

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

Funding information

National Research Foundation of South Africa, Grant/Award Numbers: UID 92524, 85756, 91496; South African Research Chair in Animal Infectious Diseases (Zoonoses), Grant/Award Number: 98339; Swiss National Science Foundation, Grant/Award Numbers: 31003A-179378, P500PB_206888

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.

Permits: We obtained permission to conduct research under Section 20 of the Animal Disease Act (Act No. 35 of 1984) from the Department of Agriculture, Land Reform and Rural Development of South Africa. This research was conducted with the approval of the University of Pretoria Animal Ethics committee (Project no. EC054-14 and EC059-14). Permits were obtained for bat sample collection from the South African provinces involved: the Department of Economic Development, Environment and Tourism Limpopo province directorate- wildlife permit no. CPM006806.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) 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 *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 gltA sequences from bat flies (Figure 1). *Bartonella* sequences shared together 83.9% to 100% nucleotide pairwise identity between each other.

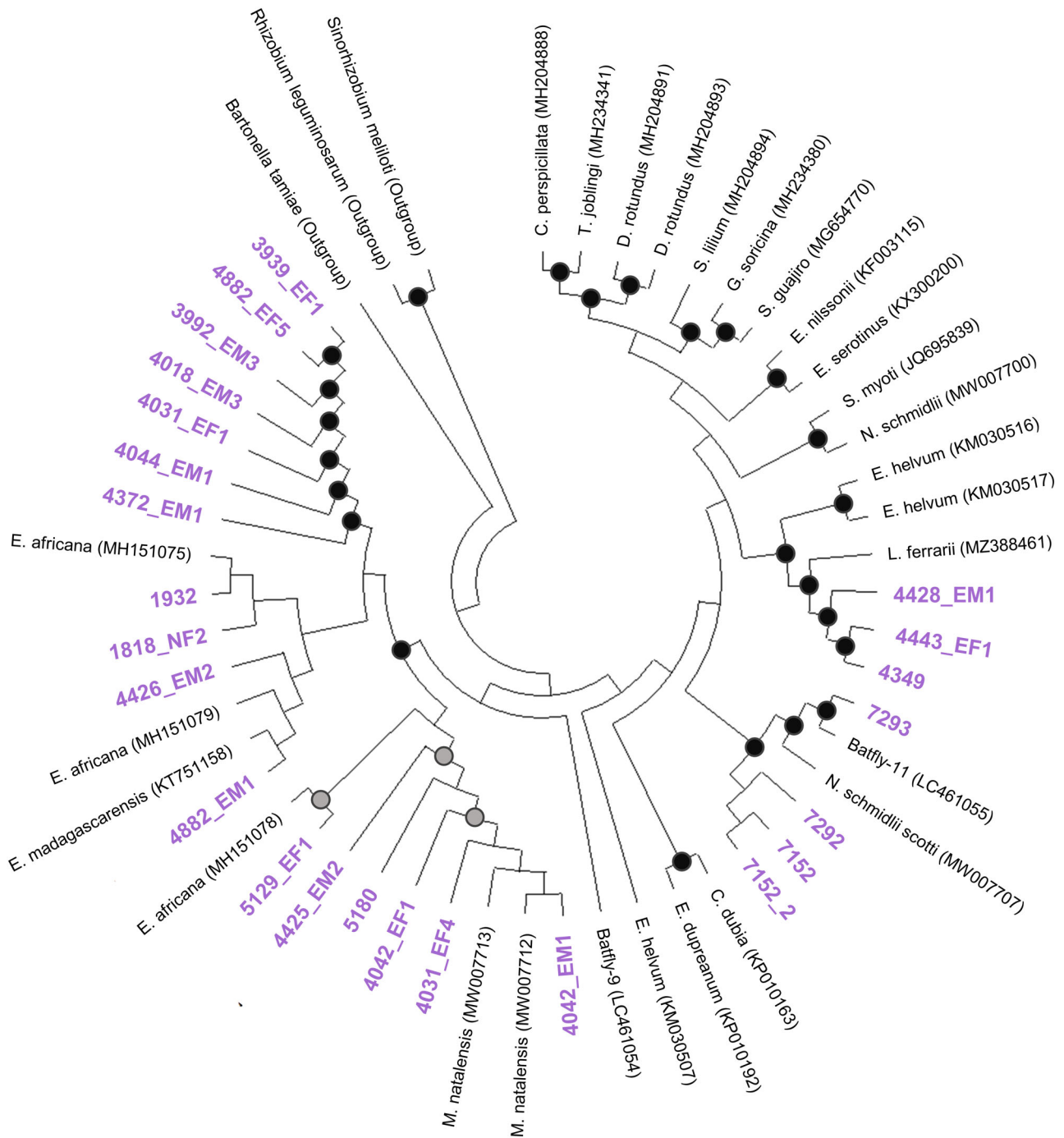


FIGURE 1 Phylogenetic tree of bat associated *Bartonella* species. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The bootstrap consensus tree inferred from 1000 replicates. Grey dots indicate bootstrap values 0.7–0.9, whereas black dots indicate values >0.9. Purple codes indicate samples from this study. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Phylogenetic tree was rooted from Rhizobiaceae.

