

Exploring the presence of coronavirus in
bats and in the air from bat caves in
Portugal: a one health approach
Mahima Hemnani

D

2025



2025



D.I.C.B.A.S

Mahima Hemnani. Exploring the
presence of coronavirus in bats and in the
air from bat caves in Portugal: a one
health approach

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR



Mahima Hemnani

Exploring the presence of coronavirus in bats and in the air from bat's caves in Portugal: a one health approach

Thesis submitted for a Doctoral Degree in Veterinary Sciences

Doctoral Programme of the University of Porto (School of Medicine and Biomedical Sciences).

Supervisor: **João Rodrigo Gonçalves Goiana Mesquita**

Category: Associate Professor

Affiliation: School of Medicine and Biomedical Sciences (ICBAS), Porto University

Co-supervisor – **Hugo Emanuel Vitorino Rebelo**

Category – Auxiliar Professor

Affiliation – Faculty of Sciences, Lisbon University

Co-supervisor – **Patrícia Alexandra Curado Quintas Dinis**

Poeta

Category – Full Professor

Affiliation – Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro

Co-supervisor – **Gertrude Averil Baker Thompson**

Category – Associate Professor

Affiliation – School of Medicine and Biomedical Sciences (ICBAS), Porto University

Acknowledgments

Início esta seção agradecendo ao meu eterno orientador Prof. Dr. Marcelo Menin (*in memoriam*), vitimado pela pandemia da COVID-19, que foi e sempre será amado por todos! Agradeço por todos os ensinamentos durante a graduação e o mestrado e que me ajudaram a chegar a este doutoramento.

Ao meu orientador, Prof. Dr. João Mesquita, agradeço imensamente por todo o apoio que sempre me proporcionou, pela orientação, pela compreensão, pela liberdade intelectual, pelo encorajamento, e por ter acreditado no meu trabalho desde a primeira vez que entrei no laboratório. Apesar de todos os percalços ocorridos durante o doutoramento, você sempre tentou me ajudar da melhor forma possível, não só procurando soluções, mas principalmente nunca desistindo de mim! Muito obrigada por tudo.

Ao meu coorientador Prof. Dr. Hugo Rebelo, muitíssimo obrigada por tudo, pelo suporte e colaboração em muitos pontos do desenvolvimento deste trabalho, pelo feedback constante ao longo da pesquisa, por todas as conversas aleatórias durante o campo, por todo o encorajamento para eu entrar nas cavernas e por todo o conhecimento que compartilhou comigo sobre os morcegos e sobre a história de Portugal. Fazer campo com você e coletar morcegos com certeza está entre as minhas melhores experiências que tive em Portugal.

As minhas coorientadoras, Prof. Dra. Patrícia Poeta e Prof. Dra. Gertrude Thompson, toda a minha gratidão por investir tempo na minha formação científica e por todo o apoio ao longo desta jornada.

A Fundação para a Ciência e a Tecnologia (FCT) pelo financiamento das atividades de pesquisa e pela bolsa de estudos concedida a mim (Ref. 2021.09380.BD).

Agradeço imensamente aos meus pais, Jasmine e Dev, por sempre apoiarem meus sonhos e estudos. Se não fosse por acreditarem que a educação é a maior herança que pode ser deixada a um filho, eu não estaria aqui. Obrigada por me mostrarem, com exemplos e gestos, que desistir nunca foi e nunca será uma opção. Nos momentos em que pensei em desistir do doutoramento, por ter mudado de país, de área de pesquisa e por enfrentar tantas transformações ao mesmo tempo, foram vocês que me lembraram da minha força e me encorajaram a continuar. Sem esse apoio constante, eu não teria encontrado coragem para seguir em frente. Eu sei que não sou a filha mais fácil do mundo, e justamente por

isso admiro ainda mais a forma como vocês nunca deixaram de lutar por mim e comigo. Vocês me ensinaram a sonhar alto, a acreditar que o impossível pode ser alcançado, e espero levar esse ensinamento comigo para sempre, em tudo o que eu fizer. Este trabalho, assim como cada conquista da minha vida, sempre terá um pedaço de vocês. Espero, um dia, conseguir deixá-los orgulhosos de mim e ser ao menos metade da força que vocês são.

À minha querida irmãzinha mais nova, Jordana Maria (ou Jogan), que toda vez tem que me aguentar quando estou querendo “surtar” e com isso acabar brigando com todo mundo. Ela é única, pois me atura de uma forma inimaginável. Ela é minha melhor amiga, meu guia, minha confidente e sem ela eu não teria mais sanidade mental (ou um trabalho constantemente corrigido diz ela). Obrigada por todas as conversas intermináveis, por me ouvir sem (com) julgamentos, por me fazer sentir perto mesmo quando estamos longe. Obrigada por cuidar de mim de um jeito silencioso e profundo, como só você sabe fazer. Eu tenho muito orgulho de quem você é e de tudo o que ainda vai conquistar. A sua presença é um dos maiores presentes que a vida me deu, e eu carrego comigo todos os dias a sorte de poder chamar você de irmã.

Não poderia deixar de agradecer à minha filha gatinha Zara Maria, que esteve presente em tantas longas noites de escrita. Sempre ao meu lado, do jeitinho dela, me apoiou, mesmo que isso significasse receber uma chuva de beijos e carinhos em troca (algo que ela odeia). Sua companhia fez cada noite mais leve e aconchegante, e me fazendo lembrar que o amor mais simples e inocente é o que nos fortalece.

Um agradecimento especial à Priscilla, que há cinco anos resolveu vir falar comigo no Twitter, quando percebeu minha vontade de estudar fora. Desde então, você esteve presente em todos os momentos e pouco a pouco, quando se tornou uma amiga tão essencial que já não sei viver sem. Eu me inspiro demais em você e na sua força de conquistar tudo aquilo que deseja. Mas não se esqueça: para mim, você sempre será a menina do fichário rosa.

Obrigada também à Luísa por ser essa amiga incrível, pelas risadas e choros no laboratório, por ser minha parceira de ginásio e fora dele, seja para irmos às compras ou para ficarmos vendo os meninos bonitos do TikTok; Sérgio por ter me ajudado no início, quando eu não sabia nem segurar uma pipeta, por me passar todos os seus ensinamentos e por repetir a mesma frase mil vezes até que meu ouvido se acostumasse ao sotaque tuga; Sara por ser essa amiga indispensável, por baixar livros para mim, por me ajudar em

praticamente tudo o que preciso e por estar sempre ao meu lado; Guilherme e Jaqueline, por todas as risadas e até pelo bullying, que eu sei que no fundo é sempre cheio de amor e ternura; Joana por aguentar meus surtos no lab, cantarolar Luan Santana comigo e dividir todos os desabafos que só uma amiga como você entende. Obrigada a todos pelas idas ao café constantes, pelas idas à adega e por tudo em geral. Vocês fazem meus dias no laboratório muito mais leves e felizes.

Aos meus amigos que tornaram meus anos em Portugal inesquecíveis — Aline, Bianca, Fon, Heverton, Kizzy, Leo, Luna, Rafaela, Sia e Patrick. Obrigada por sempre estarem do meu lado, nos momentos tristes e felizes, entre risadas e choros. Aline, minha amiga “alcoólatra”, inimiga do fim como eu. Nossa amizade começou do nada, mas agora não vivo sem você. Bianca, minha primeira amiga em Portugal. Mesmo depois de se mudar para Lisboa e agora para a Irlanda, nada entre a gente mudou: seguimos unidas e sempre apoiando uma à outra. Fon e Leo, meus amigos das trevas, que conseguiram gostar de morcegos ainda mais do que eu. Vocês são únicos e quero vocês comigo para a vida inteira. Heverton, um amigo maravilhoso, sempre presente, seja em conversas sérias ou nas nossas conversas mais aleatórias. Kizzy, minha personal trainer particular e aquela amiga que sempre tem o conselho certo na hora certa e com o vinho ou o café certo. Luna, que iluminou minha vida em tantos momentos e tornou os dias mais leves só por estar presente. Rafaela, minha amiga grossa e fofa ao mesmo tempo, que no fundo é uma das pessoas mais amorosas que conheço. Sia, que esteve comigo na minha jornada de autodescoberta e me deu forças quando eu mais precisei. Patrick, meu amigo conterrâneo que sempre sabe me fazer rir, pelos rolês aleatórios pelo Porto e por todos os ensinamentos (e risadas) que só você sabe proporcionar. Vocês são minha família escolhida em Portugal, e tenho a maior sorte do mundo de ter cada um de vocês na minha vida.

