

Vector-borne protozoan and bacterial pathogen occurrence and diversity in ectoparasites of the Egyptian Rousette bat

Tamara Szentiványi^{1,2,3}  | Anne-Caroline Heintz³ | Wanda Markotter⁴  | Jérôme Wassef³ | Philippe Christe³  | Olivier Glaizot^{1,3} 

¹Museum of Zoology, Lausanne, Switzerland

²Centre for Ecological Research, Institute of Ecology and Botany, Vácrátót, Hungary

³Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

⁴Department of Medical Virology, Centre for Viral Zoonoses, University of Pretoria, Pretoria, South Africa

Correspondence

Tamara Szentiványi, Centre for Ecological Research, Institute of Ecology and Botany, Alkotmány ut 2-4, HU-2163, Vácrátót, Hungary.

Email: tamaraszentivanyi@gmail.com

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Abstract

Bats are known reservoir hosts for a wide variety of parasites and pathogens, including bacteria and protozoans. Some of these pathogens are vector-borne, and although their role is poorly studied, ectoparasites may contribute significantly to their transmission. The aim of this study was to molecularly detect the presence of vector-borne microorganisms in bat-associated ectoparasites to explore their diversity and distribution in these insects. We tested the presence of *Bartonella* spp., *Polychromophilus* spp., and *Trypanosoma* spp. in bat flies and bat fleas collected from 56 Egyptian Rousette bats (*Rousettus aegyptiacus*), using conventional PCR. We found a high prevalence of 43.9% (47/107) of *Bartonella* spp. in bat flies, but a low prevalence of 6.6% (4/61) in bat fleas. *Polychromophilus* and *Trypanosoma* DNA were absent in both bat flies and bat fleas. Furthermore, we found novel gltA *Bartonella* sequences, as well as genotypes that are highly similar to recently described and potentially zoonotic ones. Our results show high diversity of *Bartonella* in bat flies, however, their role in pathogen transmission is still unknown and should be further explored.

KEYWORDS

Bartonella, ectoparasite, Ischnopsyllidae, Nycteribiidae, vector

INTRODUCTION

With about 1400 species worldwide, bats (Mammalia: Chiroptera) are the second most diverse mammalian taxa after rodents (Mammalia: Rodentia) (Simmons & Cirranello, 2019). Their high morphological and physiological diversification allow them to live in many different environments and have a wide geographical distribution. Bats also show a

unique resistance to intracellular pathogens, and their ability to fly is indirectly involved in their capacity to mediate those pathogens (Brook & Dobson, 2015; Mougari et al., 2022). Moreover, the presence of brown adipose tissue, which is present in all mammals including bats (Cannon & Nedergaard, 2004), has been suggested to play an immunological role in their ability to control the proliferation of pathogens (Brook & Dobson, 2015). Bats can form highly dense and large colonies, although colony size can significantly vary between and within species. In addition, they have an exceptionally long lifespan, up to 40 years, compared to other small mammals (Munshi-South & Wilkinson, 2010). All these ecological, immunological, and physiological traits may contribute to making bats ideal candidates for being reservoir hosts of many pathogenic groups without showing clinical signs of the disease itself (Dobson, 2005; Calisher et al., 2006; Moratelli & Calisher, 2015; Olival et al., 2017; Irving et al., 2021).

Tamara Szentiványi and Anne-Caroline Heintz shared first authorship.

Philippe Christe and Olivier Glaizot equally contributed to this study.

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In this study, we focused on the Egyptian Rousette bat, *Rousettus aegyptiacus* (Chiroptera: Pteropodidae). This species is occasionally consumed in rural areas, along with other species such as *Eidolon* and *Pteropus* spp., which are more frequently targeted for hunting and trading (Leroy et al., 2009; Mickleburgh et al., 2009; Bai et al., 2018). Due to the possibility of human contact with these species, pathogen surveillance of these bats is highly important (Ramanantsalama et al., 2022) and has previously been explored in the case of *R. aegyptiacus* (Table S1). *R. aegyptiacus* can form colonies of up to thousands of individuals and is frequently found in mixed colonies with other cave-dwelling species, such as *Miniopterus* spp. and *Rhinolophus* spp. (Amman et al., 2012, W. Markotter, Personal communication), which potentially contributes to interspecific parasite and pathogen flow.

