

Research Article

Pathogen detection success and trends in lethal sampling during bacterial pathogen research in bats

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Abstract

Pathogen surveillance is crucial to understanding the current occurrence and emergence of diseases. Nevertheless, detection success can greatly depend on collection method, source of samples, and diagnostic approach. In this work, we gathered data from published literature on zoonotic bacterial pathogen sampling and detection in bats from studies conducted globally, with a focus on molecular detection methods and specific sample types (e.g., tissue, blood, guano, or urine). Additionally, we explored geographic and taxonomic patterns of lethal collection risks to bats. We found that sample type did not affect pathogen detection, with blood or urine samples from live-caught individuals being equally successful for bacterial pathogen surveillance compared to tissues from lethal sampling across several pathogenic groups. Furthermore, our data showed a strong taxonomic and geographical bias, indicating that certain bat groups and regions are most affected by lethal sampling. Overall, since non-lethal sampling appears to be as effective as invasive and lethal approaches, we recommend minimally invasive sample collection whenever possible. However, lethal sampling of bats for pathogen surveillance appears to be a common practice in published literature despite the availability of less invasive methods. An understanding of how bat collection affects populations and their conservation status is still limited. More sustainable sampling methods are a viable option of minimizing our negative impact on wild and threatened populations, while still conducting effective pathogen surveillance.

Key words: bacterial pathogen, Chiroptera, conservation, disease surveillance, lethal sampling

Pathogen surveillance in wildlife is critical for understanding disease ecology, transmission, and spread, which can help improve measures related to veterinary and public health (Watsa 2020). By facilitating the early detection of pathogens, surveillance helps identify emerging threats and forecast outbreaks that could endanger both human and animal health (Bird and Mazet 2018). Efficient surveillance is particularly critical in identifying zoonotic diseases caused by pathogens such as Filoviruses, Henipaviruses, and rabies (Shiferaw et al. 2017; Becker et al. 2019). Pathogen surveillance also supports public health preparedness, conservation efforts, and biodiversity protection while mitigating economic losses caused by disease outbreaks (Bernstein et al. 2022). Additionally, monitoring and early detection of pathogens provides insights into ecosystem health and reduces the risk of disease spillovers to livestock, domestic animals, and humans (Thumbi et al. 2019; Vora et al. 2023).

Bats have long been recognized as reservoirs and hosts for various pathogenic groups, not only those with significant zoonotic spillover but also those posing conservation threats to bat populations (Brook and Dobson 2015; Hoyt et al. 2021; Szentiványi et al. 2023a). Pathogen surveillance in bats is often biased towards viral pathogens, particularly those of zoonotic importance (Lei and Olival 2014; Szentiványi et al. 2023a). However, bats can host a wide range of pathogens that are frequently neglected during surveillance, such as bacterial

pathogens like *Bartonella*, *Borrelia*, *Leptospira*, and *Rickettsia* species whose impacts on bat populations and human health often remain uncertain (Mühldorfer et al. 2011; Mühldorfer 2013; Szentiványi et al. 2023a). Additionally, fungal pathogens such as *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, are considered emerging threats to bat populations and conservation efforts (Hoyt et al. 2016; Hoyt et al. 2021).

Several approaches are used in pathogen surveillance of wildlife, including minimally invasive methods such as swabbing, blood sampling, and collecting guano or urine, as well as the more invasive methods of lethal sampling and tissue or organ collecting (Ryser-Degiorgis 2013). Collecting voucher specimens for research is a common and often necessary procedure for faunal surveys, disease surveillance studies, and building museum collections (Russo et al. 2017; Thompson et al. 2021). However, this practice might pose an additional threat to the conservation of bat populations if not done carefully. Previous study has highlighted the problematic aspects of voucher specimen collection, in which the authors found that 14% of the studies reported euthanasia of bats including individuals assessed as critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT), and data deficient (DD) according to the IUCN Red List (Russo et al. 2017). That study proposed alternative sampling methods that can replace invasive voucher specimen

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collection with less invasive methods such as fecal sampling, wing biopsy, and blood sampling (Russo et al. 2017). Hematophagous ectoparasite sampling has also been successfully used in pathogen surveillance as a less invasive alternative to blood sampling in bats (Szentiványi et al. 2020). Still, voucher specimen collection remains essential for museum collections and accurate host identification because these samples carry valuable information for pathogen research. Proper host identification is crucial for reliably identifying reservoir species, planning targeted surveillance, and conducting eco-epidemiological studies (Thompson et al. 2021). Moreover, post-mortem tissue samples are often needed for studying the distribution of pathogens within tissues and understanding the pathology of diseases (Mühldorfer et al. 2011). Nonetheless, euthanasia might not always be necessary during research studies and could be problematic for bat conservation.

