

Report for the project NKFI 132794

Title: Host-vector-pathogen coevolution strategies: role of pathogens in shaping bat and bat-parasite population dynamics

Duration: 2019-2024

Our aim with this project was to elucidate the main strategies used by parasites to increase their chances to infest/colonize new hosts and to decode the hosts' responses to avoid or reduce this by behavioural mechanisms. We used experimental and descriptive studies in a three-actor system, relying on the model of the bat-vector-pathogen cycle. Using both in-situ, as well experimental settings we tested several hypotheses on host choice, pathogen/parasite avoidance and host-manipulation. In addition, in order to establish the importance of individual vector species in pathogen transmission, we implemented comparative assessments of phylogenetically close pathogens.

We experimentally tested soft ticks for pathogen transfer, and tried to characterize bat flies for their capacity in vectoring zoonotic bacteria, as well haemosporidians, such as *Polychromophilus* spp. Our studies targeted the presence of zoonotic pathogens in five arthropod parasite clades (bat flies, bat bugs, fleas, ticks and gamasid mites) of Palearctic bats. As such we managed to provide new data on the importance of ticks and flies for bat-malaria, to elucidate the role played by different bat flies, gamasid acarids and fleas in *Bartonella* spp. transfer, but also on the importance of individual microbiome may have in reducing the intensity of host-parasite immune conflicts. Below we present the results reached of each category of proposed objectives.

5.1 Investigating parasite mediated behaviour in vertebrate hosts

Through a series of observational data, we managed to prove that parasite-avoidance shapes the roosting behaviour of social bats (submitted MS), parasite-pressure not only influences individual condition (submitted MS), but also triggers behavioural response (MS in preparation on early emergence of highly parasitized individuals). Bat flies are greatly influenced by the presence of entomopathogenic fungi, resulting in clustered distribution (10.3390/jof6040361, 10.3389/fevo.2022.795020) as well morphological peculiarities (MS in preparation on impact of entomopathogenic fungi on fluctuating asymmetry of parasitized individuals). The results of our experiments were inconclusive in deciding the role played by infection in a series of direct behavioural choices by bats or ectoparasites, likely due to the reduced metabolic pressure exercised by the pathogens on their hosts.

5.2 Vectorial capacity of bat ectoparasites

5.2.1 Transmission of *Babesia vesperuginis* by *Argas vespertilionis*

While we tried to perform several experiments, we failed to prove experimentally the role played by the soft tick *Argas vespertilionis* in the transmission cycle of bat-specific *Babesia*. Our failures were caused by the destruction of our indoor colonies of infected and uninfected *Argas vespertilionis* due to an accidental power outage during COVID lockdown. We were unable to replace these colonies later.

5.2.2 Transovarial transmission of *Bartonella* spp. by nycteribiid flies

While we are not able to rule out, our efforts were not able to detect the presence of *Bartonella* spp. in selected, unfed bat flies (112 individuals of three species tested), thus suggesting a reduced role of transovarial transmission for the maintenance of *Bartonella* spp. infection in bat flies, at least in those bat fly species we tested (*Nycteribia pedicularia*, *Phthiridium biarticulatum*, *Penicilidia dufourii*).

5.2.3 Vectorial capacity of nycteribiid flies for *Bartonella* spp.

We detected *Bartonella* spp. in four different species of bat flies and proved their importance in the maintenance of host-specific cycles. In addition, we managed to find these bacteria deeply imbedded into the microbiome of both bats and bat flies, suggesting a long evolutionary relationship (10.1016/j.cimid.2020.101605, 10.1128/spectrum.01531-23 and also submitted MS).

5.2.4 Elucidating the importance of individual bat fly species in the maintenance of *Bartonella* strain diversity

While our studies detected several lineages of *Bartonella* strains, we failed yet to link these to individual bat fly species, likely due to our reduced access to bat flies in geographically distinct areas (our planned visits were abandoned due to COVID-related travel restrictions). While we have some promising results, these are yet inconclusive and likely require extended future sampling.

5.2.5 Vectorial transmission of *Polychromophilus murinus* by nycteribiid flies

Polychromophilus spp. are haemosporidians associated with bat malaria, being a regularly detected in bats all over the world. We managed to prove the vectorial role for several bat fly species for two *Polychromophilus* spp. (*P. melanipherus* and *P. murinus*) in several regions (10.3390/microorganisms9020230). In addition, our experiments proved the importance of malarial infection for hosts' roost emergence timing (MS in preparation on early emergence of highly parasitized individuals), as well the importance of host-choice for ectoparasite infection (MS in preparation on experimental host choice of bat flies).

