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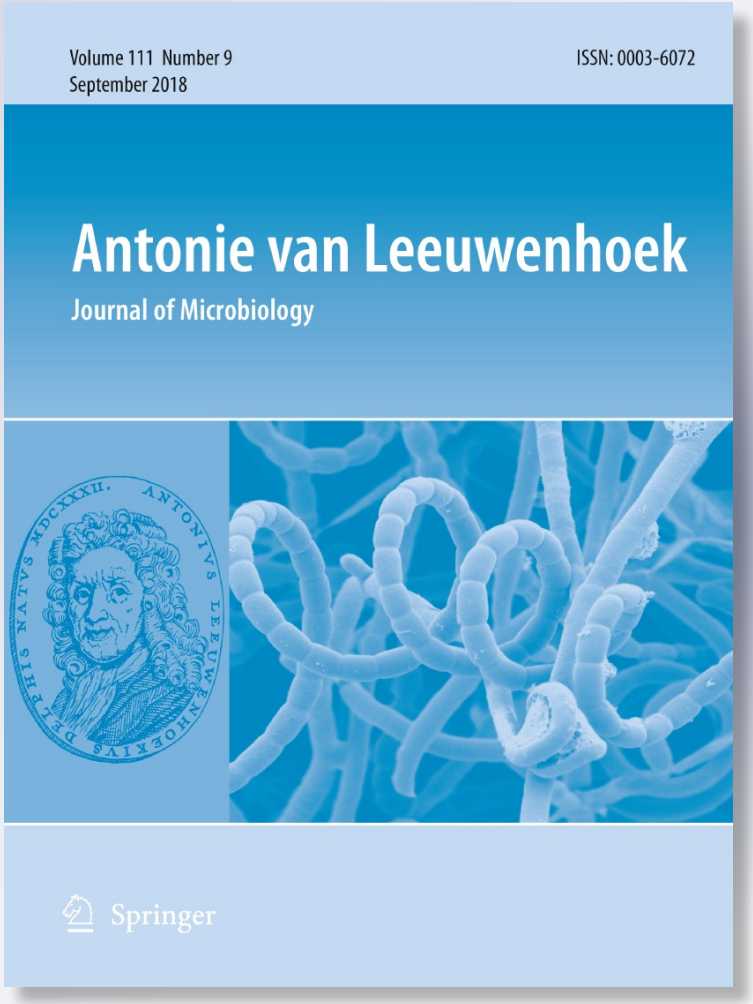

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SHORT COMMUNICATION

Assessing bat droppings and predatory bird pellets for vector-borne bacteria: molecular evidence of bat-associated *Neorickettsia* sp. in Europe

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Abstract In Europe, several species of bats, owls and kestrels exemplify highly urbanised, flying vertebrates, which may get close to humans or domestic animals. Bat droppings and bird pellets may have epidemiological, as well as diagnostic significance from the point of view of pathogens. In this work 221 bat faecal and 118 bird pellet samples were screened for a broad range of vector-borne bacteria using PCR-based methods. *Rickettsia* DNA was detected in 13 bat faecal DNA extracts, including the sequence of a rickettsial insect endosymbiont, a novel *Rickettsia* genotype and *Rickettsia helvetica*. Faecal samples of the pond bat (*Myotis dasycneme*) were positive for a *Neorickettsia* sp. and for haemoplasmas of the haemofelis group. In addition, two bird pellets (collected from a Long-eared Owl, *Asio otus*, and from a

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Common Kestrel, *Falco tinnunculus*) contained the DNA of a *Rickettsia* sp. and *Anaplasma phagocytophilum*, respectively. In both of these bird pellets the bones of *Microtus arvalis* were identified. All samples were negative for *Borrelia burgdorferi* s.l., *Francisella tularensis*, *Coxiella burnetii* and Chlamydiales. In conclusion, bats were shown to pass rickettsia and haemoplasma DNA in their faeces. Molecular evidence is provided for the presence of *Neorickettsia* sp. in bat faeces in Europe. In the evaluated regions bat faeces and owl/kestrel pellets do not appear to pose epidemiological risk from the point of view of *F. tularensis*, *C. burnetii* and Chlamydiales. Testing of bird pellets may provide an alternative approach to trapping for assessing the local occurrence of vector-borne bacteria in small mammals.