Optimizing pathogen detection methods and adopting minimally invasive collection approaches can improve the efficiency of sampling and monitoring bat-associated pathogens while reducing disturbance and stress on vulnerable bat populations. To evaluate this approach, we conducted wide-scale data collection from published studies to assess: (i) pathogen detection rates from different sample types; (ii) pathogen detection rates from individuals handled differently, including salvaged, euthanized, and released individuals; and (iii) trends in bat euthanasia practices during bacterial disease studies from 2000 to 2022. We chose to focus exclusively on potentially zoonotic bacterial pathogens, excluding viruses, as it is often a neglected area in disease surveillance and ecological studies compared to viruses (Szentiványi et al. 2023a), and bacterial detectability across sampling types has rarely been explored in the past.

Methods

Data collection.

We focused on bat-associated bacterial pathogens of zoonotic importance, including species from the following 10 genera: *Anaplasma*, *Bartonella*, *Borrelia*, *Brucella*, *Coxiella*, *Ehrlichia*, *Leptospira*, *Mycoplasma*, *Neorickettsia*, and *Rickettsia*. Data came from a previously published review (Szentiványi et al. 2023a). For estimating bat specimen collection for lethal sampling, we included studies using different detection methods, including culture, PCR, and quantitative PCR. For analyzing pathogen detectability success, we used only results obtained from molecular screening (PCR and qPCR). Data were collected from peer-reviewed articles published from 2000 through 2022 (Supplementary Data SD1). For tissue samples, we combined data (e.g., spleen, kidney, heart, or liver) because data were either reported combined or were too few to analyze each type separately. For each species, we applied the global conservation status according to the 2020 International Union for Conservation of Nature (IUCN) Red List (Red List of Threatened Species; <http://www.iucnredlist.org>).

Data analysis.

For data handling, analyses, and visualization we used R statistical software (R Core Team 2021). Data on the number of pathogens detected in the surveyed studies were structured so that the number of available records was collected for each combination of study paper, bat species, and pathogen. Analyses were done utilizing Beta-binomial generalized linear mixed-effects models (GLMMs) of the R-package “glmmTMB” (Brooks et al. 2017). Model diagnostics for the fitted GLMMs were done using the “glmmTMB” package’s “diagnose” function, as well as by visual inspection of the model residuals. In the models, the response variable was the composite of the number of positive and negative records (i.e., the number of records in which the given pathogen was present, and the number of records in which

the given pathogen was absent, respectively). The 2 main advantages of this modeling approach are that: (i) the model implicitly takes into account the number of records (i.e., an estimate of sampling effort); and (ii) the composite Beta-binomial model family allows for variation around the expected probability of success (pathogen being present), effectively controlling for over- or under dispersion in the data due to unobserved factors. Studies and bat species were included in the models as random effects. In addition, an observation-level random factor was created as well, which we used to enable us to approximate the residual variance from the fitted models. Furthermore, one can then calculate an approximation for a variance partition coefficient (VPC), using the observation-level random effect in addition to the random factors of studies and bat species. This approach makes it is possible to quantify an approximate proportion of variance in the response variable (i.e., pathogen presence probability) explained by between-study, or between-species variation; since it is a proportion, VPC can range between 0 and 1, and the larger the value, the more variance is explained by the between-group variability (e.g., large VPC for bat species indicates a large proportion of variation explained by bat species alone; Supplementary Data SD2–SD6). Notably, however, this estimate should be interpreted with caution because it is just an approximation to unexplained variation estimated by the GLMMs. Also, this approach sometimes can substantially underestimate the proportion of unexplained variation, hence inflating the proportion of variance explained by other random factors. Nevertheless, in the presented case, it is still informative when we want simply to compare the variation explained by between-study differences vs. between-bat-species differences. VPC values were calculated as:

$$VPC_{study} = \frac{V_{study}}{V_{study} + V_{bat\ sp.} + V_{residual}},$$

$$VPC_{bat\ sp.} = \frac{V_{bat\ sp.}}{V_{study} + V_{bat\ sp.} + V_{residual}},$$

where V_{study} , $V_{bat\ sp.}$, and $V_{residual}$ are the random intercept variances between studies, bat species, and the approximate residual variance, respectively.

