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

Tamara Szentiványi1,2,3 | Anne-Caroline Heintz3 | Wanda Markotter4 | Jérôme Wassef3 | Philippe Christe3 | Olivier Glaizot1,3

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; Mogari 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).

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. Medical and Veterinary Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society.
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 Eucampsiopoda africana (Diptera: Nycteribiidae) and in the bat flea Thaumapsylla breviceps (Siphonaptera: Ischnopsyllidae). Both species are highly specialized and frequently found on African origin (Clément et al., 2020). To date, Polychromophilus通话 is widely regarded as a vector of bartonellosis in various mammals and to a lesser extent, reptiles (Gardner & Molyneux, 1988a). However, it is currently acknowledged that M. henselae and B. quintana bacteria are also capable of infecting E. breviceps (Sartori et al., 2012). In addition, 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 hemathophagous 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 trypanosomases 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 Table 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 gltA sequences from bat flies (Figure 1). Bartonella sequences shared together 83.9% to 100% nucleotide pairwise identity between each other.
BLAST analysis of the gltA sequences showed 94% to 99.7% similarity to different bat associated *Bartonella* sequences. We found sequences that exhibited 99.85% identity to sequences found in *Miniopterus natalensis* and its bat fly, *Nycteribia schmidlii scotti* from South Africa (MW007702-MW007711). In addition, we retrieved a single sequence with 99% identity from *Eucampsipoda madagascariensis* (KT751158) collected from *Rousettus madagascariensis* in Madagascar (Wilkinson et al., 2016). Some sequences showed 98.8% identity with the potentially zoonotic *Bartonella rousetti* (HM363764), which was recently described from *R. aegyptiacus*, and >99% identity with *Bartonella* isolated from its bat flies, *E. africana* from Nigeria (Bai et al., 2018). Some of our sequences showed 94% identity (highest match) with a *Bartonella* strain isolated from the straw-coloured fruit bat (*Eidolon helvum*) from Ghana (KM030516, KM030517).
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 myoti) 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 para sites (Morse et al., 2012; McKee et al., 2016), which our results further confirm. Previous work has found six major bat-associated Bartonella clades (Corduneau 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 (Rosskopf 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 Miniopteridae (Witsenburg et al., 2015a; Obame-Nkoche 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).
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.

**ACKNOWLEDGMENTS**

We thank all the staff and students from the Biosurveillance and Ecology of Emerging Zoonoses (BEEZ) Research Group in the Centre for Viral Zoonoses of University of Pretoria (UP-CVZ), Centre for Emerging Zoonotic and Parasitic Diseases (CEZPD) at the National Institute for Communicable Diseases (NICD), who assisted with field work pertaining to this research project. We would also like to thank the Ga Mafefe community in the Limpopo Province for supporting our research at Matlapitsi cave. We are grateful to the reviewers for their insights and for their constructive comments.

**FUNDING INFORMATION**

This work was also financially supported in part by the National Research Foundation of South Africa under grant numbers UID 92524, 85756, and 91496 (held by Prof W. Markotter) as well as the DSI-NRF South African Research Chair in Animal Infectious Diseases (Zoonoses) held by Prof W. Markotter, grant no. 98339. Additional support was received by the Swiss National Science Foundation under grant numbers 31003A–179378 (held by Prof P. Christie), and P500PB_206888 (held by Dr Tamara Szentivanyi).

**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 Szentiványi https://orcid.org/0000-0001-8123-0374  
Wanda Markotter https://orcid.org/0000-0002-7550-0080  
Philippe Christie https://orcid.org/0000-0002-8605-7002  
Olivier Glaizot https://orcid.org/0000-0001-9116-3355

**REFERENCES**


from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector-Borne and Zoonotic Diseases, 9, 723–728.


Qu, Y., Kajiha, M., Nakao, R., Mulenga, E., Harima, H., Hangombe, B.M. et al. (2020) Isolation of candidatus bartonella rousetti and other bat-associated bartonellae from bats and their flies in Luebo, Democratic Republic of Congo, 2007. Vector-borne pathogens (excluding viruses) tested (based on culture and/or nucleic acid based testing) and recorded in Table S1. Additional supporting information can be found online in the Supporting Information section at the end of this article.


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 Rouset- tussaegyptiacus and its ectoparasites.

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

Table S3.