Keywords Rickettsiales · Anaplasmataceae · *Francisella* · *Coxiella* · *Mycoplasma* · *Chlamydia*

Introduction

Bats (Mammalia: Chiroptera) indigenous to Europe are insectivorous, nocturnal mammals, which are well known for their urban populations. During the past decades the epidemiological significance of bats has become increasingly recognised because they have been reported to harbour a broad range of pathogens (Klimpel and Mehlhorn 2014). On the other hand, certain predatory birds, such as owls (Aves: Strigiformes) and kestrels (Aves: Falconiformes) also invade cities but appear to be less studied from an epidemiological point of view, despite the fact that their most important prey, rodents, harbour a "disproportionate number" of zoonotic pathogens (Han et al. 2015).

These three categories of highly urbanised, flying vertebrates have ample occasions to get close to humans or their pet and livestock animals. For instance, several bat species are known to roost in buildings (Klimpel and Mehlhorn 2014) or in stables (Dekker et al. 2013). Barn owls (*Tyto alba*) also frequently choose stables and other man-made structures for nesting (Milchev and Gruychev 2014). Eurasian kestrels live even in the centre of large cities (Sumasgutner et al. 2014). In such scenarios, direct contact of humans and domestic animals with bats, owls and kestrels is less likely than indirect contact through contamination of their common environment with droppings (faeces) and pellets (i.e., the regurgitated portion of prey items of predatory birds). For instance, bat faeces or owl pellets may contaminate the food of stable-kept animals (entailing the risk of consequent oral infection) or may become gradually aerosolised (promoting air-borne transmission of pathogens with this infectious potential).

While the epidemiological risks posed by bat droppings and bird pellets might be highest in the case of extracellular pathogens, it was also shown to be relevant to facultative and obligate intracellular bacteria (as exemplified by *Bartonella* spp. and *Coxiella burnetii*, respectively: Dietrich et al. 2017). Nevertheless, detection of bacterial DNA in these excreta may have yet another, i.e., diagnostic significance. In the case of vertebrates, which are hard to access for blood sampling, and/or are highly protected and possibly vulnerable to invasive sampling methods, molecular analysis of their "products", such as faeces, may prove to be a valuable diagnostic method in surveys. Accordingly, a major step in the identification of microorganisms associated with apes was achieved when their faeces were shown to contain the DNA of blood-borne pathogens (Keita et al. 2013). More relevant to this study, the DNA of vector-borne protozoa (Hornok et al. 2015a) and vector-borne bacteria (Veikkolainen et al. 2014) are present in bat droppings. The advantage of this method is that results are obtained with non-invasive sampling but it also has a drawback: the DNA in bat faeces may originate either from the host itself (e.g., from cells crossing the gut barrier towards the lumen, or if there is intestinal bleeding), from bat intestinal parasites (e.g., flukes or their eggs) or from gut food contents, e.g., ingested insects (Hornok et al. 2015a; Veikkolainen et al. 2014). In this context, to the best of the authors' knowledge, no scientific reports have described bird pellets as sources of rodent-borne pathogen DNA.

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Thus, the aim of this study was to assess the epidemiological and diagnostic importance of bat faeces and bird pellets focusing on vector-borne bacteria. Bats and urban predatory birds were sampled, taking into account their synanthropic presence and scarcity or lack of literature data on their epidemiological significance in the context of vector-borne bacteria. Vector-borne bacteria were chosen as the target group, because bats have access to these in their food (Hornok et al. 2015a), and synanthropic rodents (which are among the most frequent prey items of predatory birds in cities) are significant reservoirs of vector-borne bacteria (Hornok et al. 2015b).

Materials and methods

Sample collection

Between May and September 2014, 196 individual and 25 pooled bat faecal samples were collected from 19 bat species (Table 1) at 38 locations in Hungary, and at 10 locations in the Netherlands. These bats were caught for monitoring purposes. After identification of their species, they were held individually in sterile paper bags until sufficient defecation occured. Pooled bat faecal samples were collected from the top of accumulated droppings under bat colonies. Faecal pellets were stored frozen at − 20 °C until evaluation (Hornok et al. 2015a). In addition, 118 owl and kestrel pellet samples were collected from the resting sites of 4 bird species (Table 2) between February and October 2015. Bones of prey animals in bird pellets were identified by a zoologist expert (Péter Estók).