To test the effects of sampling source, a Beta-binomial GLMM was fitted on the number of positive (given pathogen being present) and negative (given pathogen being absent) observations, in which fixed predictors were the pathogen taxon and the sampling source (blood, tissue), also controlling for the two-way interaction between them. Testing the difference between tissue and urine samples was only possible in the *Leptospira* taxon due to the sparsity of observations on urine samples in the data; therefore, we fitted a separate GLMM on a subset of the data containing tissue and urine samples from *Leptospira*.

When testing the effect of bat handling (whether a bat was euthanized, released, or salvaged in the study) separate GLMMs were fitted for each pathogen because there was substantial variation in the records of bat handling across the available studies, leading to sparsity in the observations rendering it difficult to use a large model with an interaction term between pathogen taxon and bat handling methods. In other words, the number of records for specific combinations varied substantially, and some combinations were rank-deficient. Estimated marginal mean (EMM) probability of the surveyed pathogens to be present in the samples, as well as the estimated marginal differences (contrasts: EMC) between method groups (e.g., blood vs. tissue in sample source) were extracted using the R-package “emmeans” (Lenth 2022). Data visualization was performed in R 4.0.3, using the packages, “ggplot2”, “maturalearth”, “leaflet”, and “ggspatial” (Wickham and Chang 2016; South 2017; Cheng et al. 2021; Dunnington and Thorne 2021).

Results

Pathogen detection comparison from different sample sources.

We compared the pathogen detection rate of all individuals (including released, salvaged, and euthanized) in the dataset based on the sample collection source (i.e., blood, tissue, and urine) used during surveillance. We found no significant difference in pathogen detection success between blood and tissue samples in the case of *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Mycoplasma*, and *Rickettsia* testing (Fig. 1; Table 1). Furthermore, no significant difference was found between urine and tissue samples during *Leptospira* testing (Table 1).

Pathogen detection rate from euthanized, released, and salvaged individuals.

We compared pathogen detection rates between euthanized, released, and salvaged individuals across all tested bats. No difference was found in pathogen detection success between euthanized and released individuals (Fig. 2; Table 2). For *Rickettsia* sampling, salvaged individuals had a significantly higher infection rate compared to euthanized and released individuals.

Distribution of euthanized individuals between bat families and IUCN categories.

A total of 18,975 bacterial pathogen tests were performed in 103 published studies. The reported tests were associated with the following bat handling categories, not excluding multiple tests that were done on the same individual: euthanized ($n=9,279$, 48.9%); released ($n=5,306$, 27.9%); unknown/not reported ($n=2,854$, 15.0%); found dead/salvaged ($n=1,258$, 6.6%); and not captured (non-invasive sampling, e.g., guano; $n=278$, 1.4%). A total of 8,264 bats were reported as euthanized (excluding multiple tests done on the same individual),

with the following categories based on their IUCN threat status: Least Concern (LC, $n=6,079$, 73.6%); Near Threatened (NT, $n=1,031$, 12.5%); Vulnerable (VU, $n=290$, 3.5%); Data Deficient (DD, $n=295$, 3.6%); and Endangered (EN, $n=81$, 1%; Fig. 3). Additionally, the threat status of 5.9% ($n=488$) of the euthanized individuals could not be determined because species level identifications were missing or the species has not yet been assessed by IUCN (Fig. 3). We did not find bats classified as Critically Endangered (CR) among the euthanized individuals.

Euthanized bats came from 14 bat families and 204 species. The 3 most frequently collected families were Phyllostomidae ($n=2,380$, 28.8%), Pteropodidae ($n=1,712$, 20.7%), and Vespertilionidae ($n=1,459$, 17.7%) (Fig. 3). The most frequently collected species were *Eidolon helvum* ($n=724$, NT), *Desmodus rotundus* ($n=390$, LC), and *Myotis davidii* ($n=338$, LC). The highest proportion of euthanized individuals from an imperilled group was reported from the family Pteropodidae, with about 71.1% of individuals belonging to NT or VU categories (Fig. 3; Supplementary Data SD1).

Spatial distribution of euthanized individuals.

Geographic sampling was relatively broad—reported bacterial pathogen studies with euthanized bats from 2000 to 2020 came from 25 countries (Fig. 4). By continent, reports came from South America ($n=2,665$, 32.2%), Africa ($n=2,414$, 29.2%), Asia ($n=2,010$, 24.3%), North America ($n=770$, 9.3%), Australia and Oceania ($n=270$, 3.3%), and Europe ($n=135$, 1.6%; Fig. 4).