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).

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 Miniopteridae (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.

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. Christe), 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 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>

REFERENCES

- Amman, B.R., Carroll, S.A., Reed, Z.D., Sealy, T.K., Balinandi, S., Swanepoel, R. et al. (2012) Seasonal pulses of marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. *PLoS Pathogens*, 8, e1002877.
- Anderson, B.E. & Neuman, M.A. (1997) *Bartonella* spp. as emerging human pathogens. *Clinical Microbiology Reviews*, 10, 203–219.
- Atama, N., Manu, S., Ivande, S., Rosskopf, S.P., Matuschewski, K. & Schaer, J. (2019) Survey of hepatocystis parasites of fruit bats in the Amurum forest reserve, Nigeria, identifies first host record for *Rousettus aegyptiacus*. *Parasitology*, 146, 1–5.
- Bai, Y., Osinubi, M.O.V., Osikowicz, L., McKee, C., Vora, N.M., Rizzo, M.R. et al. (2018) Human exposure to novel bartonella species from contact with fruit bats. *Emerging Infectious Diseases*, 24, 2317–2323.
- Brook, C.E., Bai, Y., Dobson, A.P., Osikowicz, L.M., Ranaivoson, H.C., Zhu, Q. et al. (2015) *Bartonella* spp. in fruit bats and blood-feeding ectoparasites in Madagascar. *PLoS Neglected Tropical Diseases*, 9, 1–9.
- Brook, C.E. & Dobson, A.P. (2015) Bats as “special” reservoirs for emerging zoonotic pathogens. *Trends in Microbiology*, 23, 172–180.
- Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V. & Schountz, T. (2006) Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews*, 19, 531–545.
- Cannon, B. & Nedergaard, J. (2004) Brown adipose tissue: function and physiological significance. *Physiological Reviews*, 84, 277–359.
- Chomel, B.B., Boulouis, H.J., Maruyama, S. & Breitschwerdt, E.B. (2006) *Bartonella* spp. in pets and effect on human health. *Emerging Infectious Diseases*, 12, 389–394.
- Clément, L., Dietrich, M., Markotter, W., Fasel, N.J., Monadjem, A., López-Baucells, A. et al. (2020) Out of Africa: the origins of the protozoan blood parasites of the *Trypanosoma cruzi* clade found in bats from Africa. *Molecular Phylogenetics and Evolution*, 106705, 106705.
- Corduneanu, A., Sándor, A.D., Ionică, A.M., Hornok, S., Leitner, N., Bagó, Z. et al. (2018) *Bartonella* DNA in heart tissues of bats in central and eastern Europe and a review of phylogenetic relations of bat-associated bartonellae. *Parasites and Vectors*, 11, 1–7.
- Dick, C.W. & Patterson, B.D. (2006) Bat flies: obligate ectoparasites of bats. In: Morand, S., Krasnov, B.R. & Poulin, R. (Eds.) *Micromammals and macroparasites*. Tokyo, Tokyo: Springer, pp. 179–194.
- Dietrich, M., Kearney, T., Seamark, E.C.J. & Markotter, W. (2017) The excreted microbiota of bats: evidence of niche specialisation based on multiple body habitats. *FEMS Microbiology Letters*, 364, 1–7.
- Dietrich, M., Tjale, M.A., Weyer, J., Kearney, T., Seamark, E.C.J., Nel, L.H. et al. (2016) Diversity of *Bartonella* and *Rickettsia* spp. in bats and their blood-feeding ectoparasites from South Africa and Swaziland. *PLoS One*, 11, 1–9.
- Dobson, A.P. (2005) What links bats to emerging infectious diseases? *Science*, 310, 628–629.
- Duval, L., Mejean, C., Maganga, G.D., Makanga, B.K., Mangama Koumba, L. B., Peirce, M.A. et al. (2012) The chiropteran haemosporidian *Polychromophilus melanipherus*: a worldwide species complex restricted to the family Miniopteridae. *Infection, Genetics and Evolution*, 12, 1558–1566.
- Frank, H.K., Boyd, S.D. & Hadly, E.A. (2018) Global fingerprint of humans on the distribution of *Bartonella* bacteria in mammals. *PLoS Neglected Tropical Diseases*, 12, 1–17.
- Gardner, R.A. & Molyneux, D.H. (1988a) *Polychromophilus murinus*: a malarial parasite of bats: life-history and ultrastructural studies. *Parasitology*, 96, 591–605.
- Gardner, R.A. & Molyneux, D.H. (1988b) *Trypanosoma* (Megatrypanum) incertum from *Pipistrellus pipistrellus*: development and transmission by cimicid bugs. *Parasitology*, 96, 433–447.
- Hamilton, P.B., Teixeira, M.M.G. & Stevens, J.R. (2012) The evolution of *Trypanosoma cruzi*: the “bat seeding” hypothesis. *Trends in Parasitology*, 28, 136–141.
- Hoare, C. (1972) *The trypanosomes of mammals*. Oxford and Edinburgh: Blackwell Scientific Publications.
- Irving, A.T., Ahn, M., Goh, G., Anderson, D.E. & Wang, L.F. (2021) Lessons from the host defences of bats, a unique viral reservoir. *Nature*, 589, 363–370.
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
- Kosoy, M., Bai, Y., Lynch, T., Kuzmin, I.V., Niezgodza, M., Franka, R. et al. (2010) *Bartonella* spp. in bats, Kenya. *Emerging Infectious Diseases*, 16, 1875–1881.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.
- Leroy, E.M., Epelboin, A., Mondonge, V., Pourrut, X., Gonzalez, J.P., Muyembe-Tamfum, J.J. et al. (2009) Human ebola outbreak resulting

