

## Vector-Borne Agents Detected in Fleas of the Northern White-Breasted Hedgehog

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### Abstract

This is the first large-scale molecular investigation of fleas from a geographically widespread and highly urbanized species, the northern white-breasted hedgehog. In this study, 759 fleas (the majority were *Archaeopsylla erinacei*) collected from 134 hedgehogs were molecularly analyzed individually or in pools for the presence of three groups of vector-borne pathogens. All flea samples were positive for rickettsiae: In two samples (1.5%) *Rickettsia helvetica* and in 10% of the others a novel rickettsia genotype were identified. Additionally, *Bartonella henselae* (the causative agent of cat scratch disease in humans) was demonstrated in one flea (0.7%), and hemoplasmas of the hemofelis group were identified in seven other samples (5.2%). The findings of vector-borne agents not detected before in *A. erinacei* fleas broaden the range of those diseases of veterinary–medical importance, of which hedgehogs may play a role in the epidemiology.

**Key Words:** Hedgehog—Flea—Zoonosis—Vector-borne—Rickettsia—*Bartonella*—Hemoplasma.

### Introduction

**H**EDGEHOGS (EULIPOTYPHILA: ERINACEIDAE) ARE SMALL, primarily nocturnal, insectivorous mammals. Their veterinary–medical significance is related to their urban habitats and to the numerous zoonotic pathogens they may harbor (Riley and Chomel 2005). In addition, hedgehogs are pet animals in several parts of the world, including the United States, where they are kept in approximately 40,000 households (Riley and Chomel 2005).

The northern white-breasted hedgehog (*Erinaceus roumanicus*) is a geographically widespread species, being native to central-eastern Europe and western Siberia (longitude, E20–80°). It is highly urbanized, providing an opportunity for its fleas (most importantly *Archaeopsylla erinacei*) to infest dogs and cats (Gilles et al. 2008), or even human beings (e.g., Pomykal 1985). The chances for this are particularly high when hedgehogs enter hibernation or die in or near animal-keeping facilities and human dwellings, and their fleas depart in search of a new host (Pomykal 1985).

Recently, the hedgehog flea, *A. erinacei* was reported to be a frequent carrier of *Rickettsia felis*, the causative agent of human flea-borne spotted fever (Gilles et al. 2008, Khaldi et al. 2012,

Marié et al. 2012). Because this rickettsia was shown to be present in Hungary (Hornok et al. 2010), the main purpose of the present study was to evaluate if hedgehog fleas have a similarly high infection prevalence with *R. felis* as reported abroad. At the same time molecular investigation of the samples was extended to include hemotropic *Mycoplasma* spp. (hemoplasmas) and bartonellae.

### Materials and Methods

A total of 759 fleas were included in the present study. They were collected from 134 northern white-breasted hedgehogs in the capital city of Hungary, Budapest. The animals were hand-captured after sunset (once a month from May to October, 2009, and from March to November, 2010), and examined for ectoparasites as reported (Földvári et al. 2011). The sampling site was the urban park situated on the Margaret Island of the Danube river (near the city center), with up to 50,000 daily visitors during the summer months. Fleas were identified on the species level according to standard morphological keys. Specimen(s) collected from the same host were stored (in 70% ethanol) and processed together (i.e., there were individual and pooled samples). DNA was

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TABLE 1. VECTOR-BORNE BACTERIA DETECTED IN FLEAS FROM NORTHERN WHITE-BREADED HEDGEHOGS

Flea species	Sample type	Number of positives/all tested			
		<i>Rickettsia helvetica</i>	Other <i>Rickettsia</i> sp.	<i>Bartonella henselae</i>	<i>Hemoplasma</i> ( <i>hemofelis</i> group)
<i>Archaeopsylla erinacei</i>	Individual (male)	0/24	24/24	0/24	2/24
	Individual (female)	2/47	45/47	1/47	3/47
	Pooled (male, female)	0/62	62/62	0/62	2/62
<i>Ctenocephalides canis</i>	Pooled (male, female)	0/1	1/1	0/1	0/1
	Total	2/134	132/134	1/134	7/134

Individual samples are shown separately to get a more precise estimate of prevalence, whenever possible. Separation of individual samples by gender may have relevance to the potential of transovarial transmission of bacterial agents (e.g., certain rickettsiae) by female fleas.