Aos meus amigos de Manaus: Rubia, não tenho palavras para descrever o quão importante você é para mim. Dez anos de amizade e nada nos separa! Se um dia você ficar brava comigo, vai dormir que passa! Ainda me impressiona como passei quatro anos longe de você e, mesmo assim, sempre estive perto. Obrigada por estar comigo, mesmo que seja só para fazermos vários “nadas”, por, no início, ficar horas ao telefone me ouvindo chorar e me dando forças, por todas as risadas aleatórias, e por me fazer sentir especial como ninguém. Você é minha alma gêmea de melhor amiga, e já percebi que nem a distância consegue nos separar. Tio Pedroca (Pedro das cobras), obrigada por ser aquele amigo que, mesmo se não conversarmos por dias, está sempre presente quando eu preciso, me fazendo rir e compartilhando do mesmo neurônio que eu. Joana, minha gêmea na

Austrália, que mesmo longe nunca deixou de estar presente. Amo como sempre nos apoiamos, compartilhamos nossos momentos e nos entendemos mesmo à distância, parece que nada muda entre a gente, e tenho muita sorte de ter você na minha vida. Vocês me mostraram que amizade verdadeira não é sobre falar o tempo todo, mas sobre estar lá quando importa.

Por fim, aos meus avós. A minha avó por me guiar e me apoiarem mesmo estando distantes. Em especial, ao meu avô. Saudades de você. Sei que está cuidando de mim onde quer que esteja.

Agradeço a todas as forças, divinas ou não, que possibilitaram a conclusão com êxito desse trabalho. Por fim, muito obrigada a todos que colaboraram com essa jornada científica e humanística. Suas contribuições nunca serão esquecidas.

Table of Contents

<i>Acknowledgments</i>	<i>iii</i>
<i>List of abbreviations</i>	<i>viii</i>
<i>List of figures</i>	<i>ix</i>
<i>List of publications</i>	<i>x</i>
<i>Abstract</i>	<i>xi</i>
<i>Resumo</i>	<i>xiii</i>
Chapter 1- General Introduction	15
1.1.1 Bat Diversity and Unique Biological Traits.....	17
1.1.2 Bats as Natural Reservoirs of Viruses	21
1.1.3 Environmental Change, Zoonotic Spillover, and Bat Conservation Challenges.....	24
1.2. Coronaviruses	27
1.2.1. Overview of Coronaviruses	27
1.2.2. Coronavirus genome and structure	27
1.2.3. Taxonomy of Coronavirus	30
1.3 Bats and the Origin of Coronavirus	35
1.3.1 Presence of CoVs in European bats	36
<i>References</i>	<i>40</i>
<i>Scientific aims</i>	<i>69</i>
Chapter 2- First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal	70
Chapter 3- Presence of Alphacoronavirus in Tree- and Crevice-Dwelling Bats from Portugal	92
Chapter 4- Metagenomic Analysis of Viral Diversity in Portuguese Bats	115
Chapter 5- General Discussion and Conclusions	172
General discussion	173
Conclusions	213

List of abbreviations

APN: Aminopeptidase N

COVID-19: Coronavirus Disease 2019

CoVs: Coronaviruses

ENSO: El Niño–Southern Oscillation

HSPs: Heat-Shock Proteins

MERS: Middle East Respiratory Syndrome

NGS: Next-Generation Sequencing

ORF: Open Reading Frame

PEDV: Porcine Epidemic Diarrhea Virus

PP1a/PP1ab: Polyprotein 1a/ Polyprotein 1ab

RBD: Receptor Binding Domain

RdRp: RNA-Dependent RNA Polymerase

ROS: Reactive Oxygen Species

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

SARS: Severe Acute Respiratory Syndrome

WHO: World Health Organization

List of figures

Figure 1: Schematic representation of the CoV virion structure. highlighting key structural proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. This diagram was created using BioRender 23

Figure 2: Schematic representation of the four recognized CoV genera, respective subgenera, and their associated host ranges according to ICTV. The relationships shown do not reflect actual phylogenetic distances or topology and were created using BioRender for illustrative purposes. Icons indicate common host taxa associated with each subgenus based on current knowledge 29

Figure 3: Bats genera that were found carrying CoVs in European countries..... 32

List of publications

This dissertation is based on research published in international SCI-indexed journals and presented at scientific meetings. In accordance with the regulations of *Regulamento Geral dos Terceiros Ciclos de Estudos* of the University of Porto, the author participated in the conception, execution, data analysis, and manuscript writing. The included full manuscripts are original to this thesis.

Review articles:

1. Hemnani, M., da Silva, P. G., Thompson, G., Poeta, P., Rebelo, H., & Mesquita, J. R. (2024). Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *EcoHealth*, 21(2), 125-140. <https://doi.org/10.1007/s10393-024-01688-5>

Research articles:

1. Hemnani, M., Silva, P. G. D., Thompson, G., Poeta, P., Rebelo, H., & Mesquita, J. R. (2023). First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses*, 15(7), 1521. <https://doi.org/10.3390/v15071521>
2. Hemnani, M., da Silva, P. G., Thompson, G., Poeta, P., Rebelo, H., & Mesquita, J. R. (2024). Presence of Alphacoronavirus in Tree-and Crevice-Dwelling Bats from Portugal. *Viruses*, 16(3), 434. <https://doi.org/10.3390/v16030434>
3. Hemnani, M., Karatas, M., Cruz, A. V., da Silva, P. G., Thompson, G., Poeta, P., ... & Mesquita, J. R. (2025). Metagenomic analysis of viral diversity in Portuguese bats. *Veterinary Research Communications*, 49(6), 319. <http://dx.doi.org/10.1007/s11259-025-10888-5>

Abstract

This doctoral thesis, entitled "**Exploring the presence of coronavirus in bats and in the air from bat caves in Portugal: a one health approach**" investigates the complex interplay between bats, coronaviruses (CoVs), and the environment within an One Health framework. The objectives were to understand the presence, diversity, and distribution of CoVs in Portuguese bat populations while also assessing the potential for environmental circulation of these viruses in bat roosting sites, thereby contributing to the understanding of viral maintenance, transmission, and potential zoonotic spillover at the human-animal-environment interface. To achieve these aims, the study adopted a multi-layered methodology that combined fieldwork, molecular biology, bioinformatics, and *in silico* modelling. Field sampling encompassed a wide geographical range across central and southern Portugal and targeted bats roosting in ecologically distinct habitats, including caves, trees, and crevices. Biological samples (oral and rectal swabs, faeces, and blood) were collected alongside environmental air samples within cave systems to evaluate both direct host-associated viral presence and airborne transmission potential. Molecular detection strategies included conventional and real-time PCR screening for coronaviruses, complemented by metagenomic next-generation sequencing to capture the broader viral diversity present. In addition, *in silico* protein docking analyses were performed to examine the binding interactions between the spike proteins of the discovered bat CoVs and aminopeptidase N (APN) receptors from different host species (bats, pigs, and humans), with the aim of inferring possible cross-species transmission risks. Key findings revealed the circulation of AlphaCoVs in Portuguese bat populations, with a clear habitat-associated gradient in CoV prevalence, indicating higher prevalence in cave-dwelling bats (8.87%) compared to tree- and crevice-dwelling bats (1.15%). While CoVs were detected in bat samples, they were not found in environmental air samples, suggesting the need for longer sampling periods and improved methodologies for airborne virus detection. Notably, most positive detections occurred in faecal samples (nine), compared to a single positive buccal swab and one positive anal swab, suggesting gastrointestinal replication and a potential faecal–oral transmission route, which can somehow explain why no bat CoVs were found in air samples. The study also uncovered significant viral diversity beyond CoVs, including picornaviruses and mastadenoviruses, highlighting the rich gut virome of these animals. Protein docking studies indicated strong binding affinity of bat CoV spike protein with bat APN, but also comparable affinities with human and pig APNs, suggesting a potential for cross-species transmission, although further experimental validation is required. In conclusion, this thesis provides crucial

insights into the ecology and diversity of bat-borne viruses in Portugal, reinforcing the role of bats as important reservoirs for a wide range of viruses. The findings underscore the importance of continued surveillance and research to monitor viral dynamics, assess associated risks to public health and wildlife conservation, and inform strategies for mitigating potential zoonotic outbreaks within the One Health paradigm.