DNA extraction and molecular analyses

DNA was extracted from bat faeces with the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was extracted from the soft parts within owl/kestrel pellets with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (tissue protocol). All procedures included an extraction control to monitor cross-contamination of samples. The amount and purity of DNA in bird pellet extracts were measured with a Thermo Scientific Multiscan GO spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). DNA concentrations and purities of bird pellet extracts were broadly variable (i.e., concentration of 3–295 ng/µl and purity of 1–2.23, respectively). All samples were tested for the quantity and quality of DNA contents with a TaqMan real-time PCR specific for the 18S rRNA gene (Thermo Fisher Scientific, Vantaa, Finland; Boretti et al. 2009). Samples were consequently used in the dilution (undiluted, 1:10 or 1:100) yielding the lowest Ct value.

Both bat faecal and bird pellet samples were screened for the presence of DNA from *Anaplasma phagocytophilum*, *Neorickettsia risticii*, *Rickettsia* spp., *Francisella tularensis*, *C. burnetii* and Chlamydiales, according to the methods summarised in Table 3. In addition, bat faecal DNA extracts were also analysed for haemoplasmas, and bird pellets for *Borrelia burgdorferi* s.l. (Table 3). The presence of tick DNA in the *Rickettsia helvetica*-positive bat faecal sample was excluded by a PCR, which amplifies part of the 18S rRNA gene of Ixodidae (Hornok et al. 2015a).

Each PCR was run with positive and negative controls (i.e., sequence-verified DNA extract of the relevant agent and non-template reaction mixture, respectively). Positive controls were always PCR positive, whereas negative controls and extraction controls remained PCR negative.

Sanger-dideoxy sequencing was attempted from samples yielding the lowest Ct values. Sequences were aligned and compared to reference GenBank sequences using the nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). Representative sequences were submitted to GenBank (accession numbers: KP862896 [*Neorickettsia* sp.], MF347694 and MF347695 [*Rickettsia* spp.]). Phylogenetic analysis was conducted according to the Tamura–Nei model and maximum likelihood method by using MEGA 6.0.

Results

Bat droppings

Among bat faecal DNA extracts, 13 were real-time PCR positive for rickettsiae (Table 1). In one sample the sequence of a rickettsial endosymbiont (reported from the fly species *Medetera jacula*: JQ925589) was

Table 1 Results of bat faeces analyses

Bat genus (number of spp.)	Number of samples	*Rickettsia* spp.		*Neorickettsia risticii*		*Mycoplasma* spp. (haemofelis group)
		Real-time PCR positive bat species (country)	Identified rickettsiae[a] (accession number)	Real-time PCR positive bat species (country)	Sequence identity (accession number)	Real-time PCR positive bat species (country)[b]
Myotis (9)	135	2 × *M. daubentonii* (HU)	–	4 × *M. dasycneme* (NL)	100% (KP862896)	3 × *M. dasycneme* (NL)
		M. alcathoe (HU)	Novel genotype (MF347695)			
		M. alcathoe (HU)	Fly endosymbiont (MF347694)			
Pipistrellus (3)	18	*P. pipistrellus* (NL)	–	–	–	–
		P. pipistrellus (HU)	–	–	–	–
Nyctalus (2)	30	–	–	–	–	–
Barbastella (1)	6	*B. barbastellus* (HU)	–	–	–	–
Plecotus (1)	1	–	–	–	–	–
Rhinolophus (2)	5	–	–	–	–	–
Miniopterus (1)	1	–	–	–	–	–
Unknown (pooled)	25	2 × Unknown (NL)	Novel genotype (MF347695)	–	–	Unknown (NL)
		4 × Unknown (HU)	*R. helvetica*			

Data of vector-borne bacteria in a field are relevant to a single sample, unless otherwise indicated

HU Hungary, *NL* The Netherlands

[a]Sequencing of rickettsiae was performed from samples with the lowest Ct values

[b]Sequencing of haemoplasmas was unsuccessful

identified (341/341 bp, i.e., 100% identity; Table 1). From three further samples (collected in Hungary and the Netherlands) a novel rickettsia genotype was amplified, which had the highest similarity (333/341 bp, i.e., 97.7%) to a *Rickettsia* genotype recently detected in a rodent species (*Apodemus flavicollis*) in Poland (KY488187) but was also relatively closely related to *Rickettsia felis* (332/341 bp, i.e., 97.4% identity). In addition, *R. helvetica* was identified with a species-specific real-time PCR (Table 3) in one pooled sample collected in Hungary.