Discussion

Even though bats are considered important members of ecosystems as seed dispersers, flower pollinators, and predators of insect pests, they are under continuing and emerging threats worldwide, with

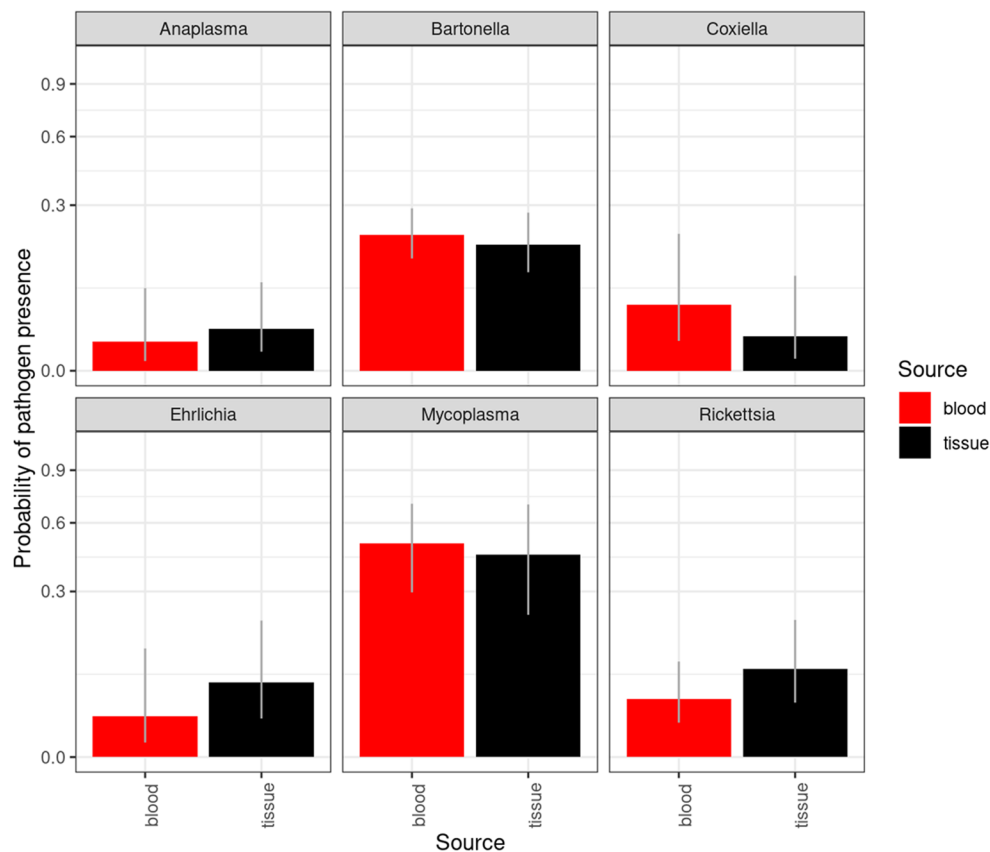


Fig. 1. GLMM-estimated bacterial pathogen detection probabilities ($\pm 95\%$ confidence intervals) from bat blood (red) and tissue (black). See additional details in Table 1 and Supplementary Data SD1.

about 18% of all species deemed to be threatened according to the IUCN Red List (Frick et al. 2020). Additionally, about 15% of bats are categorized as “data deficient,” meaning that information is lacking about population sizes, distributional ranges, and basic biology. Furthermore, about 57% of described bat species currently have unknown population trends (Frick et al. 2020). Threats that negatively impact bat populations include habitat loss, global warming, insecticide use, and predation by domesticated animals such as cats (Ancillotto et al. 2013; Frick et al. 2020; Oedin et al. 2021; Szentivanyi et al. 2023b). Most of these threats are considered direct or indirect results of anthropogenic activities. Intentional culling of bats appears to be the third most common reason that results in the death of a huge number of animals, after wind turbine-caused deaths and mortality by white-nose syndrome (O’Shea et al. 2016). Culling of bats seems to be decreasing since 2000; however, it is still a common practice in certain areas, such as in Brazil for rabies control and in Mauritius for crop protection, despite being proven to be ineffective in both cases (Stoner-Duncan et al. 2014; Florens and Baider 2019).

Table 1. Estimated marginal contrasts in pathogen detection between different sample types.