extracted, and its quality/quantity was assessed as previously reported (Boretti et al. 2009, Hornok et al. 2010). Molecular analyses for three groups of vector-borne agents were performed in the following ways: (1) The presence of rickettsiae was evaluated in two steps with TaqMan real-time PCRs (Boretti et al. 2009). For *R. helvetica* this was done by amplifying a portion of its 23S gene, and for other rickettsiae by detecting part of their citrate synthase (*gltA*) gene. From 10% of the positive samples (with the lowest threshold cycle =  $C_t$  values) in the latter test, a 476-bp-long portion of the *gltA* gene was sequenced. The sequence was submitted to GenBank. (2) *Bartonella henselae* was evaluated by the method of Molia et al. (2004). (3) General screening for hemoplasma DNA was done with a SYBR Green real-time PCR (Willi et al. 2009), and positive samples were then tested with two hemoplasma group-specific TaqMan real-time PCRs (hemofelis vs. hemominutum groups; Tasker et al. 2010).

## Results and Discussion

The fleas were analyzed as 71 individual and 62 pooled samples of *A. erinacei* (collected from 133 hedgehogs) and one pool (five specimens) of *Ctenocephalides canis* (from one hedgehog). Results of molecular analyses are summarized in Table 1.

All flea samples were positive for rickettsiae. Two female *A. erinacei* individuals harbored *R. helvetica*. This spotted fever group rickettsia is transmitted by *Ixodes ricinus*, and may cause mild-to-severe (e.g., neurological) disease in humans (Nilsson et al. 2010). Most recently, it has been reported from bat fleas (Hornok et al. 2012) and hedgehog ticks (Speck et al. 2013). However, to the best of our knowledge, this is the first identification of *R. helvetica* in the hedgehog flea *A. erinacei*. Hedgehogs are thought to play a role in the epidemiology of *R. helvetica* (Speck et al. 2013). Although the vector competence of hedgehog fleas in its transmission has yet to be proven, on the basis of these results humans may come into contact with hedgehog-derived *R. helvetica* from fleas.

The presence of other *Rickettsia* sp./spp. was demonstrated by PCR positivity of the remaining samples. From among these, sequencing of 13 individual samples indicated a novel *Rickettsia* genotype (accession no. KC822951), having the highest (99%, 98%) sequence identity to African rickettsia isolates, i.e., endosymbionts from soft ticks and tsetse flies, with 1.1% (5 out of 438) and 2.3% (10 out of 438) mismatches of nucleotides, respectively. Interestingly, the newly discovered rickettsial endosymbiont of tsetse flies was reported to be

phylogenetically close to *R. felis* (Mediannikov et al. 2012), a species highly prevalent in *A. erinacei* fleas collected from other European and African hedgehog species (Gilles et al. 2008, Khaldi et al. 2012, Marié et al. 2012).

One female hedgehog flea harbored *B. henselae*, the causative agent of cat scratch disease of humans (Molia et al. 2004). Fleas play an important role in the transmission of *B. henselae* (Chomel et al., 1996); in particular, flea feces are the main source of infection for humans. To the best of our knowledge, this is the first account of this species in *A. erinacei*, in which flea species (when collected from African hedgehogs) only *B. clarridgeiae* and *B. elizabethae* were hitherto identified (Bitam et al. 2012). Therefore, in light of the present findings, further investigation is warranted if northern white-breasted hedgehogs play a role in the epidemiology of cat scratch disease.

In the general hemoplasma screening assay, 14% (19 out of 134) of samples were positive, of which 5% (7 out of 134) could be confirmed with the group-specific PCRs (Table 1). All these samples contained a member of the hemofelis group, which could not be identified further on the species level as reflected by high  $C_t$  values. The remaining samples may have been hemoplasmas with very low loads, or spiroplasmas, which are also weakly amplified by the primers of the SYBR Green assay as reported earlier (Hornok et al. 2010). To the best of our knowledge this is the first indication that hedgehogs and/or their fleas may harbor a hemotropic *Mycoplasma* sp.

In summary, this is the first molecular study of fleas from a geographically widespread and highly urbanized species, the northern white-breasted hedgehog. The present findings of new vector-borne agents in *A. erinacei* fleas—shared between this and other hedgehog species—broaden the range of those diseases of veterinary–medical importance, of which hedgehogs may play a role in the epidemiology.

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#### Author Disclosure Statement

No competing financial interests exist.

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