All four samples of the pond bat (*Myotis dasycneme*, collected in the Netherlands) were real-time PCR positive for *N. risticii*. The 16S rRNA gene sequence from these samples, designated *Neorickettsia* sp. BF87, was 100% identical (273/273 bp) with horse-derived isolates of *N. risticii* (e.g., AF380258) and closely related *Neorickettsia* genotypes (e.g., KX818101 from bat-associated flukes), whereas it had one nucleotide difference (272/273 bp, i.e., 99.6 identity) from *N. risticii* reported from bats (e.g., KX986616) and *Neorickettsia* isolates from flukes (e.g., KX818100). These geographically diverse sequences clustered together in the phylogenetic analysis (Fig. 1).

Three of these *M. dasycneme* samples also contained haemotropic *Mycoplasma* DNA, the species of which could not be identified with sequencing (Table 1). All samples were negative for *F. tularensis*, *C. burnetii* and Chlamydiales.

Table 2 Collection data and contents of owl and kestrel pellets used in this study

Bird category	Bird species	Location in Hungary (number of samples)	Prey taxa identified according to bones in pellets			
			Rodentia	Soricomorpha	Chiroptera	Aves
Owls	*Asio otus*	Budapest (27)	***Microtus arvalis***, *Mi. subterranus*, *Micromys minutus*, *Myodes glareolus*, *Apodemus* sp., *Mus* sp.	*Crocidura suaveolens*	–	+
		Kisújszállás (14)	*Mus musculus*	–	–	–
		Túrkeve (20)	n.a.	n.a.	n.a.	n.a.
		Kiskunlacháza (15)	*Microtus arvalis*, *Apodemus agrarius*, *Mus* sp.	–	–	+
		Hajdúböszörmény (14)	*Microtus arvalis*, *Apodemus agrarius*, *Mus* sp.	–	–	–
		Kelemér (1)	n.a.	n.a.	n.a.	n.a.
	Tyto alba	Szőlősardó (9)	*Microtus arvalis*, *Arvicola amphibius*	*Crocidura suaveolens*, *C. leucodon*	*Myotis blythii*, *M. myotis*	–
		Viszló (1)	*Mus* sp.	*Crocidura suaveolens*	–	–
	Strix aluco	Budapest (5)	*Apodemus* sp.	–	*Eptesicus serotinus*	–
Diurnal predators	*Falco tinnunculus*	Budapest (12)	***Microtus arvalis***, *Apodemus* sp.	–	–	–

Prey species name identified in the two pellets, which were PCR positive for *Rickettsia* sp. or *Anaplasma phagocytophilum*, is written in bold. The signs "+" or "−" indicate, respectively, the presence or absence of bones from a certain taxon

n.a. not available

Owl and kestrel pellets

Only two pellets were real-time PCR positive. One sample, collected from a Long-eared Owl (*Asio otus*), contained *Rickettsia* DNA, whereas the other, obtained from a Common Kestrel (*Falco tinnunculus*), was positive for *A. phagocytophilum*. Sequencing was not possible due to the relatively high Ct values. Both PCR-positive pellets were collected in Budapest (Hungary) and contained the bones of the same rodent species, *M. arvalis* (Table 2). All samples were negative for *B. burgdorferi* s.l., *F. tularensis*, *C. burnetii* and Chlamydiales.

Discussion

This is the first report of molecular analyses of a broad range of vector-borne bacteria in bat faeces and bird pellets. For the interpretation of the present results it can be hypothesised that the DNA of vector-borne bacteria in bat faeces may originate either from the arthropod food of bats (having passed through the entire gastrointestinal tract), from bat intestinal parasites (such as digenean flukes or their eggs) or from the bats themselves (Hornok et al. 2015a), whereas vector-borne bacteria in the inner parts of owl and kestrel pellets derive from their prey (i.e., mostly small mammals), taking into account that regurgitated pellets are less digested than faeces and only their surface comes into contact with the foregut.

