Veterinary Microbiology 159 (2012) 541–543

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Letter to the Editor

First detection of bartonellae in a broad range of bat ectoparasites

Bats (Mammalia: Chiroptera) are increasingly recognized as reservoirs of emerging – mostly zoonotic – viral diseases (Calisher et al., 2006). Features underlying their epidemiological significance include ubiquitous occurrence, long life-span, social behaviour (close contacts in colonies) and tendency for persistent infections (Calisher et al., 2006). Even more importantly, bats frequently fly into human settlements, where they roost in buildings (attics, cellars), sometimes introducing disease agents indoors (Jaenson et al., 1994). In such situations direct contact with bats is not a prerequisite for humans to contract bat-associated pathogens or to become infested with bat ectoparasites (Jaenson et al., 1994; De Serres et al., 2008).

In the New World certain bat ectoparasites (soft ticks, bugs and fleas) were shown to contain bacterial pathogens (Billeter et al., 2008). However, such data are scarce or absent from other regions of the globe. For instance, although the long-legged bat tick (*Ixodes vespertilionis*) is widely distributed across the Old World – from Europe to Australia (Arthur, 1956) –, there are no reports on vector-borne agents it may carry. Recently bartonellae were isolated from one Asian and four European chiropteran species (Concannon et al., 2005; Lin et al., 2012). Here we provide a plausible explanation on how bartonellae may spread amongst bats and possibly to other hosts.

We collected ninety hard ticks (*I. vespertilionis*) in a peri-urban area (Pilis Mountains, Hungary) from cave walls near colonies of Palaearctic bat species (*Rhinolophus hipposideros*, *Rhinolophus ferrumequinum*, *Myotis myotis*) in January, 2011. Other ectoparasites (including 436 mites, three fleas and two flies) were removed from eight living bats found in an urban environment (Budapest): *Nyctalus* and *Eptesicus* in January, whereas *Myotis* in May, 2011 (Table 1). Bat ticks were processed individually, whereas other ectoparasites in pools according to their species and host individuals. DNA extraction was done as described elsewhere (Hornok et al., 2010a). Molecular methods applied for the detection of vector-borne bacteria included TaqMan real-time PCRs in case of *Bartonella* spp. (Molia et al., 2004) and *Rickettsia helvetica* (Boretti et al., 2009). After the PCR for Anaplasmataceae (Goodman et al., 1996) the products were cloned into PCR4-Topo plasmid (Invitrogen), followed by isolation and sequencing three clones. Data were submitted to the GenBank (accession number JQ478427).

Concerning groups of arthropods analysed here, mites and nycteribid flies are host-specific, permanent bat ectoparasites that do not usually infest other species than their typical hosts; however, ticks and fleas are less host-specific, temporary ectoparasites with peculiar mechanisms of host-finding and host transfer (Balashov, 2006). Justifying higher importance of the latter category in the transmission of zoonotic agents, bat-adapted soft ticks are known to bite humans (Jaenson et al., 1994).

In questing populations of *I. vespertilionis* (Ixodida: Ixodidae) on cave walls males predominated (Table 1), in line with previous observations that males are usually not seen on their host (Arthur, 1956). Regarding exposure risks for humans to encounter this tick species, its habitat is not restricted to caves, as it was reported to occur in attics and cellars of houses, as well as in tree holes (Ševčík et al., 2010). Accordingly, not only does it differ in its spatial distribution from other *Ixodes* spp., but also it has a unique seasonality, being most active (in its protected environment) during winter time (Arthur, 1956; Ševčík et al., 2010).

Bartonella-positivity was shown in a female *I. vespertilionis* (Table 1), implying an association of bartonellae with *Rhinolophus* spp. and/or *M. myotis*. These bacteria were detected by others in several *Ixodes* spp. (Billeter et al., 2008). However, to the best of our knowledge, this is the first PCR-positivity to bartonellae in the winter-active long-legged bat tick (*I. vespertilionis*), and in any members of the genus outside the *Ixodes ricinus* complex.

Further ectoparasites that contained *Bartonella* DNA include the eight-combed bat flea (*Ischnopsyllus octactenus*), two species of mites and nycetribid flies (Table 1). Taking into account that the cat flea (*Ctenocephalides felis*) is a known vector of *Bartonella henselae* (Chomel et al., 1996), and bartonellae were also identified in one New World bat flea (Billeter et al., 2008), the present finding of these bacteria in yet another bat flea genus and species supports the vector potential of bat fleas in transmitting bartonellae. Similarly, *Steatonyssus* sp. mites (Mesostigmata: Macronyssidae) have been reported to harbour *Bartonella* DNA in the New World (Billeter et al., 2008) and keds or forest flies (Diptera: Hippoboscidae) – closely related to nycteribid bat flies analysed here – are also known carriers/vectors of these bacteria (Billeter et al., 2008). Therefore, based on literature data, all ectoparasite

0378-1135/$ – see front matter
http://dx.doi.org/10.1016/j.vetmic.2012.04.003

Table 1
Summary of samples and results of molecular analyses.