Pathogen	Sample type	SE	Z ratio	P value
<i>Anaplasma</i>	Blood/tissue	0.6366	−0.55605	0.578
<i>Bartonella</i>	Blood/tissue	0.4478	0.50057	0.616
<i>Coxiella</i>	Blood/tissue	5.0663	1.00614	0.314
<i>Ehrlichia</i>	Blood/tissue	0.3565	−1.00432	0.315
<i>Leptospira</i>	Urine/tissue	1.0917	0.47036	0.638
<i>Mycoplasma</i>	Blood/tissue	0.8481	0.30028	0.763
<i>Rickettsia</i>	Blood/tissue	0.3036	−1.20409	0.228

Continuous disease surveillance is crucial to recognize conservation threats to host populations and to predict and prevent the spill-over of zoonotic wildlife diseases to humans, livestock, and domestic animals. Various surveillance methods have been developed throughout the past decades, some of which are highly invasive and therefore might further affect negatively the conservation efforts of the targeted species. Non-invasive sampling of wildlife for pathogen surveillance has been on the rise in the past 2 decades, including the use of environmental DNA, non-invasive blood or guano sampling, or using camera-trap photos to detect signs of diseases (Soto-Azat et al. 2009; Acevedo-Whitehouse et al. 2010; Vos et al. 2010; Oleaga et al. 2011; Evans et al. 2015; Szentiványi et al. 2020; Bajić et al. 2023; Walker et al. 2024). Generally, non-lethal sampling methods are preferred to prioritize host conservation whenever possible, especially in the case of threatened species. Blood sampling and swabbing might be viable alternatives to tissue sampling during pathogen surveillance; however, occasionally these can be operationally difficult to obtain from bats during fieldwork. For instance, while blood samples can be used to detect bacteria, the volume of blood required may exceed what can be safely taken from small individuals, and blood drawn from bats are technically challenging. It is possible that in some cases, detecting bacteria in the animal might only be possible after euthanasia. Furthermore, researchers are required to meet animal care standards, which may prioritize minimizing individual animal suffering over evaluating the broader effects of research on population trends. This focus may, in some cases, justify lethal sampling. However, researchers should carefully assess the risks at both individual and population levels when estimating harm and animal welfare. Field wildlife anesthesia could offer a safer alternative, potentially supporting non-lethal sampling while reducing stress on the animals (Soulsbury et al. 2020). Overall, our results indicate that

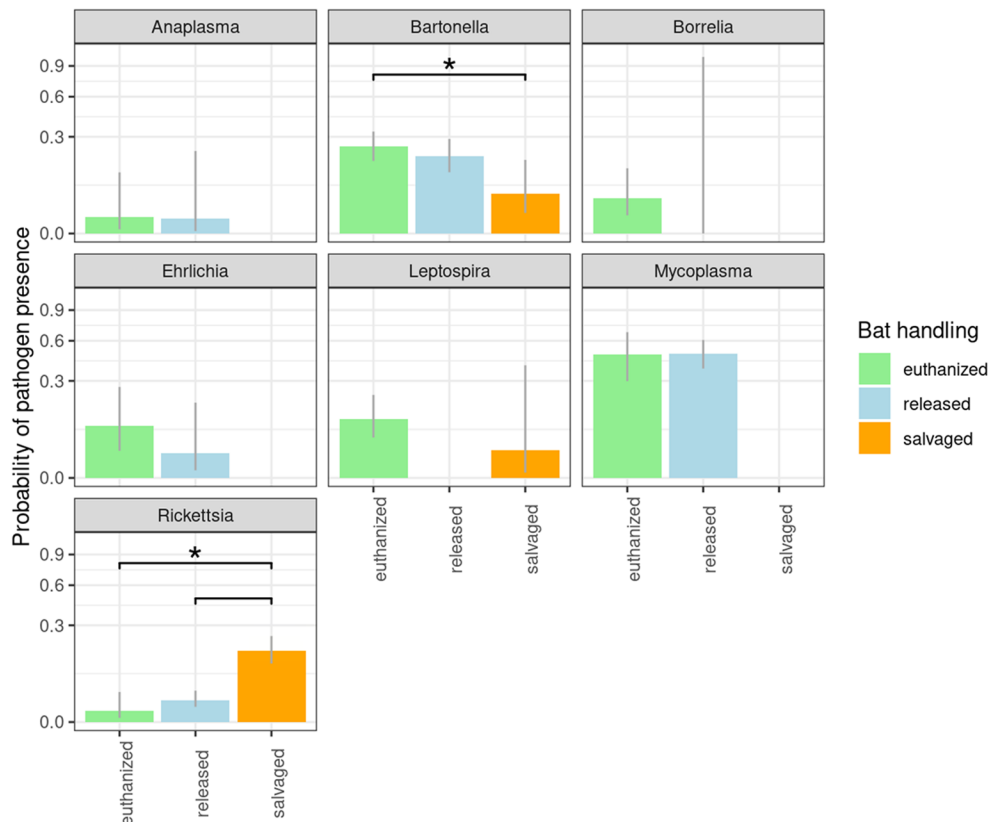


Fig. 2. GLMM-estimated bacterial pathogen detection probabilities (±95% confidence intervals) from bats undergoing different sampling treatment, that is, euthanized (green), released after sampling (blue), and salvaged or naturally deceased (orange). See additional details in Table 2 and Supplementary Data SD1.