- from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector-Borne and Zoonotic Diseases*, 9, 723–728.
- McKee, C.D., Hayman, D.T.S., Kosoy, M.Y. & Webb, C.T. (2016) Phylogenetic and geographic patterns of bartonella host shifts among bat species. *Infection, Genetics and Evolution*, 44, 382–394.
- Megali, A., Yannic, G. & Christe, P. (2011) Disease in the dark: molecular characterization of *Polychromophilus murinus* in temperate zone bats revealed a worldwide distribution of this malaria-like disease. *Molecular Ecology*, 20, 1039–1048.
- Mickleburgh, S., Waylen, K. & Racey, P. (2009) Bats as bushmeat: a global review. *Oryx*, 43, 217–234.
- Moratelli, R. & Calisher, C.H. (2015) Bats and zoonotic viruses: can we confidently link bats with emerging deadly viruses? *Memorias do Instituto Oswaldo Cruz*, 110, 1–22.
- Morse, S.F., Olival, K.J., Kosoy, M., Billeter, S., Patterson, B.D., Dick, C.W. et al. (2012) Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). *Infection, Genetics and Evolution*, 12, 1717–1723.
- Mougari, S., Gonzalez, C., Reynard, O. & Horvat, B. (2022) Fruit bats as natural reservoir of highly pathogenic henipaviruses: balance between antiviral defense and viral tolerance. Interactions between Henipaviruses and their natural host, fruit bats. *Current Opinion in Virology*, 54, 101228.
- Munshi-South, J. & Wilkinson, G.S. (2010) Bats and birds: exceptional longevity despite high metabolic rates. *Ageing Research Reviews*, 9, 12–19.
- Obame-Nkoghe, J., Rahola, N., Bourgarel, M., Yangari, P., Prugnolle, F., Maganga, G.D. et al. (2016) Bat flies (Diptera: Nycteribiidae and Streblidae) infesting cave-dwelling bats in Gabon: diversity, dynamics and potential role in *Polychromophilus melanipherus* transmission. *Parasites and Vectors*, 9, 1–12.
- Olival, K.J., Hosseini, P.R., Zambrana-Torrel, C., Ross, N., Bogich, T.L. & Daszak, P. (2017) Host and viral traits predict zoonotic spillover from mammals. *Nature*, 546, 646–650.
- Qiu, Y., Kajihara, M., Nakao, R., Mulenga, E., Harima, H., Hangombe, B.M. et al. (2020) Isolation of candidatus bartonella roussetti and other bat-associated bartonellae from bats and their flies in Zambia. *Pathogens*, 9, 1–14.
- Ramanantsalama, R.V., Goodman, S.M., Dietrich, M. & Lebarbenchon, C. (2022) Interaction between Old World fruit bats and humans: from large scale ecosystem services to zoonotic diseases. *Acta Tropica*, 231, 106462.
- Reeves, W.K., Rogers, T.E., Durden, L.A. & Dasch, G. A. (2007) Association of Bartonella with the fleas (Siphonaptera) of rodents and bats using molecular techniques. *Journal of Vector Ecology: Journal of the Society for Vector Ecology*, 32, 118–122.
- Roskopf, S.P., Held, J., Gmeiner, M., Mordmüller, B., Matsiégué, P.B., Eckerle, I. et al. (2019) Nycteria and Polychromophilus parasite infections of bats in Central Gabon. *Infection, Genetics and Evolution*, 68, 30–34.
- Sándor, A.D., Földvári, M., Krawczyk, A.I., Sprong, H., Corduneanu, A., Barti, L. et al. (2018) Eco-epidemiology of novel Bartonella genotypes from parasitic flies of insectivorous bats. *Microbial Ecology*, 76, 1–13.
- Segerman, J. (1995) *Siphonaptera of southern Africa: handbook for the identification of fleas*. Johannesburg, South Africa: South African Institute for Medical Research.