Resumo

Esta tese de doutoramento, intitulada '*Exploring the presence of coronavirus in bats and in the air from bat caves in Portugal: a one health approach*', investiga a complexa interação entre morcegos, coronavírus (CoVs) e o ambiente no âmbito do paradigma *One Health*. Os objetivos foram compreender a presença, diversidade e distribuição de CoVs em populações de morcegos em Portugal, avaliando igualmente o potencial de circulação ambiental destes vírus em abrigos de morcegos, contribuindo assim para a compreensão da manutenção viral, das vias de transmissão e do possível *spillover* zoonótico na interface humano–animal–ambiente.

Para atingir estes objetivos, o estudo adotou uma metodologia multidimensional que combinou trabalho de campo, biologia molecular, bioinformática e modelação *in silico*. A amostragem de campo abrangeu uma vasta área geográfica do centro e sul de Portugal e teve como alvo morcegos a utilizar habitats ecologicamente distintos, incluindo cavernas, árvores e fendas. Foram recolhidas amostras biológicas (zaragatoas orais e retais, fezes e sangue), bem como amostras de ar ambiental no interior de sistemas cársticos, com o objetivo de avaliar tanto a presença viral diretamente associada ao hospedeiro como o potencial de transmissão aérea. As estratégias de deteção molecular incluíram rastreio por PCR convencional e em tempo real para coronavírus, complementado por sequenciação metagenómica de nova geração para captar a diversidade viral mais ampla. Além disso, realizaram-se análises de *docking* proteico *in silico* para examinar as interações de ligação entre as proteínas *spike* dos CoVs detetados em morcegos e os recetores aminopeptidase N (APN) de diferentes espécies hospedeiras (morcegos, suínos e humanos), com o objetivo de inferir possíveis riscos de transmissão entre espécies.

Os principais resultados revelaram a circulação de AlphaCoVs nas populações de morcegos em Portugal, com um claro gradiente de prevalência associado ao habitat, indicando maior prevalência em morcegos cavernícolas (8,87%) em comparação com morcegos arborícolas e de fenda (1,15%). Embora os CoVs tenham sido detetados em amostras de morcegos, não foram encontrados em amostras de ar ambiental, sugerindo a necessidade de períodos de amostragem mais longos e metodologias mais robustas para a deteção de vírus transportados pelo ar. Notavelmente, a maioria das deteções positivas ocorreu em amostras fecais (nove), em contraste com uma única zaragatoa bucal e uma

zaragatoa anal positivas, sugerindo replicação gastrointestinal e uma potencial via de transmissão faecal–oral, o que poderá, de certo modo, explicar a ausência de CoVs de morcego nas amostras de ar. O estudo também revelou uma diversidade viral significativa para além dos CoVs, incluindo picornavírus e mastadenovírus, evidenciando a riqueza do viroma intestinal destes animais.

As análises de *docking* proteico indicaram uma forte afinidade de ligação da proteína *spike* de CoVs de morcego com APN de morcego, mas também afinidades comparáveis com APNs humanos e suínos, sugerindo um potencial de transmissão entre espécies, embora sejam necessárias validações experimentais adicionais.

Em conclusão, esta tese fornece contributos essenciais para a compreensão da ecologia e diversidade de vírus associados a morcegos em Portugal, reforçando o papel destes animais como reservatórios importantes para uma vasta gama de vírus. Os resultados sublinham a importância de manter a vigilância e a investigação contínuas para monitorizar a dinâmica viral, avaliar os riscos associados para a saúde pública e a conservação da vida selvagem, bem como para orientar estratégias de mitigação de potenciais surtos zoonóticos no âmbito do paradigma *One Health*.

.

Chapter 1-

General Introduction

Contents from this Chapter were adapted from the following review paper:

Hemnani, M., da Silva, P. G., Thompson, G., Poeta, P., Rebelo, H., & Mesquita, J. R. (2024). Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *EcoHealth*, 21(2), 125-140. <https://doi.org/10.1007/s10393-024-01688-5>

1.1 Bat ecosystem, role in virus transmission and conservation challenges

1.1.1 Bat Diversity and Unique Biological Traits

Across the mammalian class, bats are one of the most diverse (Burgin et al. 2018), with the bat order (Chiroptera) consisting of more than 1,470 species in over 20 families (Bokelmann and Balkema-buschmann 2021a; Hao et al. 2024), being of great relevance in a One Health context (Figueiroa et al. 2025).

Bats possess unique evolutionary adaptations that have enabled the development of true, sustained flight, an ability unmatched among mammals (Racey 2015), and the capacity to exploit a wide variety of roost environments and food resources. The evolution of flight in bats represents one of the most remarkable innovations in mammalian history (Kunz 1982a; Kasso and Balakrishnan 2013; Anderson and Ruxton 2020). Unlike gliding species such as flying squirrels, bats developed powered flight through extensive modification of the forelimb (Cooper et al. 2012), in which elongated finger bones support a thin, elastic membrane of skin known as the patagium. This structure, coupled with flexible wing joints, allows for far greater manoeuvrability than the rigid, feathered wings of birds (Anderson and Ruxton 2020). Wing morphology varies considerably among species and is closely tied to ecological niche (Kunz and Fenton 2003a; De Oliveira et al. 2020): bats with long, narrow wings are adapted for fast, energy-efficient flight in open habitats, while those with short, broad wings excel in manoeuvrability, enabling them to forage in cluttered forests or roosting environments (Findley and Wilson 1982; De Oliveira et al. 2020).

The energetic demands of sustained flight are exceptionally high, and bats have evolved specialized physiological traits to meet them (Kunz and Fenton 2003b; Shen et al. 2010). Elevated metabolic rates, high cardiac output, and efficient oxygen transport systems enable bats to maintain the endurance required for aerial foraging (Cao and Jin 2020). These traits not only support flight but also influence life-history strategies, such as long-distance migration and unusual longevity for mammals of their size. Along bats' evolutionary history, flight opened profound ecological opportunities by granting access to aerial insect prey, facilitating dispersal across broad geographic regions, and enabling the colonization of roosting habitats inaccessible to non-flying mammals (Racey 2015; Anderson and Ruxton 2020; Ingala et al. 2025).

Equally transformative was the evolution of echolocation, a sensory system that allows bats to orient, navigate, and forage in darkness (Jones and Teeling 2006; Collen 2012).

Echolocation calls are generally produced in the larynx and emitted either orally or nasally, with returning echoes received by highly specialized auditory structures (Metzner and Müller 2016). Echolocation call design varies with ecological demands: open-space foragers emit low-frequency, long-range calls (Denzinger and Schnitzler 2013; Schnitzler and Denzinger 2024). Beyond prey capture, echolocation plays a vital role in navigation, roost detection, and even social communication within colonies (Schnitzler et al. 2003; Page and Bernal 2020; Stidsholt 2025). From an evolutionary perspective, the origin of echolocation remains debated, with evidence suggesting it may have evolved once in the common ancestor of bats or independently in multiple lineages (Jones and Teeling 2006; Teeling et al. 2016). Regardless of its evolutionary pathway, echolocation, together with flight, forms the foundation of the extraordinary ecological and evolutionary success of Chiroptera.