(Number) sample source (species)	Sample category (species or genus)	Specimens per pool*	PCR positive/all	(Endosymbiont) or pathogen detected
Cave wall	Hard tick (*Ixodes vespertilionis*)	Males: 1	**1/31**	(*Wolbachia* sp.)
		Females: 1	**1/7**	*Bartonella* sp.
		Nymphs: 10	0/5	–
		Larvae: 2	0/1	–
(3×) Bat (*Nyctalus noctula*)[a,b,c]	Flea (*Nicteridopsylla eusarca*)	1[a]/2[c]	**1[c]/2**	*Rickettsia helvetica*
	Flea (*Ischnopsyllus octactenus*)	1[b]	0/1	*Bartonella* sp.
	Mite (*Macronyssus kolenati*)	29[a]/45[c]	0/2	–
	Mite (*Spinturnix* sp.)	1[b]	0/1	–
(1×) Bat (*Eptesicus serotinus*)	Mite (*Steatonyssus occidentalis*)	100	0/1	–
(4×) Bat (*Myotis myotis*)[e,f,g,h]	Mite (*Steatonyssus occidentalis*)	23[e]/28[f]/35[g]/90[h]	**1/4**	*Bartonella* sp.
	Mite (*Spinturnix myoti*)	17[f]/20[h]/48[g]	**3/3**	*Bartonella* sp.
	Fly (*Nycteribia* sp.)	1[f]/1[d]	**2/2**	*Bartonella* sp.

* The number of specimens collected from different individuals of the same host species are separated by slash mark horizontally; those from the same host individual have the same superscript vertically.

species that showed positivity in the *Bartonella* PCR (Table 1) are new potential vectors in the context of the Old World, and also taxonomically on either the species (ticks, mites), genus (flea) or family level (flies). Mean threshold cycle (Ct) value of samples from *Spinturnix* mites (33.5 ± 2) was much lower, than in case of dermanyssoid mites and larger nycteribid flies (42.4 ± 1.5) in the same test, indicating a *Bartonella* DNA quantity difference of three orders of magnitude on behalf of the former. As Ct values in the present study were consistently above 30, species identification (sequencing) in PCR-positive samples could not be achieved.

In addition, PCR analysis for *Borrelia burgdorferi* s.l., *Francisella tularensis*, *Coxiella burnetii*, haemoplasmas and *Anaplasma phagocytophilum* yielded negative results for all bat ectoparasites, despite the regional occurrence of these pathogens or groups (methods and data not shown). However, from one male *I. vespertilionis*, positive for Anaplasmataceae, a 452 bp-long sequence was obtained (JQ478427) which had 99% similarity to *Wolbachia* sp. This is the first report of this group of endosymbiotic bacteria in bat ectoparasites.

One five-combed bat flea (*Nicteridopsylla eusarca*) sample (Siphonaptera: Pulicidae) was positive in the *R. helvetica* PCR (Table 1). Interestingly, 45 blood-containing mites from the same bat yielded negative results (Table 1), suggesting that relevant fleas carried *R. helvetica* prior to their blood-sucking on the host from which they have been removed. *R. helvetica* was already detected in fleas of other animals, e.g. those from cats (*C. felis*: Hornok et al., 2010b), but this is its first report from bats fleas, and in any bat ectoparasites. The present finding also raises the possibility that bats are reservoirs of *R. helvetica*, similarly to other rickettsiae (Billeter et al., 2008).

The bat harbouring the rickettsia-positive flea was found weak (on the ground) near the city centre, where no vectors of *R. helvetica* (most notably *I. ricinus*) occur. To illustrate implications of similar situations, fleas adapted to a certain animal host and normally rare on humans can cause massive flea infestation on the latter, if their type hosts dwell, hybernate or die in or near inhabited buildings (Pomykal, 1985). Furthermore, the flea species found *R. helvetica*-positive in the present study is winter-associated (Rupp et al., 2004), unlike other potential vectors from which these rickettsiae were isolated.

In summary, these findings broaden the potential vector range of bartonellae and *R. helvetica*, including two bat ectoparasites that are winter-associated, unlike known vectors of these agents under continental climate in Europe. Blood-sucking arthropods having stricter host-specificity may be responsible for the spread of relevant agents between bats, whilst less host-specific vector candidates pose the risk of transmission to other mammals, including humans. First-time identification of these zoonotic agents in association with synanthropic bat species in an urban environment also indicates that relevant bacteria can be quickly introduced into and maintained in cities by flying bat reservoirs.

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12 March 2012