2006: Co-detection of *Bartonella henselae*, *Borrelia burgdorferi*, and *Anaplasma phagocytophilum* in *Ixodes pacificus* ticks from California, USA. Vector Borne Zoonotic Dis. 6: 99–102.

ISHIDA C., TSUNEOKA H., IINO H., MURAKAMI K., INOKUMA H., OHNISHI T., TSUKAHARA M. 2001: [*Bartonella henselae* infection in domestic cat and dog fleas.] Kansenshogaku Zasshi 75: 133–136. (In Japanese.)

JACOMO V., KELLY P.J., RAOULT D. 2002: Natural history of *Bartonella* infections (an exception to Koch's postulate). Clin. Diagn. Lab. Immunol. 9: 8–18.

KÁLMÁN D., SRÉTER T., SZÉLL Z., EGYED L. 2003: *Babesia microti* infection in anthropophilic ticks (*Ixodes ricinus*) in Hungary. Ann. Trop. Med. Parasitol. 97: 317–319.

KELLY P.J., MEADS N., THEOBALD A., FOURNIER P., RAOULT D. 2004: *Rickettsia felis*, *Bartonella henselae*, and *Bartonella clarridgeiae*, New Zealand. Emerg. Infect. Dis. 10: 967–968.

KIM C.M., KIM J.Y., YI Y.H., LEE M.J., CHO M.R., SHAH D.H., KLEIN T.A., KIM H.C., SONG J.W., CHONG S.T., O'GUINN M.L., LEE J.S., LEE I.Y., PARK J.H., CHAE J.S. 2005: Detection of *Bartonella* species from ticks, mites and small mammals in Korea. J. Vet. Sci. 6: 327–334.

LAPPIN M.R., GRIFFIN B., BRUNT J., RILEY D., HAWLEY J., BREWER M.M., JENSEN W.A. 2006: Prevalence of *Bartonella* species, haemoplasma species, *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Neorickettsia risticii* DNA in the blood of cats and their fleas in the United States. J. Feline Med. Surg. 8: 85–90.

LA SCOLA B., HOLMBERG M., RAOULT D. 2004: Lack of *Bartonella* sp. in 167 *Ixodes ricinus* ticks collected in central Sweden. Scand. J. Infect. Dis. 36: 305–306.

LOFTIS A.D., REEVES W.K., SZUMLAS D.E., ABBASSY M.M., HELMY I.M., MORIARITY J.R., DASCH G.A. 2006: Surveillance of Egyptian fleas for agents of public health significance: *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Rickettsia*, and *Yersinia pestis*. Am. J. Trop. Med. Hyg. 75: 41–48.

PAROLA P., DAVOUST B., RAOULT D. 2005: Tick- and flea-borne rickettsial emerging zoonoses. Vet. Res. 36: 469–492.

PAROLA P., RAOULT D. 2001: Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. 32: 897–928.

PAROLA P., SANOGO Y.O., LERDTHUSNEE K., ZEAITER Z., CHAVANCY G., GONZALEZ J.P., MILLER R.S., TELFORD S.R. 3RD, WONGSRICHANALAI C., RAOULT D. 2003: Identification of *Rickettsia* spp. and *Bartonella* spp. in fleas from Thai-Myanmar border. Ann. N. Y. Acad. Sci. 990: 173–181.

PAROLA P., SHPYNOV S., MONTOYA M., LOPEZ M., HOUPIKIAN P., ZEAITER Z., GUERRA H., RAOULT D. 2002: First molecular evidence of new *Bartonella* spp. in fleas and a tick from Peru. Am. J. Trop. Med. Hyg. 67: 135–136.

RAR V.A., FOMENKO N.V., DOBROTVORSKY A.K., LIVANOVA N.N., RUDAKOVA S.A., FEDOROV E.G., ASTATIN V.B., MOROZOVA O. 2005: Tick-borne pathogen detection, Western Siberia, Russia. Emerg. Infect. Dis. 11: 1708–1715.

ROLAIN J.M., 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.

ROLAIN J.M., BROUQUI P., KOEHLER J.E., MAGUINA C., DOLAN M.J., RAOULT D. 2004: Recommendations for treatment of human infections caused by *Bartonella* spp. Antimicrob. Agents Chemother. 48: 1921–1933.

ROLAIN J.M., BOURRY O., DAVOUST B., RAOULT D. 2005: *Bartonella quintana* and *Rickettsia felis* in Gabon. Emerg. Infect. Dis. 11: 1741–1744.

SANOGO Y.O., ZEAITER Z., CARUSO G., MEROLA F., SHPYNOV S., BROQUI P., RAOULT D. 2003: *Bartonella henselae* in *Ixodes ricinus* ticks (Acari: Ixodidae) removed from humans, Belluno province, Italy. Emerg. Infect. Dis. 9: 329–332.

SCHOULS L.M., VAN DE POL S.G., RIJPKEMA S.G., SCHOT C.S. 1999: Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. J. Clin. Microbiol. 37: 2215–2222.

SHAW S.E., KENNY M.J., TASKER S., BIRTLES R.J. 2004: Pathogen carriage by the cat flea *Ctenocephalides felis* (Bouche) in the United Kingdom. Vet. Microbiol. 102: 183–188.

SRÉTER T., KOVÁCS G., DA SILVA A.J., PIENIAZEK N.J., SZÉLL Z., DOBOS-KOVÁCS M., MÁRIALIGETI K., VARGA I. 2000: Morphologic, host specificity, and molecular characterization of a Hungarian *Cryptosporidium meleagridis* isolate. Appl. Environ. Microbiol. 66: 735–738.

SRÉTER T., SZÉLL Z., VARGA I. 2003: Ectoparasite infestations of red foxes (*Vulpes vulpes*) in Hungary. Vet. Parasitol. 115: 349–354.

STEVENSON H.L., BAI Y., MONTENIERI J.A., LOWELL J.L., CHU M.C., GAGE K.L. 2003: Detection of novel *Bartonella* strains and *Yersinia pestis* in prairie dogs and their fleas (Siphonaptera: Ceratophyllidae and Pulicidae) using multiplex polymerase chain reaction. J. Med. Entomol. 40: 329–337.

SZABÓ I. 1975: Fauna Hungariae, vol. XV. Diptera II, No. 18. Siphonaptera. Academic Press, Budapest, pp. 1–97. (In Hungarian.)

SZÉLL Z., SRÉTER-LANCZ Z., MÁRIALIGETI K., SRÉTER T. 2006: Temporal distribution of *Ixodes ricinus*, *Dermacentor reticulatus* and *Haemaphysalis concinna* in Hungary. Vet. Parasitol. 377–379.

TOKODI I., MÁJ C., DEÁK J., GYETVAI B., LAKATOS B., SIMON G. 2001: [Unusual manifestations of *Bartonella* infections.] Orv. Hetil. 142: 2197–2200. (In Hungarian.)

ZEAITER Z., FOURNIER P.E., RAOULT D. 2002: Genomic variation of *Bartonella henselae* strains detected in lymph nodes of patients with cat scratch disease. J. Clin. Microbiol. 40: 1023–1030.

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