Thus, the DNA of a fly endosymbiont *Rickettsia* sp. in bat droppings can be explained by the presence of those flies as food items in the diet of the relevant bat species (*M. alcathoe*). On the other hand, the novel *Rickettsia* genotype demonstrated here from bats both in Hungary and the Netherlands may as well originate from the tissues of bats, especially taking into account that the *Rickettsia* sp. closest to this genotype was formerly amplified from rodent blood (KY488187). In

Table 3 Technical data and references of molecular methods used in this study

Target taxa (target gene, aim of analysis)	Oligonucleotides (sequence 5′–3′)	References: original (modified) method
Anaplasma phagocytophilum (Msp2, screening)	ApMSP2f (ATG GAA GGT AGT GTT GGT TAT GGT ATT) ApMSP2r (TTG GTC TTG AAG CGC TCG TA) ApMSP2p (TGG TGC CAG GGT TGA GCT TGA GAT TG-HEX)	Courtney et al. (2004) (Hornok et al. 2016)
Neorickettsia risticii (16S rRNA, screening)	ER. 133f (GTT ATT CCC TAC TAC CAG GCA AGT TC) ER. 54r (AAC GGA ATC AGG GCT GCT T) ER. 77p (FAM-ACG CAC CCG TCT GCC ACG GGA-TAMRA)	Pusterla et al. (2000)
Anaplasmataceae (16S rRNA, sequencing)	EHR16SD (5′-GGT ACC YAC AGA AGA AGT CC-3′) EHR16SR (5′-TAG CAC TCA TCG TTT ACA GC-3′)	Parola et al. (2000) (Hornok et al. 2008)
R. helvetica (23S rRNA, screening)	Rickhelv.147f (TTT GAA GGA GAC ACG GAA CAC A) Rickhelv.211r (TCC GGT ACT CAA ATC CTC ACG TA) Rickhelv.170p (6FAM-AAC CGT AGC GTA CAC TTA-MGBNFQ)	Boretti et al. (2009)
Rickettsiaceae (gltA, screening)	CS-F (TCG CAA ATG TTC ACG GTA CTT T) CS-R (TCG TGC ATT TCT TTC CAT TGT G) CS-P (FAM-TGC AAT AGC AAG AAC CGT AGG CTG GAT G-BHQ)	Stenos et al. (2005)
Rickettsiaceae (gltA, sequencing)	Rp877p (GGG GAC CTG CTC ACG GCG G) Rp1258n (ATT GCA AAA AGT ACA GTG AAC A)	Roux et al. (1997)
Haemoplasmas (16S rRNA, screening)	Sybr_For (AGC AAT RCC ATG TGA ACG ATG AA) Sybr_Rev1 (TGG CAC ATA GTT TGC TGT CAC TT) Sybr_Rev2 (GCT GGC ACA TAG TTA GCT GTC ACT)	Willi et al. (2009) (Hornok et al. 2014)
Bat haemoplasmas (16S rRNA, sequencing)	HemMycop16S-41 s (GYA TGC MTA AYA CAT GCA AGT CGA RCG) HemMyco16S-938as (CTC CAC CAC TTG TTC AGG TCC CCG TC) HemMycop16S-322 s (GCC CAT ATT CCT ACGGGA AGC AGC AGT) HemMycop16S-1420as (GTT TGA CGG GCG GTG TGT ACA AGA CC)	Mascarelli et al. (2014)
Borrelia burgdorferi s.l. (flagellin, screening)	B.398f (GGG AAG CAG ATT TGT TTG ACA) B.484r (ATA GAG CAA CTT ACA GAC GAA ATT AAT AGA) B.421p (FAM-ATG TGC ATT TGG TTA TAT TGA GCT TGA TCA GCA A-TAMRA)	Leutenegger et al. (1999)
Francisella tularensis (tul4, screening)	Tul4F (ATT ACA ATG GCA GGC TCC AGA) Tul4R (TGC CCA AGT TTT ATC GTT CTT CT) Tul4P (FAM-TTC TAA GTG CCA TGA TAC AAG CTT CCC AAT TAC TAA G-BHQ)	Versage et al. (2003)
Coxiella burnetii (IS1111a, screening)	IS1111F (CCG ATC ATT TGG GCG CT) IS1111R (CGG CGG TGT TTA GGC) IS1111P (FAM-TTA ACA CGC CAA GAA ACG TAT CGC TGT G-MGB)	Loftis et al. (2006)
Chlamydiales (16S rRNA, screening)	panCh16F2 (CCG CCA ACA CTG GGA CT) panCh16R2 (GGA GTT AGC CGG TGC TTC TTT AC) panCh16S (FAM-CTA CGG GAG GCT GCA GTC GAG AAT C-BHQ1)	Lienard et al. (2011)