Some bats have successfully exploited caves as permanent shelters (Zagmajster 2019). Caves that provide a broad thermal range combined with structural and elevational complexity support the greatest diversity of bat species (Perry 2013). Narrow crevices may also provide refuge, often for smaller maternity colonies (Kunz 1982b; Kunz and Fenton 2003b). Tree cavities in the trunks and branches of both dead and living trees are especially important in tropical regions, where they offer protection from ambient temperature fluctuations, humidity changes, predators, and adverse weather (Kunz and Fenton 2003a). However, tree cavities present certain disadvantages: they offer limited space for colonial species and, because they eventually rot and collapse, they require periodic relocation of inhabitants (Lindenmayer and Laurance 2017).

Roosting habits have also influenced the morphological evolution of bats (Santana et al. 2011; Marinello and Bernard 2014). Selective pressures related to shelter exploitation have shaped wing and skull morphology, producing well-rounded skulls with marked dorsoventral flattening. Other modifications include the development of thickened pads or adhesive disks on the thumbs and feet, adaptations that allow some species to cling to smooth or moist surfaces such as leaves or the internal cavities of bamboo (Riskin and Racey 2010). These roosting strategies illustrate the high degree of specialization present within different lineages.

Beyond structural adaptations, bats actively modify their microhabitats. By clustering together, depositing feces and urine, or generating metabolic heat, they can alter the physical properties of roosts, increasing temperature and humidity (Korine et al. 2022; Conenna et al. 2025). Such manipulations are especially beneficial during periods of high energetic

demand, such as reproduction. In many cases, interspecific associations occur within shared shelters outside the breeding season, with individuals benefiting from the thermal advantages of roosting in proximity to other species or large aggregations (Russo et al. 2017). Most species, however, segregate into different roosts during the maternity period, although exceptions exist where partitioning within the same shelter occurs.

Bats also demonstrate remarkable adaptability to anthropogenic environments (Voigt and Kingston 2015). Many species successfully exploit man-made structures as substitutes for natural roosts, including barns, houses, bridges, wells, tombs, crypts, ancient ruins, and cellars. These structures often mimic the protective features of caves or tree cavities, highlighting the opportunistic nature of bat roost selection.

Together, these behavioral and morphological adaptations have allowed bats to exploit an exceptionally wide range of ecological niches. Consequently, bats inhabit diverse ecosystems worldwide, except for the Arctic and Antarctic regions (Dacheux et al. 2014; Hu et al. 2017). Roosts are central to their survival, offering not only protection from predators and environmental extremes but also serving as sites of intense social interaction. Within these shelters, bats engage in communication, thermoregulation, and group cohesion behaviors, all of which are fundamental to reproduction and the persistence of populations (Kunz 1982; Senthilkumar and Marimuthu 2012; Chaverri et al. 2018a).

From an evolutionary perspective, the diversity of roosting strategies has been a key driver of bat radiation and persistence. By exploiting structurally and thermally diverse habitats—from caves and tree cavities to man-made structures—bats have secured ecological opportunities that reduce competition and buffer populations against environmental change. Roost plasticity not only enhances survival in variable environments but also underpins social complexity, reproductive strategies, and interspecific interactions. These dynamics, together with the twin innovations of flight and echolocation, have likely contributed to the extraordinary diversification of Chiroptera, making them the second most speciose order of mammals. In this way, roosting ecology, flight, and echolocation stand as both consequences of bat evolution and fundamental forces shaping their evolutionary trajectory.

Bats exhibit a remarkably diverse diet, consuming arthropods, insects, fruits, nectar, pollen, small mammals, fish, frogs, and even blood (Gloza-Rausch et al. 2008a). This dietary flexibility, combined with their ability to navigate and hunt in darkness using echolocation, has allowed bats to thrive in numerous environments (Woo and Lau 2019; Nojiri et al. 2021). Furthermore, bats play essential roles in ecosystem stability by serving as seed dispersers,

pollinators, insect population suppressors, and nutrient recyclers, providing critical services that support environmental balance (Kunz et al. 2011; Goffard et al. 2015; Ramírez-Fráncel et al. 2022).

They are also known as significant potential bioindicators due to their well studies taxonomy, ease of monitoring population trends, and sensitivity to both short- and long-term environmental changes (Jones et al. 2009; Zukal et al. 2015). Bats' global distribution, combined with their ecological roles, enhances their utility as bioindicators of environmental health. As insectivores at higher trophic levels, bats are particularly susceptible to the accumulation of pesticides and other toxins (Torquetti et al. 2021). Fluctuations in bat populations can also serve as indicators of changes in the abundance of their arthropod prey species (Jones et al. 2009).

Bats are widely regarded as valuable bioindicators due to their well-documented taxonomy, the relative ease of monitoring their population dynamics, and their sensitivity to both short- and long-term environmental disturbances (Jones et al. 2009; Zukal et al. 2015). Their broad global distribution and diverse ecological functions further strengthen their relevance as indicators of ecosystem health. As insectivorous mammals occupying higher trophic levels, bats are especially vulnerable to the bioaccumulation of pesticides and other environmental contaminants (Torquetti et al. 2021). Moreover, changes in bat population trends may reflect shifts in the availability and abundance of arthropod prey, providing additional insights into ecosystem dynamics (Jones et al. 2009).

1.1.2 Bats as Natural Reservoirs of Viruses

Bats represent a unique group of mammals that have evolved to coexist with a vast diversity of viruses (Tiwarei et al. 2020; Gupta et al. 2021a), a phenomenon that fascinates virologists and immunologists. Over the past few years, the interest in bat research has increased because of the range of viruses that they harbor and the potential of spillover to humans and other vertebrates (O'Shea et al. 2014; Hayman 2016). In certain cases, there is direct evidence of viral spillover events from bats to other vertebrate hosts. Notably, Nipah virus has transmitted from bats to humans and pigs, causing outbreaks in Southeast Asia, while Hendra virus continues to spill over into horses and occasionally humans in Australia (Field et al. 2001; Luby et al. 2006; Edson et al. 2015a, b; Islam et al. 2016).

The order Chiroptera plays a role as a reservoir for members of many virus families such as *Coronaviridae*, *Adenoviridae*, *Parvoviridae*, *Picornaviridae*, *Rhabdoviridae*, *Herpesviridae*, *Filoviridae*, *Paramyxoviridae*, *Astroviridae* (Calisher et al. 2006a; Drexler et al. 2012; Smith and Wang 2013; Moreno et al. 2017; Wang and Anderson 2019; Gupta et al. 2021b) and members of a total of 66 genera from 15 families of RNA and five families of DNA viruses have been detected from 75 bat genera (Openshaw et al. 2017a) with new viruses constantly being identified through next-generation sequencing (NGS). They are also natural reservoirs for coronaviruses (CoVs) related to those that have caused outbreaks in humans, such as for SARS, MERS and SARS-CoV-2, even though these specific human pathogens have not been directly isolated from bats.

A recent systematic review reported that, to date, there are 30 studies in Europe that have examined the presence of CoVs in bats, covering countries such as Italy, Germany, the Netherlands, Ukraine, Belgium, Bulgaria, Denmark, Finland, France, Hungary, Luxembourg, Romania, Slovenia, Spain, Sweden, Switzerland, Poland, the United Kingdom, and Portugal (Hemnani et al. 2024b). These distinctive ecological traits, paired with bats' longevity and gregariousness, have laid the foundation for their intriguing relationship with viruses, one that continues to be unravelled by virologists and ecologists.

Pathogen shedding by bats is a complex phenomenon regulated by host antiviral defenses and viral factors (Subudhi et al. 2019). Over millions of years, these animals have developed a specialized immune system that allows them to harbor many viruses (Apoorva and Singh 2024), several of which have zoonotic potential such as rabies viruses (Soler-Tovar and Escobar 2025), Nipah virus (Pujol et al. 2025), and Marburg virus without displaying severe symptoms (Irving et al. 2021a). This apparent tolerance suggests that bats

have evolved unique immune strategies that enable them to maintain persistent viral infections without exhibiting clinical signs, supporting a state of balanced virus–host coexistence.