Table 2. Estimated marginal contrasts in pathogen detection between different sample types.

Pathogen	Sample type	SE	Z ratio	P value
Bartonella	Euthanized/released	0.485365	0.856169	0.667
	Euthanized/salvaged	4.271529	2.46819	0.036 ^a
	Released/salvaged	3.204706	2.005925	0.110
Borrelia	Euthanized/released	2368875	0.142902	0.886
Ehrlichia	Euthanized/released	5.965564	1.171802	0.241
Leptospira	Euthanized/salvaged	8.66868	0.93279	0.350
Mycoplasma	Euthanized/released	0.455271	-0.06612	0.947
Rickettsia	Euthanized/released	0.281654	-1.24541	0.426
	Euthanized/salvaged	0.021892	-3.69116	<0.005 ^a
	Released/salvaged	0.036403	-5.54488	<0.005 ^a

^aSignificant P values at $\alpha = 0.05$ are denoted by an asterisk.

there was no significant difference in pathogen detection between blood samples and tissue samples for several pathogens, including *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Mycoplasma*, and *Rickettsia* species. To our knowledge, only a few studies have assessed pathogen detection success from different tissue types in bats. One study demonstrated that *Leptospira* detection is generally more successful from kidney tissue by PCR when compared to urine or kidney culture or blood serum PCR in small mammals, including bats (Tulsiani et al. 2011a; Tulsiani et al. 2011b). Overall, more studies are needed to explore reliable alternative sampling methods, especially those that are more sensitive than lethal sampling methods.

Detection of pathogens from naturally deceased individuals is an alternative for disease surveillance (Szekeres et al. 2019; Corduneanu et al. 2021), though after death, the microbiome undergoes rapid changes that might alter pathogen detection success and therefore do not necessarily reflect the presence of pathogens circulating in natural populations. *Rickettsia* had the highest detectability rate in naturally deceased individuals, which might indicate that these pathogens are persistent and easy to detect after death, or their presence might have been the cause of death. Nevertheless, the impact of these pathogens on mortality events in bats is unclear. Overall, pathogen surveillance appears to be as successful from released or salvaged individuals as from euthanized bats; therefore, non-lethal sampling methods might be preferred during disease surveillance studies.

From our collation of literature, it is often unclear whether euthanized individuals are collected solely for the purpose of scientific research and disease surveillance or if researchers take the opportunity to explore pathogen diversity in already culled individuals. In the latter case, disease surveillance could benefit from reusing these samples. Nevertheless, culling solely for population management or pathogen-spread reduction has been shown to be unsuccessful (Streicker et al. 2012; Florens and Baider 2019) and therefore should be carefully evaluated in the future and regarded primarily as a policy issue.

Overall, some patterns can be recognized in the literature regarding bat euthanasia, even though the purpose of mass culling is not always evident. One of the most frequently euthanized groups was the Old World fruit bats, family Pteropodidae, which also showed an exceptionally high proportion of threatened bats that have been collected, with 71% of the euthanized individuals belonging to one of the threatened IUCN categories. We found that the most commonly euthanized bat species in our study was the Straw-colored Fruit Bat, *E. helvum*. This species is endemic to Africa and has a wide geographical distribution, though it has been categorized as near threatened on the IUCN Red List and has a declining population trend (Cooper-Bohannon et al. 2020). *Eidolon helvum* has been under rigorous disease surveillance because it is hunted and consumed in certain