We examined the presence of vector-borne blood parasites (*Polychromophilus* spp. and *Trypanosoma* spp.) and bacterial pathogens (*Bartonella* spp.) in the bat fly *Eucampsipoda africana* (Diptera: Nycteribiidae) and in the bat flea *Thaumapsylla breviceps* (Siphonaptera: Ischnopsyllidae). Both species are highly specialized and frequently found on *R. aegyptiacus* (Theodor, 1967; Segerman, 1995). In mixed species colonies in Gabon, *E. africana* occasionally infects *Hipposideros caffer*, *Hipposideros gigas*, and *Miniopterus inflatus* (Obame-Nkoghe et al., 2016).

Bat flies and fleas are known or suspected vectors of several pathogens. Bat flies transmit the haemosporidian malaria-like protozoan, *Polychromophilus* spp., which exclusively infects bats (Gardner & Molyneux, 1988a). It has been shown that infections by this blood protozoan occur only in insectivorous bats (Gardner & Molyneux, 1988a; Megali et al., 2011; Duval et al., 2012). Additionally, the presence of this pathogen can contribute to weaker body condition in bats (Witsenburg et al., 2014) and a shorter life span of bat flies (Witsenburg et al., 2015b).

Trypanosoma spp. are intra- and extracellular protozoan blood parasites and have been reported to be transmitted to bats by hematophagous insects, such as Cimicidae bugs (Hemiptera) (Gardner & Molyneux, 1988b); nevertheless, *Trypanosoma* parasites have been detected in parasitic bat flies, and it has been suggested that they may also play a role in the transmission of these blood parasites (Hoare, 1972; Szentiványi et al., 2019, 2020). *R. aegyptiacus* infection by *Trypanosoma* has only been reported once in Gabon (Stevens et al., 1999). A previous work supports that bat trypanosomes are involved in the evolution of the clade of *Trypanosoma cruzi*, causing the Chagas disease in humans (Hamilton et al., 2012), and have an African origin (Clément et al., 2020).

In addition, both bat flies and bat fleas are suspected vectors of *Bartonella* species (Reeves et al., 2007; Morse et al., 2012; Sándor et al., 2018). *Bartonella* spp. are Gram-negative bacteria and are facultative intracellular parasites. *Bartonella* species are known to cause human diseases, cat scratch disease and trench fever, which are caused by *Bartonella henselae* and *B. quintana*, respectively, but can also cause additional diseases, both in humans and animals (Anderson & Neuman, 1997; Chomel et al., 2006).

The aims of this study were to test for the presence and diversity of three different vector-borne pathogens (*Bartonella* spp., *Polychromophilus* spp., and *Trypanosoma* spp.) in both bat flies and fleas collected from *R. aegyptiacus*.

MATERIAL AND METHODS

Collection of ectoparasites

Bat ectoparasites (bat flies: $n = 107$; bat fleas: $n = 61$) were collected from a single colony of *R. aegyptiacus* in Matlapitsi cave, Limpopo, South Africa (24°11'49.7"S, 30°12'15.1" E), between February 2013 and September 2016. After collection, ectoparasites were individually stored in 98% ethanol. Bat fly and flea identification was done by T. Szentiványi based on several identification keys (Theodor, 1967; Segerman, 1995). All flies were identified as *E. africana* (Diptera: Nycteribiidae) and fleas as *T. breviceps* (Siphonaptera: Ischnopsyllidae).

Voucher samples (DNA extractions) are deposited at the Museum of Zoology, Lausanne, Switzerland, under the accession numbers: SMA 852_SF1-UP 7002_SM2 (Table S3).

Pathogen detection and analysis

Ectoparasites genomic DNA was extracted using DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany) based on the protocol provided by the manufacturer. PCR primers, protocols, and annealing temperatures are detailed in Data S1. Positive controls for each pathogen were obtained from previous work (Szentiványi et al., 2020).

Positive PCR products were sent to Microsynth (Switzerland) for Sanger sequencing (HTS). Multiple sequence alignments were done using ClustalW software (Thompson et al., 1994). Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). We performed the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). Reference sequences were obtained from GenBank (Table S2).