5.2.6 The potential role of spinturnicid mites as vectors of zoonotic pathogens

Spinturnicid mites are the ever-present ectoparasites of most bat species. Using an extensive collection of mites from Europe and Asia, we managed to detect several *Bartonella* strains in four different mite species (three Macronyssidae and one Spinturnicidae, see 10.1111/mve.12757). We also proved that larger pools were more likely to harbour *Bartonella* sp. DNA, than smaller ones. In addition, cave-dwelling bat hosts and host generalist mite species are more associated with *Bartonella* spp. presence. Mites with ubiquitous presence may facilitate the long-term maintenance (and even local recurrence) of *Bartonella*-infestations inside local bat populations, thus acting as continuous reservoirs for *Bartonella* spp. in bats.

5.2.7 The potential role of cimicid bugs as vectors of protozoan parasites

We tested two different cimicid bug species (*Cimex lectularius* and *C. pipistrelli*) collected at eleven geographical locations from four countries for the presence of protozoan DNA. We detected presence of *Trypanosoma* spp. DNA (sequencing didn't provide definite identification) in a sole bug. Unfortunately, the rareness of bugs (most sites provided a single bug individual) prevented us to reach conclusive results on the importance of cimicid bugs as vectors of protozoan parasites.

5.2.8 The potential role of bat fleas as vectors of zoonotic pathogens

Bat fleas are neglected study objects of bat-associated parasitological studies. This may be caused by several methodological challenges. First of all, access to samples (fleas show host-specific association to roosts, rarely present on the bats' themselves), but also identification pitfalls. As flea ID relies on exoskeleton prepared on microscope slide, after soft tissue was chemically destroyed, it is hard to access DNA from known individuals. To overcome this problem, we developed a new identification guide using intact fleas and backed the morphological keys with molecular barcodes (MS in preparation on morphological and molecular identification of bat specific fleas). The identified bat fleas (1087 individuals of 13 species from eight countries) were later used for assessing presence of bacterial pathogens. We detected low prevalence of *Mycoplasma* sp. (<1%), *Borrelia* sp. (<1%) and Anaplasmataceae (<1%) DNA, while 11% of fleas harbored *Bartonella* spp.

5.3. Pathogen-mediated behavior in arthropod ectoparasites

Long-lasting or debilitating infectious diseases are known to cause behavioural modifications in most vertebrate hosts. However, the impact of pathogens is rarely investigated on arthropod ectoparasites. We tested the impact of several (bacterial, parasitic and fungal) pathogens on ectoparasitic insects. We showed that fungal infection may impact bat flies (10.3390/jof6040361, 10.3389/fevo.2022.795020, also MS in preparation on impact of entomopathogenic fungi on fluctuating asymmetry of parasitized individuals). In addition, presence of malarial pathogen reduces life expectancy of two different fly species, while we found no such relationship in the case of *Bartonella* spp. in bat flies (MS in preparation on impact of malarial and bacterial infection presence on survival of individual bat flies).

Although this project was greatly impacted by the COVID-19 epidemic, which caused several direct (die-out of our laboratory colonies of ticks and bat flies during lockdown) and indirect problems (the shortage and later delay in procurement of lab materials), we managed to reach most of the proposed objectives. Our results (published in 17 papers, with three more submitted and also five more prepared for submission) are important in the light of the known reservoir status of bats for high number of pathogenic bacteria, kinetoplastid and apicomplexan parasites, as well the infamous vectorial status for a series of similar arthropod species. Further to these we managed to publish several papers on geographical distribution (10.12905/0380.sydwia72-2020-0231, 10.3897/BDJ.9.e57680, 10.1515/mammalia-2021-0130, 10.1007/s11686-023-00732-8), ecology (10.3390/jof6040361, 10.3389/fvets.2021.684737, 10.11646/zootaxa.5120.1.7, 10.1111/mam.12283, 10.1186/s12866-023-02836-7) and zoonotic importance of several parasite and pathogen species (10.1186/s13071-021-04592-x, 10.1016/j.ttbdis.2021.101722). In addition, our deep involvement within the study of bat/ectoparasite populations provided data for a series of results in biodiversity conservation or impact of climate-change may have (or may have in the future) on these species (two papers, both submitted and under evaluation).

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