The route of viral shedding is closely linked to the site of replication within the host. For instance, viral replication in the kidneys may result in shedding via urine, while replication in the intestinal tract typically leads to faecal shedding (Subudhi et al. 2019). Caution is warranted when interpreting findings on bat immune defenses, as the order *Chiroptera* comprises over 1,470 species. Given this considerable diversity, insights gained from a single species may not be representative or applicable across the entire order (Clark et al. 2011).

The remarkable ability of bats to tolerate viral infections without triggering excessive inflammation, while still suppressing viral replication, raises a fundamental question: what makes bats so unique? This phenomenon has been linked to the evolutionary demands of flight. Sustained flight requires a high metabolic rate, which increases the production of reactive oxygen species (ROS) (Thomas and Suthers 1972; Maina 2000) and consequently raises the risk of DNA damage (Cadet and Richard Wagner 2013). To cope with this constant oxidative stress, bats have evolved enhanced DNA damage response pathways and dampened inflammatory signalling, adaptations that not only protect them from self-inflicted cellular damage but also reduce harmful immune overreactions during viral infections (Ge et al. 2013; Ahn et al. 2019). Their ability to fly also enables bats to migrate across vast distances, which greatly increases the potential for both inter- and intra-species viral transmission (Drexler et al. 2014a; Gupta et al. 2021b).

These features collectively position bats as highly mobile and immunologically tolerant reservoirs for a wide range of viruses. Some bat species also undergo periods of hibernation, during which their physiology and immune responses are significantly altered. In big brown bats, for instance, gammaherpesviruses can persist in a latent state throughout hibernation (Subudhi et al. 2019). Upon emergence from hibernation, this latent infection may be reactivated, resulting in detectable viral DNA in the blood (Gerow et al. 2019). Such reactivation has been associated with temporarily reduced levels of antiviral antibodies. As the bats recover from hibernation, however, their immune system strengthens, leading to increased antibody production and a return of the virus to latency. In non-hibernating bats, enhanced antiviral immunity contributes to lower viral loads, thereby limiting viral persistence in circulation (Lee and McCracken 2002; Gerow et al. 2019).

Since mounting an immune response is energetically costly (Thomas and Suthers 1972; O'Shea et al. 2014) and could be detrimental in the presence of frequent self-DNA damage, it is hypothesized that bats have evolved mechanisms to suppress immune activation in response to such damage. This likely contributes to their dampened inflammatory responses and may help explain the unusually long lifespans observed in some bat species despite their small body size and elevated metabolic rates (Jebb et al. 2018). Fever in mammals is also accompanied by an initially high metabolic rate associated with beneficial immune responses (Hasday et al. 2000; Blatteis 2003).

Bats also express unusually high levels of heat-shock proteins (HSPs), which are molecular chaperones that enhance cellular resilience to elevated temperatures and oxidative stress (Phillips et al. 2017). Beyond protecting bat cells under such conditions, HSPs also influence virus–host interactions in multiple ways. They can tolerate certain viral mutations and modulate viral proteins, thereby accelerating viral evolution (Phillips et al. 2017). In addition, HSPs may function as viral receptors (Reyes-del Valle et al. 2005) regulate or inhibit apoptosis (Beere et al. 2000), and play roles in the control of inflammation (Srivastava 2002) as well as cellular aging (Singh et al. 2006). Collectively, these functions highlight the importance of HSPs in shaping both bat physiology and the dynamics of viral persistence.

Their high vagility and organization in social groups also predispose them to infection and viral dissemination (Calisher et al. 2006b; Brook and Dobson 2015). Their immune response is finely tuned and prevents overactivation, enabling bats to act as natural reservoirs for many potential human pathogens (Platto et al. 2021a). This remarkable adaptation not only benefits the bats but also impacts viral evolution and transmission patterns across different species. Despite significant advancements in recent years, research on the diversity and dynamics of viral populations in bats, particularly CoVs, remains incomplete (Lucero Arteaga et al. 2023).

1.1.3 Environmental Change, Zoonotic Spillover, and Bat Conservation Challenges

Despite their critical role in maintaining ecosystem balance, and epidemiological significance, bats face a wide range of threats that jeopardize their survival and alter their interactions with other species. Habitat destruction through deforestation, mining, and urbanization reduces roosting and foraging opportunities, while pesticide exposure and other forms of pollution add additional stress (Frick et al. 2020a). Climate change, hunting, and the expansion of agriculture and wildlife markets also not only threaten bat survival but also create opportunities for the spillover of pathogens to humans and domestic animals. These stressors can suppress immune function, increase viral shedding, and combined with negative public perceptions and persecution, further threaten bat biodiversity (O’Shea et al. 2014).

Climate change further compounds these pressures by reshaping species distributions and densities, creating novel opportunities for interspecies contact and zoonotic transmission (Guthid et al. 2020; Carlson et al. 2022; Galindo-González 2024). An example of one major driver is the El Niño–Southern Oscillation (ENSO), which influences global temperature and precipitation patterns through alternating phases of El Niño (warming), La Niña (cooling), and Neutral conditions (Hanley et al. 2003). ENSO has been linked to the timing and location of several bat-related spillover events. For example, drought conditions and forest fires associated with the 1997 El Niño may have forced fruit bats to migrate from Sumatra to Malaysia in search of food, facilitating Nipah virus transmission to pigs and subsequently to humans (Chua et al. 2002). More broadly, La Niña events have been associated with outbreaks of Nipah virus, Melaka virus, SADS-CoV, and MERS-CoV, while El Niño phases coincided with the emergence of Hendra virus, SARS-CoV-1 and -2, and Menangle virus; other bat-related viruses, such as Australian bat lyssavirus, emerged during Neutral phases (Latinne and Morand 2022).

Given their high mobility, fruit bats can track resources across landscapes and colonize new areas following extreme climatic events (Boardman et al. 2020). Projections suggest that Australian fruit bats may extend their range southwards into Tasmania within the next two decades, potentially intensifying human–wildlife conflict (Diengdoh et al. 2022). Such shifts underscore how environmental change not only threatens bat populations but also increases opportunities for pathogen spillover, reinforcing the interconnectedness of bat conservation and global health.

Projections further highlight the likelihood of continued range shifts in response to

climate change. Rising temperatures may enable bat species to expand into new areas, with shifts influenced by factors such as water availability, roost quality, physiology, and phenology (Ancillotto et al. 2016; Cappelli et al. 2021). Such changes emphasize that environmental stressors not only threaten bat survival but also increase opportunities for contact with new hosts, heightening the risk of pathogen spillover.

In addition to driving the emergence of zoonotic diseases, climate change also poses direct threats to bat populations. Grey long-eared bats, among the rarest mammals in the UK with fewer than 1,000 individuals, have expanded their range northward from Spain and Portugal as rising temperatures make their native habitats less suitable. In Australia, extreme heat waves have caused mass die-offs of flying foxes, with thousands of individuals succumbing annually (Mo et al. 2021). As such events become more frequent, bat populations may experience further declines, with cascading consequences for ecosystem stability. Mass mortality events not only disrupt ecological balance but also elevate public health risks: sick or dead bats on the ground increase the likelihood of human–bat interactions, whether through attempted rescue or carcass removal, thereby heightening the chance of zoonotic spillover (Mühldorfer et al. 2011; Openshaw et al. 2017b).

The destruction of natural habitats, whether through natural disasters or human-driven land-use change, can also increase the frequency of wildlife disease outbreaks, heightening the risk of zoonotic spillover to humans (Faust et al. 2018) (Faust et al., 2018). In bats, habitat loss forces individuals to abandon natural foraging grounds and often drives them to seek refuge in human-made environments such as rural structures or urban buildings (Martínez-Abraín and Jiménez 2016). While this adaptability has allowed bats to persist under anthropogenic pressures, it also increases the likelihood of human–bat encounters and pathogen transmission. An example is the common vampire bat (*Desmodus rotundus*), a hematophagous species that serves as a major reservoir of sylvatic rabies and a source of infection for both humans and livestock across Latin America (Bergner et al. 2020). The widespread expansion of cattle ranching, coupled with large-scale deforestation, has boosted the density and range of this species, facilitating closer contact with human populations (Gupta 2005; Botto Nuñez et al. 2020). More broadly, the ability of many bat species to tolerate habitat fragmentation may be linked to their capacity to traverse open areas and exploit resources in fragmented landscapes (Martínez-Abraín and Jiménez 2016). While such ecological flexibility supports bat survival, it simultaneously increases opportunities for cross-species transmission events, reinforcing the link between environmental degradation and spillover risk.