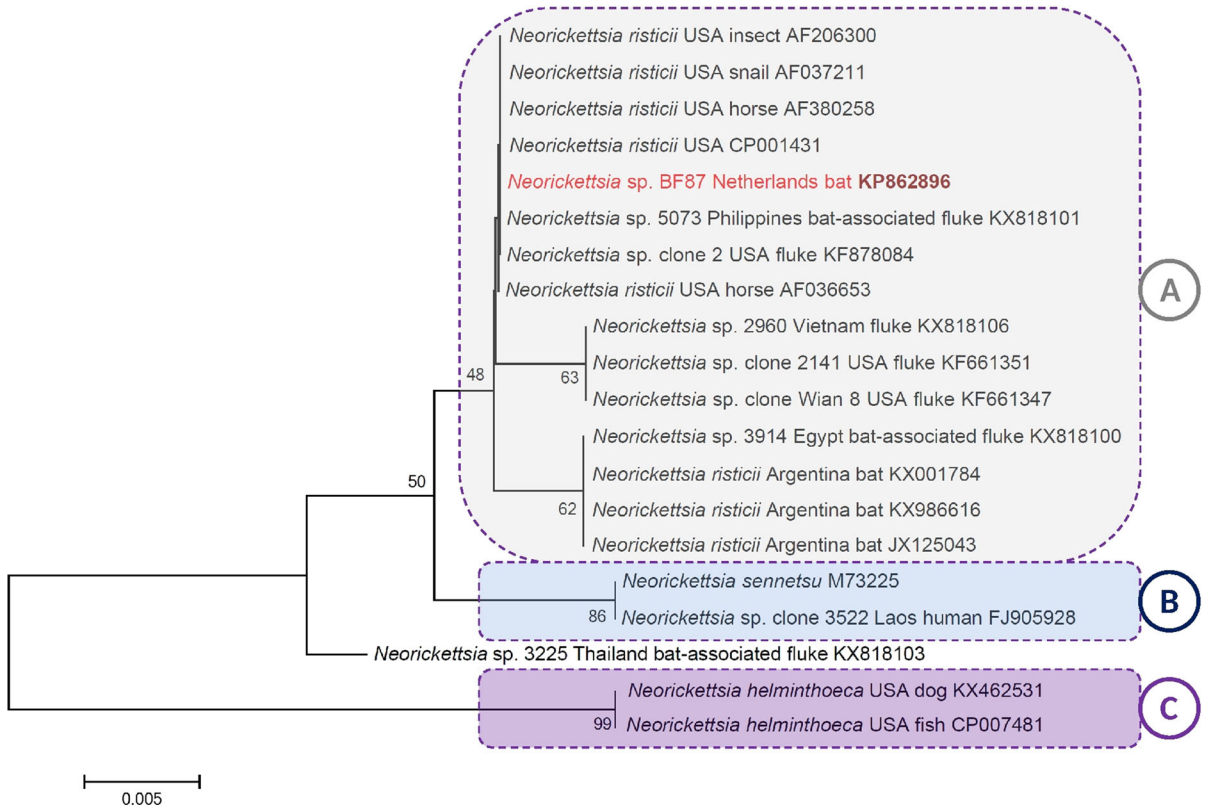

Fig. 1 Phylogenetic tree of *Neorickettsia* spp. and genotypes, based on partial 16S rRNA sequences. For each entry, GenBank data are shown in the following order: species or isolate name, country of origin, generic name of isolation source (when available), finally accession number. The bat-related *Neorickettsia* sp. identified in the present study is highlighted with red fonts and bold accession number. Three phylogenetic groups, which include their "type species" (*N. risticii*, *N. sennetsu* and *N. heminthoeca*), are also indicated (i.e., encircled with dashed line filled with different background colour, and labelled as *A*, *B* or *C*, respectively). Branch lengths represent the number of substitutions per site inferred according to the scale shown. (Color figure online)