rural areas (Mickleburgh et al. 2009), and it is an assumed or confirmed reservoir host of several zoonotic and potentially zoonotic viruses, including the Zaire ebolavirus and Marburg virus, respectively (Hayman et al. 2010; Olival and Hayman 2014; Ogawa et al. 2015). Nonetheless, its role in zoonotic pathogen spillover has not been clarified until recently (Weber et al. 2023). Old World fruit bats have been under increased anthropogenic threats because hunting disproportionately affects them when compared to other bat families, with about half of the known species being actively hunted for consumption or used in traditional medicine (Jenkins and Racey 2010; Mickleburgh et al. 2009). Human-wildlife agricultural conflict also contributes to these threats—for instance, the endangered Mauritius Flying Fox, *Pteropus niger*, has been the target of mass culling due to crop protection efforts; however, it has been proven to be an unsuccessful method to protect agricultural products (Florens and Baider 2019). Furthermore, insular bat species, mostly represented by pteropodids, are especially vulnerable to anthropogenic and natural threats such as the introduction of invasive species and severe weather phenomena (Jones et al. 2009; Welch and Leppanen 2017). Hence, less invasive sample collection methods should be strongly prioritized during pathogen surveillance when working with this group.

The second most frequently euthanized bat species in our dataset was the Common Vampire Bat, *D. rotundus*. This species has received a negative perception due to its ability to transmit rabies to humans and domestic animals (Streicker et al. 2012). Annually, large numbers of vampire bats are culled for rabies control and surveillance. However, estimating the exact number of culled bats is difficult as most of these remain unreported. The success of controlling rabies spillover by mass culling of vampire bats has been called into question, with data suggesting that culling does not reduce seroprevalence in the host population, and rabies exposure might even increase after population reduction efforts (Streicker et al. 2012). Nonetheless, it is important to protect humans and livestock from virus exposure, and new advances in the management of rabies in bats are currently under development, including reproductive control and oral vaccines for bats (Bakker et al. 2019; Gonçalves et al. 2021; Griffiths et al. 2023). Making use of policy-driven mass culling could benefit disease surveillance, and in the case of vampire bats, collection pressure during surveillance appears to be less of a concern. Lastly, spatial differences were also present in this practice; however, government and policy-driven mass culling of bats for disease control and non-restrictive or uncontrolled collection practices likely explain these patterns, although we caution that only a few countries per continent were represented in our dataset.

Re-using non-lethally collected samples to answer a wide variety of research questions, as well as retesting them for the presence of more than 1 pathogen, could be a more sustainable practice when considering host conservation. It could be especially feasible during bacterial pathogen surveillance because bacterial DNA is generally more stable when compared to viral genetic material and, therefore, easier to detect in a wide variety of samples. Yet, even viral and viral antibody detection can be highly successful using samples collected non-lethally, such as from blood, feces, urine, and saliva samples when stored properly (Young and Olival 2016).

Museums are inarguably crucial for specimen preservation for future studies; however, bats from mass euthanasia do not necessarily end up in museum collections. We suggest the deposition of euthanized individuals in public and accessible collections and adding this information to published papers and reports as well. These efforts will help future studies to avoid re-sampling the same areas

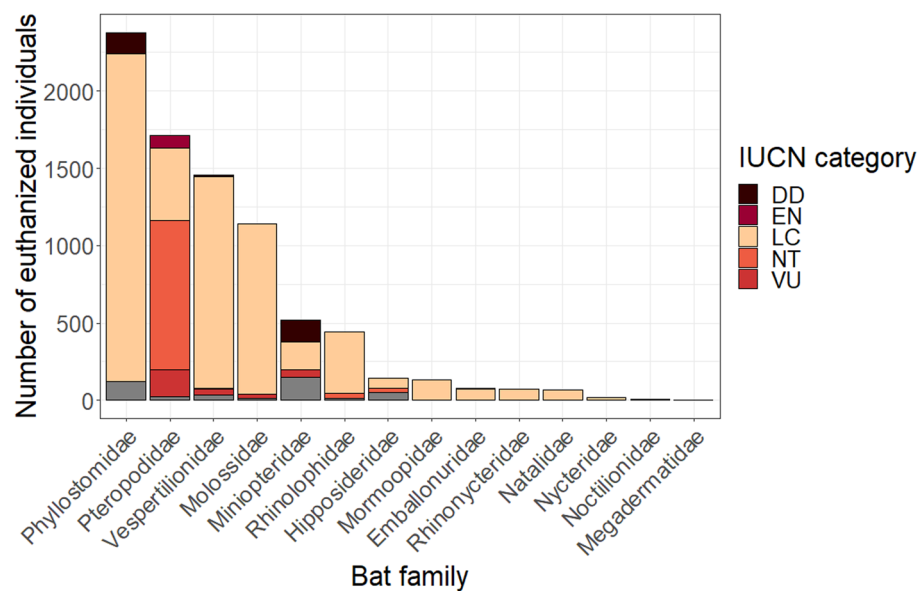


Fig. 3. Number of euthanized individuals reported in bacterial pathogen research studies 2000 to 2022. IUCN threat status includes DD (Data Deficient), LC (Least Concern), EN (Endangered), NT (Near Threatened), VU (Vulnerable), and NA (species has not been assessed by IUCN or species level not reported in study, therefore threat status cannot be determined).