RESULTS

Ectoparasites were collected from *R. aegyptiacus* ($n = 56$). We detected the presence of *Bartonella* spp. in 47 out of 107 bat flies (43.9%) and in four out of 61 bat fleas (6.6%). A single *Trypanosoma* infection was present as positive in a bat fly individual; however, sequencing was unsuccessful, therefore we omitted this from the results. In addition, *Trypanosoma* was absent in fleas. We did not observe the presence of *Polychromophilus* infection, neither in bat flies nor in bat fleas. As the presence of *Trypanosoma* and *Polychromophilus* DNA was negligible, we focused on *Bartonella* spp. in further analyses.

Phylogeny of *Bartonella* in bat flies

For the phylogenetic analysis, only the highest quality sequences were selected ($n = 21$). Seven *Bartonella* genotypes (unique sequence variants with ≥ 1 bp differences (Kosoy et al., 2010)) have been obtained from gItA sequences from bat flies (Figure 1). *Bartonella* sequences shared together 83.9% to 100% nucleotide pairwise identity between each other.

Furthermore, some sequences exhibited 90% identity with *Bartonella bovis*, obtained from cattle in Malaysia (KR733183) and France (KF199895), as well as from water buffalo in Thailand (KF199899) and Guatemala (KF199897).

DISCUSSION

During this study, we found the presence of *Bartonella* DNA in bat flies and bat fleas collected from the Egyptian Rousette bat, *R. aegyptiacus*. The presence of *Bartonella* DNA has been previously observed in several bat species and in bat flies in South Africa, including our target species, *R. aegyptiacus* and *E. africana* (Dietrich et al., 2016; Szentiványi et al., 2019, 2020; Ramanantsalama et al., 2022). Here, we found highly similar sequences to the potentially zoonotic *Bartonella rousetti* in bat flies in South Africa. In a recent study, antibodies against bat associated *Bartonella rousetti* were described in humans (Bai et al., 2018), indicating that bat-associated bacteria can potentially infect humans. However, antibodies against *Bartonella* tend to be highly cross reactive within the genus and with other non-*Bartonella* agents. Therefore, the true zoonotic potential of *B. rousetti* needs to be further explored. In some communities, such as in Nigeria, people enter caves during the annual bat festival to capture and consume bats, including *R. aegyptiacus*, in large numbers. *Bartonella rousetti* has also been found in Zambia in the same host species (Qiu et al., 2020). Transmission routes of zoonotic pathogens can be diverse, either vector-borne, or via contacting body fluids and/or faeces of the infected animals. For instance, *Bartonella* DNA has been detected in bat saliva (Dietrich et al., 2017), bat guano (Veikkolainen et al., 2014; Dietrich et al., 2017), and urine (Dietrich et al., 2017).

Even though it might be a rare scenario, bat flies are known to occasionally bite humans, which could facilitate pathogen transmission (Dick & Patterson, 2006). Close contact with bats and their ectoparasites could potentially contribute to the spillover of new and emerging infectious diseases, as in the case of certain viruses (Calisher et al., 2006); nevertheless, the occurrence of these spillover events is supposedly rare.

There is some evidence that pathogen presence might be driven by ecological factors as well, such as host habitat preference. For instance, *Spinturnix myoti* mites infecting greater mouse-eared bats (*Myotis myotis*) inhabiting caves showed a higher prevalence of *Bartonella* spp. than *S. myoti* infecting *M. myotis* inhabiting buildings (Szubert-Kruszyńska et al., 2019). Similarly, it has been found that bat flies collected from cave-dwelling species exhibited the highest *Bartonella* spp. prevalence (Sándor et al., 2018). These observations might be explained by microclimatic conditions in caves that favour ectoparasitic abundance (Szubert-Kruszyńska et al., 2019), which has been observed to positively correlate with the presence of *Bartonella* DNA in hosts (Stuckey et al., 2017).