addition, *R. helvetica* DNA was shown to be present in bat faeces. This most likely means that the relevant bat(s) harboured *R. helvetica*, because bats are not known to feed on the arthropod vector and reservoir of this *Rickettsia* species (i.e., *Ixodes ricinus*) and DNA of *I. ricinus* was not detectable in that particular sample. In support of this assumption, *R. helvetica* was formerly demonstrated from bat flea (Hornok et al. 2012). Therefore, bats should be further evaluated as potential reservoirs of *R. helvetica*.

N. risticii is the causative agent of equine neorickettsiosis (formerly called Potomac horse fever) (Vaughan et al. 2012). In digenean bat flukes this species has an endosymbiotic nature, i.e., it is transmitted vertically from the adult flukes to their eggs (Gibson et al. 2005). Subsequent developmental stages ensure the maintenance of *N. risticii* in the intermediate hosts (aquatic snails, insects) and eventually in the final hosts (insectivorous bats) of flukes (Vaughan et al. 2012). If horses inadvertently take up *Neorickettsia*-carrier insects when grazing, they become dead-end hosts in the life cycle and their infection usually leads to pathological manifestations such as acute diarrhoea, laminitis and abortion, with up to 30% mortality (Vaughan et al. 2012).

Here *Neorickettsia* DNA was identified in the faeces of the pond bat (*M. dasycneme*), which appears to be the most significant finding of the present study. Although the *Neorickettsia* sp. present in bat faeces was identified by sequencing only a short portion of its 16S rRNA gene, the real-time PCR used here for its detection is regarded as highly sensitive and specific for *N. risticii* DNA, and is therefore the current standard for the diagnosis of equine neorickettsiosis

(Taylor 2018). Coherent results of these two molecular approaches, as well as the phylogenetic clustering of this bat-related *Neorickettsia* sp. with *N. risticii* isolates (Fig. 1), suggest that it may belong to the latter species. However, to confirm this, sequencing of the complete 16S rRNA gene and/or another marker would have been necessary, but this was beyond the scope of the present study.

To the best of the authors' knowledge, this is the first molecular evidence on the occurrence of a *Neorickettsia* sp., phylogenetically clustering with *N. risticii*, in Europe. *N. risticii* and closely related genotypes are geographically widespread. Phylogenetic studies on *Neorickettsia* spp. were hitherto reported from North and South America, Australia, China and South-East Asia, as well as from North Africa, while molecular evidence on their presence in Europe has been lacking (Vaughan et al. 2012; Greiman et al. 2014, 2017). At the same time, isolated cases with seropositivity to *N. risticii* have already been published in France (Vidor et al. 1988) and the Netherlands (van der Kolk et al. 1991), where underlying epidemiological factors remained unelucidated.

Bat fluke species harbouring *N. risticii* and closely related species belong to genera *Acanthatrium* and *Lecithodendrium* (Pusterla et al. 2003), which can be found in European *Myotis* bats, including *M. dasycneme* (Frank et al. 2015). PCR positivity in the present case may have originated from fluke eggs containing *Neorickettsia* DNA (while passing with bat faeces) or perhaps from *Neorickettsia* DNA in infected cells crossing the gut barrier of relevant bats, which may also become horizontally infected (Gibson et al. 2005).

Haemoplasmas of the haemofelis group (unidentifiable to the species level) were shown to be present in the faeces of the same bat species, *M. dasycneme*. Although haemoplasmas have been reported in the blood of bats in Spain (Millán et al. 2015), to the best of our knowledge, they have never been reported from the faeces of bats. Haemoplasmas are known to pass detectable DNA in the faeces of their feline host (Willi et al. 2007), thus it is likely that relevant bats were actually infected with the detected bacteria. This implies that when/where blood sampling of bats is not possible, their faecal pellets may also provide useful data on their haemoplasma-carrier status. This is especially important from the point of view of further studies on bat haemoplasmas of the haemofelis group, which were found to be closely related to human haemoplasmas (Millán et al. 2015).