- Simmons, N.B. & Cirranello, A.L. (2019) Bat species of the world: a taxonomic and geographic database [WWW Document]. <http://www.batnames.org> [accessed on 2019]
- Stevens, J.R., Teixeira, M.M.G., Bingle, L.E.H. & Gibson, W.C. (1999) The taxonomic position and evolutionary relationships of *Trypanosoma rangeli*. *International Journal for Parasitology*, 29, 749–757.
- Stuckey, M.J., Chomel, B.B., Galvez-Romero, G., Olave-Leyva, J.I., Obregón-Morales, C., Moreno-Sandoval, H. et al. (2017) Bartonella infection in hematophagous, insectivorous, and phytophagous bat populations of Central Mexico and the Yucatan peninsula. *American Journal of Tropical Medicine and Hygiene*, 97, 413–422.
- Szentiványi, T., Christe, P. & Glairot, O. (2019) Bat flies and their microparasites: current knowledge and distribution. *Frontiers in Veterinary Science*, 6, 115.
- Szentiványi, T., Markotter, W., Dietrich, M., Clément, L., Ançay, L., Brun, L. et al. (2020) Host conservation through their parasites: molecular surveillance of vector-borne microorganisms in bats using ectoparasitic bat flies. *Parasite*, 27, 1–10.
- Szubert-Kruszyńska, A., Stańczak, J., Cieniuch, S., Podsiadły, E., Postawa, T. & Michalik, J. (2019) Bartonella and Rickettsia infections in haematophagous Spinturnix myotis mites (Acari: Mesostigmata) and their Bat Host, Myotis myotis (Yangochiroptera: Vespertilionidae), from Poland. *Microbial Ecology*, 77, 759–768.
- Theodor, O. (1967) *An illustrated catalogue of the Rothschild collection of Nycteribiidae in the British Museum (Natural History), with keys and short descriptions for the identification of subfamilies, genera, species and subspecies*. London: British Museum (Natural History).
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
- Veikkolainen, V., Vesterinen, E.J., Lilley, T.M. & Pulliainen, A.T. (2014) Bats as reservoir hosts of human bacterial pathogen, Bartonella mayotimonensis. *Emerging Infectious Diseases*, 20, 960–967.
- Wilkinson, D.A., Duron, O., Cordonin, C., Gomard, Y., Ramasindrazana, B., Mavingui, P. et al. (2016) The bacteriome of bat flies (Nycteribiidae) from the Malagasy region: a community shaped by host ecology, bacterial transmission mode, and host-vector specificity. *Applied and Environmental Microbiology*, 82, 1778–1788.
- Witsenburg, F., Clément, L., López-Baucells, A., Palmeirim, J., Pavlinić, I., Scaravelli, D. et al. (2015a) How a haemosporidian parasite of bats gets around: the genetic structure of a parasite, vector and host compared. *Molecular Ecology*, 24, 926–940.
- Witsenburg, F., Schneider, F. & Christe, P. (2014) Epidemiological traits of the malaria-like parasite Polychromophilus murinus in the Daubenton's bat Myotis daubentonii. *Parasites and Vectors*, 7, 1–8.
- Witsenburg, F., Schneider, F. & Christe, P. (2015b) Signs of a vector's adaptive choice: on the evasion of infectious hosts and parasite-induced mortality. *Oikos*, 124, 668–676.

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.

How to cite this article: Szentiványi, T., Heintz, A.-C., Markotter, W., Wassef, J., Christe, P. & Glairot, O. (2023) Vector-borne protozoan and bacterial pathogen occurrence and diversity in ectoparasites of the Egyptian Rousette bat. *Medical and Veterinary Entomology*, 1–6. Available from: <https://doi.org/10.1111/mve.12639>