Another major threat to bat populations is hunting and the consumption of bushmeat, as well as the role of wet markets in facilitating human-bat interactions (Akem and Pemunta 2020). Bat hunting is widespread, particularly for large and colony-bound species, which are easier to capture, and constitutes a significant risk to many bat populations alongside habitat loss (Fritz et al. 2009). Hunting is often driven by socioeconomic and cultural factors, with food security being a primary motivation in rural areas (Oedin et al. 2022). In tropical regions of Africa and Asia, fruit bats and other species are hunted and consumed as bushmeat (Kamins et al. 2011). Such practices carry significant risk for cross-species disease transmission, as humans who hunt, butcher, or consume bats are directly exposed to zoonotic pathogens through mucosal contact, cuts, or contamination from feces and other materials (Cantlay et al. 2017; Biol Sci et al. 2019). Domestic animals can also become involved, either through feeding on bat remains or through direct exposure during preparation, increasing the risk of spillover to livestock and humans.

Live animal markets further exacerbate this risk (Naguib et al. 2021). These markets, particularly in Southeast Asia and China, often house dense concentrations of multiple wildlife species that would not naturally interact, including bats, civets, snakes, and hedgehogs (Lynteris 2016; Naguib et al. 2021; Galindo-González 2022). Such interactions facilitate cross-species transmission to domestic animals and humans, as seen in the Nipah virus outbreak in Malaysia, where bats infected pigs, which then infected humans (Luby et al. 2006; Nahar et al. 2020) and potentially in the initial spread of COVID-19 in Wuhan (Xiao et al. 2021). These regions are recognized as global hotspots for emerging zoonotic diseases due to high wildlife trade and human-animal contact (Allen et al. 2017).

The intensive hunting of bats, combined with their naturally low reproductive rates, has led to documented population declines in several species. Nonetheless, there are promising examples of management: voluntary hunting controls and community-based conservation initiatives, particularly on islands, have successfully halted declines and preserved bat populations. Further education, awareness campaigns, and carefully monitored sustainable harvesting strategies are essential to balance human needs with bat conservation while mitigating disease risk. Together, these findings underscore the need to consider bat conservation, climate adaptation, and public health as interconnected challenges.

1.2. Coronaviruses

1.2.1. Overview of Coronaviruses

Coronaviruses (CoVs) are viruses belonging to the order Nidovirales, *Coronaviridae* family, subfamily *Orthocoronavirinae*. The family *Coronaviridae* includes three subfamilies, *Orthocoronavirinae*, *Letovirinae* and *Pitovirinae*. The transmission routes for orthocoronaviruses are mainly through contact of contaminated surfaces or objects, fomites, respiratory aerosols/droplets and faecal–oral means but are unknown for letoviruses and piroviruses (Drosten et al. 2014; Smith et al. 2020; Richard et al. 2020). Members of the subfamily *Orthocoronavirinae* display diverse receptor usage and pathogenicity in different hosts, ranging from infections being asymptomatic to causing death. Some members of the subfamily have been responsible for well-known human epidemics or pandemics such as SARS, MERS and COVID-19 (Ksiazek et al. 2003a; Zhu et al. 2020a).

1.2.2. Coronavirus genome and structure

CoVs are enveloped, roughly spherical positive-sense single-stranded RNA viruses with some of the largest RNA genomes known, ranging from approximately 26 to 32 kilobases (Delmas et al. 1992). All CoVs have a unique characteristic: their viral particles resemble a crown, which is the origin of their name (Monto et al. 2014). The virus particle consists of four key structural proteins: spike (S), envelope I, membrane (M), and nucleocapsid (N) (Figure 1). These proteins are crucial for the formation of the virus particle and its ability to infect host cells (Yuan et al. 2017). Virus enters cells by fusion at the cell surface or by an endocytotic pathway depending upon the strain of virus and the target cell (Yang and Leibowitz 2015).

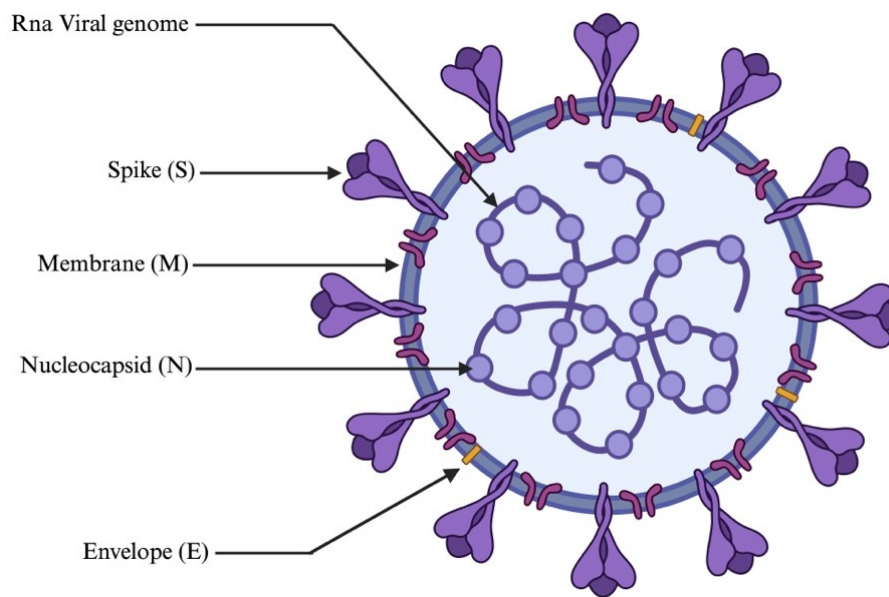


Figure 4: Schematic representation of the CoV virion structure. Highlighting key structural proteins: the spike (S), membrane (M), envelope I, and nucleocapsid (N) proteins. This diagram was created using BioRender.

Their envelope is studded with distinctive large, club-shaped spike (S) proteins that mediate host-cell entry via receptor binding and membrane fusion, which may occur either directly at the plasma membrane or through endocytosis depending on the virus strain and host cell (Delmas et al. 1992; Hofmann et al. 2005; Li et al. 2005a). The S protein is comprised of two subunits, S1 and S2. While S1 facilitates the adhesion of the virus to the host cell, S2 plays a crucial role in facilitating the fusion of the virus with the host cell membrane (Ravi et al. 2022).

Following viral entry and uncoating, the positive-sense RNA genome is released into the cytoplasm, where it functions directly as mRNA. The linear genome is capped, polyadenylated, and structurally polycistronic, allowing translation of multiple proteins from the same RNA strand. The genome of CoVs possesses at least six open reading frames (ORFs), namely ORF1a/b, S, E, M and N (Chen et al. 2020; Woo et al. 2023a). The 5' two-thirds of the genome encodes the large replicase gene comprising ORF1a and ORF1b (Bredenbeek et al. 1990; Ravi et al. 2022); translation of ORF1a produces the polyprotein pp1a, while a programmed -1 ribosomal frameshift enables translation continuation into ORF1b, yielding polyprotein pp1ab (Chen et al. 2020). These polyproteins are

proteolytically processed into 16 nonstructural proteins (nsps 1–16) (except *Gammacoronavirus* that lacks nsp1) (Chen et al. 2020), which assemble into a sophisticated replication–transcription complex that orchestrates viral RNA synthesis. Among these nsps, key conserved domains include the RNA-dependent RNA polymerase (RdRp, nsp12), helicase (nsp13), 3′–5′ exoribonuclease (nsp14), and ADP-ribose-1′-phosphatase macrodomain (nsp3).