It is relevant in this context that several bat species roost in large colonies, sometimes within buildings (such as steeples), where the droppings of many individuals can accumulate (Klimpel and Mehlhorn 2014). This may increase the epidemiological risks associated with bat faeces. At the same time, in order to assess infection prevalence of bat-borne pathogens, bat dropping have to be sampled individually.

On the other hand, the resting sites of owls tend to be more scattered and isolated from each other (Milchev and Gruychev 2014), and in such places usually the pellets of only one or two individuals accumulate. However, predatory birds feed on a variety of prey items captured in a large area and this, in turn, may increase (by "concentrating" small mammals into one place) the epidemiologic significance of bird pellets. At the same time, because prey items become mixed in bird pellets, the local prevalence of rodent-borne pathogens cannot be concluded from these.

The pellets of two owl species analysed here contained the bones of bats (Table 2). This shows that owls may come into contact with bat-borne pathogens and the epidemiological roles of bats and owls might be interrelated.

Among owl and kestrel pellets only two samples were PCR positive. This low rate of positivity was unexpected, because it was also demonstrated here that bird pellets contain detectable amount of DNA. One of the main components of owl pellets is the skin (most notably from rodents), which represents the tissue of entry for vector-borne bacteria, and a medium where they may persist. In particular, several species of tick-borne bacteria were reported to be present in the skin of rodents, as exemplified by *A. phagocytophilum* (Svitálková et al. 2015) and *Borrelia afzelii* (Szekeres et al. 2015). Although in the present study *B. burgdorferi* s.l. DNA was not detected in bird pellets, there was one sample PCR positive for *A. phagocytophilum* and another for unidentifiable rickettsiae. These findings indicate that, in places where rodents are not available from trapping, relevant information can potentially be drawn from bird pellets containing the remnants of rodents.

F. tularensis, *C. burnetii* and bacteria from Chlamydiales are known to occur in the evaluated

countries (e.g., in Hungary: Gyuranecz et al. 2012a, b; Kreizinger et al. 2015), and these bacteria may be associated with bats and the prey items of predatory birds (Gyuranecz et al. 2012a; Leulmi et al. 2016). Therefore, the PCR negativity of all samples analysed here suggests that in the evaluated regions bat droppings and bird pellets do not pose high epidemiological risk in the context of these bacteria (i.e., for the oral transmission of *F. tularensis* or for airborne transmission of *C. burnetii*; the transmission modes of many representatives of Chlamydiales remaining unknown) (Burnard et al. 2017).

In conclusion, bats were shown to pass rickettsia and haemoplasma DNA in their faeces. *Neorickettsia* DNA is present in the faeces of the pond bat (*M. dasycneme*) in Europe, suggesting that this bat species plays a final host role in the life cycle of flukes harbouring neorickettsiae. On the other hand, bat faeces and owl/kestrel pellets near human beings or pet and livestock animals (e.g., in barns) do not appear to pose high epidemiological risk from the point of view of vector-borne bacteria with alternative modes of spreading (*F. tularensis*, *C. burnetii* and bacteria from Chlamydiales). Concerning the diagnostic importance of the samples analysed here, e.g., for evaluating the presence of vector-borne bacteria in small mammals, bird pellets can be used as substitutes for trapped rodents (or at least could be evaluated in parallel with the latter sample type).

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Data availability The sequences generated during the current study are available in the GenBank Repository, under accession numbers KP862896 [*N. risticii*], MF347694 and MF347695 [*Rickettsia* spp.]. All other relevant data are contained in the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Authorisation for bat capture was provided by the National Inspectorate for Environment, Nature and Water (No. 14/2138-7/2011). Bat banding licence numbers are TMF-14/32/2010 (DK) and 59/2003 (PE). Birds were not handled during the study, therefore no ethical approval was necessary for the collection of bird pellets (i.e., these were found exclusively in the resting sites of birds).

Informed consent Informed consent was obtained from all individual participants included in the study.

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