Bartonella usually occurs in high diversity in bats and their parasites (Morse et al., 2012; McKee et al., 2016), which our results further confirm. Previous work has found six major bat-associated *Bartonella* clades (Corduneanu et al., 2018). As several highly similar

Bartonella lineages inhabit different geographical regions, host distribution, and sympatry might not be the major drivers of *Bartonella* diversification. Indeed, it has been observed that *Bartonella* diversity corresponds to host phylogeny, with different pathogen lineages likely occurring within their specific bat suborders or families (McKee et al., 2016). In addition, increasing taxonomic distance in hosts decreased the likelihood of transition rates (McKee et al., 2016). Therefore, host phylogeny is more likely to be the determinant of *Bartonella* distribution rather than host spatial distribution, although there is increasing evidence of *Bartonella* transmission between phylogenetically distant species, including domestic animals and wildlife (Frank et al., 2018). Here we found that most of our *Bartonella* sequences were highly similar to strains isolated from either *R. aegyptiacus* or their ectoparasites. Furthermore, we found 94% similarity between our sequences and *Bartonella* isolated from *Eidolon helvum* from Ghana, which belongs to the same family (Pteropodidae) as *R. aegyptiacus*, however, only a small region of the genome was targeted in this study; therefore, more and extensive molecular work is needed in the future to determine the zoonotic potential of these bat-associated pathogens.

Overall, we found a high prevalence of *Bartonella* DNA in bat flies and lower in bat fleas, of the cave-dwelling bat species, *R. aegyptiacus*. These results match the observation of a previous study, which showed a high level of pathogen prevalence in bat flies whereas bat fleas of fruit bats were not infected (Brook et al., 2015), although the sample size was relatively low to withdraw this conclusion. Nevertheless, as fleas are generally smaller compared to nycteribiid bat flies, it is possible that the smaller amount of blood-meal inside these parasites results in lower pathogen DNA detectability, hence a lower prevalence rate. However, our results indicate that different ectoparasite species and groups might harbour different infection levels of this bacterial pathogen, and therefore their vectorial or reservoir roles might differ. Nonetheless, we have no direct proof of the viability of *Bartonella* in our samples, as we did not perform culturing of these pathogens. Likewise, the vectorial capacity of the ectoparasites would require experimental studies to be demonstrated.

Similarly, to a recent study performed in Gabon (Roskopf et al., 2019), we did not find evidence of *Polychromophilus*, neither in bat flies nor in bat fleas. Nevertheless, a study showed that *P. melanipherus* infection was present in a single pool of *E. africana* flies collected on *R. aegyptiacus*, suggesting a previous blood-meal from a non-primary host, as *P. melanipherus* is only known to infect bats belonging to the family Minopteridae (Witsenburg et al., 2015a; Obame-Nkoghe et al., 2016). Based on literature records, there is no evidence of *Polychromophilus* infection in *R. aegyptiacus*, whereas the closely related haemosporidian parasite *Hepatocystis* has been found in Nigeria (Atama et al., 2019).

We detected the presence of *Trypanosoma* DNA in a single bat fly sample; however, as the quality of the acquired sequence was not satisfactory, we cannot argue if it was a genetically identical or similar sequence to the *Trypanosoma* strain identified from *R. aegyptiacus* in Gabon (Stevens et al., 1999) (Table S1). Either it is indicating the presence of *Trypanosoma* sp. in this colony of *R. aegyptiacus* or it suggests that even highly host-specific bat flies, such as *E. africana*, occasionally

feed on other bat species before returning to their main host, potentially contributing to the distribution of new pathogen species to naïve hosts. Studies targeting *Trypanosoma* infection in *Rousettus* bats need to address this question.

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CONFLICT OF INTEREST

The authors declare there are no competing interests.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Tamara Szentivanyi  <https://orcid.org/0000-0001-8123-0374>

Wanda Markotter  <https://orcid.org/0000-0002-7550-0080>

Philippe Christe  <https://orcid.org/0000-0002-8605-7002>

Olivier Glaizot  <https://orcid.org/0000-0001-9116-3355>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information

Table S1. Vector-borne pathogens (excluding viruses) tested (based on culture and/or nucleic acid based testing) and recorded in *Rousettus aegyptiacus* and its ectoparasites.

Table S2. Reference sequences of gltA region used for phylogenetic analysis, obtained from GenBank and literature.

Table S3.

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