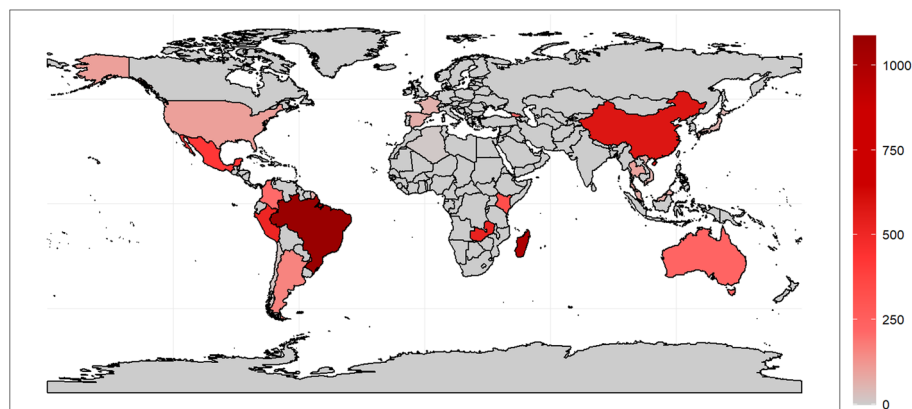


Fig. 4. Global geographic distribution of lethal bat specimen collection for bacterial pathogen studies by country in published articles from 2000 to 2020. Colored scale indicates number of individuals collected.

and species, saving funding and research effort. Additionally, deposited samples can help to detect pathogen presence and emergence retroactively. For instance, *P. destructans*, the causative agent of white-nose syndrome, was detected on a museum specimen in 2006, long after its collection in 1918 from France (Campana et al. 2017).

In previous work, it has been reported that 13% of the studies collecting voucher specimens were due to disease research in bats (Russo et al. 2017). Yet, it is important to keep in mind that most disease studies done on bats are typically related to virological surveillance, therefore bacterial research represents only a small proportion of lethally collected individuals when compared to viral surveillance. Nevertheless, even for bacterial research, we found that about half of all tested individuals in published studies had been euthanized. If we multiply this number by the proportion of the viral studies compared to bacterial studies, the number of euthanized bats is expected to be several times higher. Overall, the actual number of euthanized bats for research purposes is currently unknown.

In conclusion, we urge researchers to consider less invasive sampling methods and to evaluate the necessity of lethal sampling, when it is carried out solely for disease surveillance and is unrelated to policy-driven culling. As we have shown here, non-invasive collection methods appear to be as efficient for bacterial pathogen collection

across a wide range of pathogens as lethal methods, including tissue samples. If lethal sampling is unavoidable, we suggest limiting the number of individuals and depositing and publishing the accessibility of this material along with their results. Yet, we strongly suggest prioritizing non-lethal sampling methods and only collecting animals when it is necessary. Furthermore, we strongly recommend minimizing the sampling of animals classified as threatened, vulnerable, endangered, or critically endangered by the IUCN. The collection of data-deficient animals should be strongly limited as well because it might threaten unknown vulnerable populations.

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Author contributions

Tamara Szentiványi (Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Validation, Visualization, Writing—original draft, Writing—review & editing), Zoltan Rádai (Formal analysis, Investigation, Methodology, Visualization,

Writing—review & editing), and Muriel Dietrich (Investigation, Validation, Writing—review & editing),; Jeffrey T. Foster (Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing—review & editing)

Supplementary data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1. Infection status of bats reported in the literature, including the detected pathogens, molecular surveillance methods used, and country of sampling.

Supplementary Data SD2. Prevalence of each tested pathogen reported across studies, based on different detection methods.

Supplementary Data SD3. Table of model coefficients for each tested pathogen.

Supplementary Data SD4. Table of contrasts for each handling type during pathogen surveillance.

Supplementary Data SD5. Table of contrasts for each surveillance methods during pathogen surveillance.

Supplementary Data SD6. Table of contrasts for each source of sample during pathogen surveillance.

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Conflict of interest

None declared.

Data availability

The dataset and statistical analysis supporting the results and conclusions of this work are included within the article and in the additional files (Supplementary Data SD1–SD6).

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