CoVs rely on four key structural proteins for virion assembly and infectivity. The spike (S) protein, as mentioned before, projects from the viral envelope, is responsible for binding to host cell receptors (Delmas et al. 1992; Beniac et al. 2006). The membrane (M) protein, characterized by its three transmembrane domains, is crucial for maintaining virion shape, inducing membrane curvature, and interacting with the nucleocapsid (Nal et al. 2005; Neuman et al. 2011). The envelope I protein contributes to virion assembly and release and is also implicated in viral pathogenicity (DeDiego et al. 2007; Nieto-Torres et al. 2014). The nucleocapsid (N) protein consists of two RNA-binding domains, each engaging the viral genome through distinct mechanisms (Chang et al. 2006; Fehr and Perlman 2015). Evidence suggests that the N protein associates with the nonstructural protein nsp3 to anchor the genome to the replication-transcription complex (RTC), facilitating packaging of the RNA into new virions. Additionally, the N protein plays a role in immune evasion by antagonizing interferon responses and repressing RNA interference, enhancing viral replication efficiency.

Replication occurs entirely within the cytoplasm, associated with a reticulovesicular network derived from modified endoplasmic reticulum membranes, including double-membrane vesicles, which serve as replication factories. The 3′-proximal region of the genome encodes structural proteins in the order spike (S), envelope I, membrane (M), and nucleocapsid (N), as well as a variable number of accessory proteins that differ between genera and contribute to host adaptation or immune modulation.

The CoV replication strategy is notable for the synthesis of a nested set of subgenomic mRNAs (sg mRNAs), each sharing a common 5′ leader sequence and coterminal 3′ ends, transcribed from complementary minus-strand templates. This transcription is regulated by transcription regulatory sequences (TRSs) positioned upstream of each ORF, featuring conserved core motifs (e.g., 5′-UCUAAAC-3′ in betacoronaviruses). Packaging signals within the replicase region ensure selective incorporation of viral RNA into new virions,

exemplified by the 69-nucleotide packaging signal within ORF1b in murine hepatitis virus (MHV).

Virus assembly occurs at the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), where preformed helical nucleocapsids bud into intracellular membranes to acquire their envelope. Mature virions are subsequently transported through the secretory pathway and released from the host cell via exocytosis. Although most extensively characterized in coronaviruses, this replication mechanism is conserved among other Coronaviridae members such as toroviruses and bafiniviruses. Despite differences in host range and pathogenicity, all Coronaviridae share hallmark features including large club-shaped spikes, integral membrane M proteins, and helical nucleocapsids formed by nucleocapsid (N) protein and genomic RNA, with conserved replicase domains serving as molecular markers distinguishing them from other nidoviruses.

1.2.3. Taxonomy of Coronavirus

CoVs are classified into four genera. *Alphacoronavirus* (α -CoV) and *Betacoronavirus* (β -CoV), which typically causes diseases in mammals, and are considered pathogenic viruses. On the other hand, *Deltacoronavirus* (δ -CoV) and *Gammacoronavirus* (γ -CoV) evolved from a CoV originated from birds, and the majority of them (but not exclusively) causes diseases in birds (Mihindukulasuriya et al. 2008a; Woo et al. 2012a, 2014a), and are known as poultry viruses (Gloza-Rausch et al. 2008b).

To illustrate the diversity and host range across coronavirus taxa, a schematic representation of the CoV genera and subgenera, along with their associated hosts, is presented in Figure 2. This diagram serves as a visual summary to support the subsequent detailed discussion of each genus and its known subgenera according to ICTV.

Alphacoronavirus (AlphaCoVs)

AlphaCoVs are known to infect a broad range of mammalian hosts. According to ICTV, several subgenera within this genus, such as *Colacovirus*, *Decacovirus*, *Duvinacovirus*, *Minuacovirus*, *Myotacovirus*, *Nyctacovirus*, *Pedacovirus*, *Rhinacovirus*, and *Setracovirus*, have been frequently identified in bat species (Wong et al. 2007a; Zhou et al. 2018). Additionally, *Pedacovirus* and *Rhinacovirus* include strains responsible for enteric infections in pigs (Pensaert and de Bouck 1978; Wong et al. 2007a; Zhou et al. 2018). In

humans, two AlphaCovs are recognized: HCoV-229E and HCoV-NL63, classified under *Duvinacovirus* and *Setracovirus*, respectively (Farsani et al. 2012).

Beyond bats and humans, AlphaCoVs have been detected in various other mammals. For instance, *Luchacovirus* includes strains isolated from brown rats (Wang et al. 2015), *Minacovirus* from American mink (*Mustela vison*) (Vlasova et al. 2011), *Soracovirus* from the common shrew (Wu et al. 2018) and *Sunacovirus* from the Asian house shrew (*Suncus murinus*). The subgenus *Tegacovirus* includes *Alphacoronavirus 1*, which exhibits a broad host range, infecting dogs, cats, and pigs (Whittaker et al. 2018).

Betacoronavirus (BetaCoVs)

Similarly, BetaCoVs display extensive host plasticity, can infect a wide range of mammals, and infect humans with high transmissibility and/or fatality rate. While most embecoviruses (subgenus *Embecovirus*) have been identified in rodents, including *Betacoronavirus 1*, *China Rattus coronavirus HKU24*, *Murine coronavirus*, and *Myodes coronavirus 2JLI4* (Cheever and Daniels 1949; Woo et al. 2005, 2014b; Wu et al. 2018), no embecoviruses have yet been reported in bats, suggesting a rodent origin for this subgenus. Notably, *Betacoronavirus 1* has demonstrated cross-species transmission, with documented infections in antelopes, bovines, deer, pigs, camels, dogs, and humans (Woo et al. 2014c; Bakkers et al. 2017).

In contrast, bats remain the only known reservoir for *Hibecovirus* and *Nobecovirus*. *Merbecoviruses*, which include MERS-CoV (the causative agent of Middle East respiratory syndrome) (Zaki et al. 2012; de Groot et al. 2013) have been isolated from bats, camels, hedgehogs, and humans (Woo et al. 2007; Stalin Raj et al. 2014; Corman et al. 2014a, b; Haagmans et al. 2014; Lau et al. 2018b, 2019). The subgenus *Sarbecovirus* encompasses both SARS-CoV and SARS-CoV-2, responsible for the SARS outbreak in 2003 and the ongoing COVID-19 pandemic, respectively (Drosten et al. 2003; Ksiazek et al. 2003b; Zhu et al. 2020b).

While SARS-CoV is known to infect bats, civets, and humans (Lau et al. 2005; Li et al. 2005b), SARS-CoV-2 displays a much broader host tropism, having been detected in pangolins, felids, canids, mustelids, non-human primates, rodents, and lagomorphs (Nyberg et al. 2022). Despite this wide host range, sustained transmission appears limited to humans,

mink, and hamsters, with bi-directional zoonotic events documented (Wong et al. 2021; Yen et al. 2022). Overall, both AlphaCoVs and BetaCoVs demonstrate a significant potential for host switching and interspecies transmission, emphasizing the ecological complexity and zoonotic risk associated with these viral genera.

Deltacoronavirus (DeltaCoVs)

In contrast to the predominantly mammalian host tropism of AlphaCoVs and BetaCoVs, DeltaCoVs have been primarily identified in avian species (Woo et al. 2009b). Three subgenera are currently recognized within this genus: *Andecovirus*, *Buldecovirus*, and *Herdecovirus*. Representative viruses include *Wigeon coronavirus HKU20* (subgenus *Andecovirus*), isolated from wigeon; *Night heron coronavirus HKU19* (subgenus *Herdecovirus*), found in night herons; and several members of *Buldecovirus*, which have been detected in a variety of passerine and waterbird hosts such as bulbuls, common moorhens, munias, and white-eyes (Woo et al. 2012b; Lau et al. 2018a).

While most DeltaCoVs circulate in birds, a notable exception is *Porcine coronavirus HKU15* (species *Coronavirus HKU15*, subgenus *Buldecovirus*), which has been widely reported in swine populations across East and Southeast Asia, North America, and other regions (Wang et al. 2014; Dong et al. 2015; Madapong et al. 2016; Janetanakit et al. 2016; Lee et al. 2016; Saeng-chuto et al. 2017; Le et al. 2018). This virus, formerly known as porcine deltacoronavirus (PDCoV), is associated with respiratory and enteric disease outbreaks in pigs and is transmitted through both respiratory and faecal-oral routes. Although historically considered host-restricted to swine, *HKU15* has been sporadically identified in humans (Lednicky et al. 2021).

Notably, *human porcine deltacoronavirus* (HuPDCoV) was detected in three Haitian children presenting with mild transient symptoms such as fever, cough, or abdominal pain, raising questions about its zoonotic potential (Lednicky et al. 2021). In addition to differences in host range, δ -CoVs also exhibit variation in accessory gene content across subgenera, further highlighting their genomic and ecological diversity.

Gammacoronavirus (GammaCoVs)

Similar to DeltaCoVs, GammaCoVs are predominantly associated with avian hosts. Three subgenera have been defined within this genus: *Brangacovirus*, *Cegacovirus*, and

Igacovirus. Notable avian-associated GammaCoVs include *Canada goose coronavirus Cambridge Bay 2017* (subgenus *Brangacovirus*), identified in *Branta canadensis* (Papineau et al. 2019) and several members of *Igacovirus* such as *Avian coronavirus*, *Avian coronavirus 9203*, and *Duck coronavirus 2714*. Among these, *Infectious bronchitis virus* (IBV), a member of the species *Avian coronavirus*, was the first CoV ever described. IBV is of veterinary significance, as it targets multiple organ systems, like respiratory, gastrointestinal, renal, and reproductive, causing major economic losses in the poultry industry.

Although GammaCoVs are largely avian in distribution, rare exceptions have been reported in marine mammals. *Beluga whale coronavirus* (subgenus *Cegacovirus*) was the first γ -CoV identified in a mammalian host (Mihindukulasuriya et al. 2008b) and a related strain, *bottlenose dolphin coronavirus HKU22*, has since been detected in dolphins. Despite only 74% amino acid identity in the spike protein between these two strains, no overt clinical signs have been observed in the affected animals. As with other CoV genera, members of γ -CoV subgenera exhibit differences in accessory gene content (Vakulenko et al. 2021), further reflecting their evolutionary diversification. Overall, the genus remains primarily avian, with only limited and asymptomatic spillover into marine mammals documented to date.

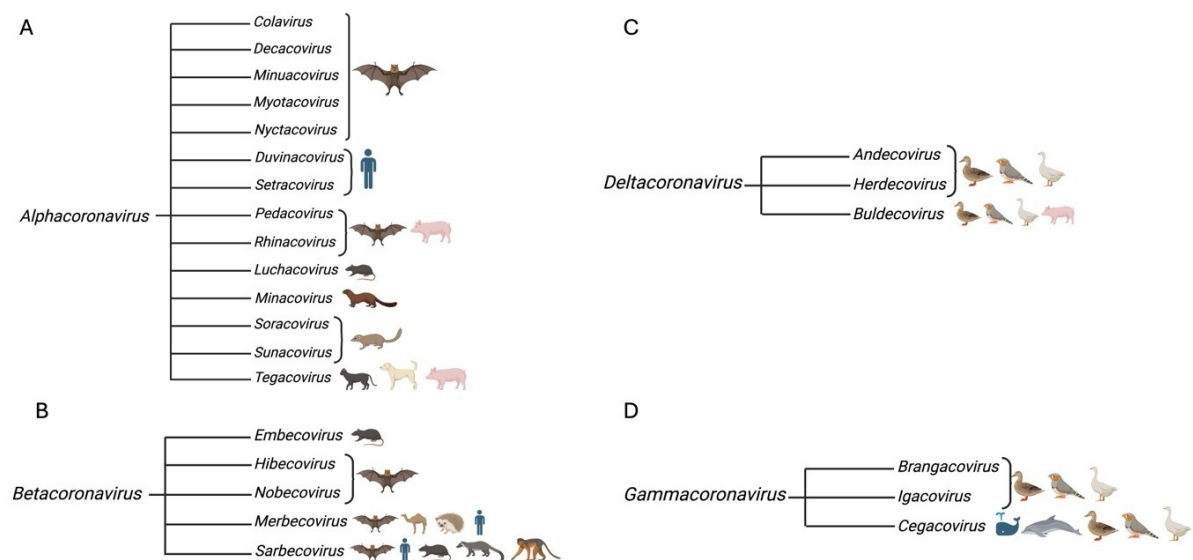


Figure 5: Schematic representation of the four recognized CoV genera, respective subgenera, and their associated host ranges according to ICTV. The relationships shown do not reflect actual phylogenetic distances or topology and were created using BioRender for

illustrative purposes. Icons indicate common host taxa associated with each subgenus based on current knowledge.

1.3 Bats and the Origin of Coronavirus

Since CoVs are found in a wide variety of hosts, including wildlife, livestock, poultry, pets, and humans, these viruses are considered to have a high potential for interspecies transmission (Reusken et al. 2010). Moreover, due to their long genomes of 30kb, high recombination frequency and high mutation rate, CoVs have the potential to adapt to new host species with novel pathogenicity and cause a wide spectrum of diseases (Woo et al. 2009a). The most iconic examples of viral spillover to humans have occurred in 2002/2003, when a highly pathogenic CoV (SARS-CoV-1) emerged in China, causing worldwide outbreaks (Peiris et al. 2004). A few years later, in 2012, an even more deadly CoV emerged in the Arabian Peninsula (MERS-CoV), with a case-fatality rate of 34.3% (Zumla et al. 2015). By the end of 2019, a new SARS-related CoV named severe acute respiratory syndrome CoV 2 (SARS-CoV-2) emerged, leading to the COVID-19 pandemic. To date, the world has paid a high toll in this pandemic in terms of human lives lost, economic losses and increased poverty (Ciotti et al. 2020).

Multiple studies suggest that bats serve as the natural and original reservoirs for various viruses closely related to mammalian CoVs, indicating that they likely evolved from ancestral bat viruses (Woo et al. 2009a; Platto et al. 2021a; Ruiz-Aravena et al. 2022a). They are also considered the group of mammals harbouring the largest number of CoVs (Bokelmann and Balkema-buschmann 2021b), and the evolutionary source for several human CoVs (Ruiz-Aravena et al. 2022a). Alpha and Beta CoVs have been detected in bats from 14 to 21 bat families in at least 69 countries across six continents (Ruiz-Aravena et al. 2022b). The absence of CoVs in particular bat taxa is most likely due to insufficient sampling rather than true absence (Anthony et al. 2017; Wang et al. 2023).

To predict risks for host transition and disease outbreak, therefore, it is important to understand the nature of CoVs hosts, their association with certain bat species and the association between bat species (Gloza-Rausch et al. 2008b). It is also important to understand colony size, density, and composition, as it could affect virus prevalence by changing transmission rates both within and between roosts. Reproductive cycles also influence prevalence and transmission of viruses in bat colonies by affecting patterns of behaviour and physiological susceptibility (Serra-Cobo and López-Roig 2017; Chaverri et al. 2018; Ruiz-Aravena et al. 2022b). Research into the origins of SARS-CoV-2 and continuing interest in CoVs ecology and evolution have highlighted the value of bat surveillance (Cohen et al. 2023)

Any microorganism has the potential to become airborne in certain environmental situations (i.e., to be present in aerosolized particles), posing serious threats to the health and well-being of both human and animal populations (Verreault et al. 2008). Phylogenetic analysis have shown that SARS-CoV-2 has 79–79.5% genetic similarity with SARS-CoV-1, making it a SARS-related CoV (Contini et al. 2020; Lu et al. 2020; Rothan and Byraredddy 2020; Zhou et al. 2020). Considering that prior research on these viruses has indicated the possibility of airborne transmission (Olsen et al. 2003; Zhao et al. 2011; Pyankov et al. 2018a; Tellier et al. 2019; Ramanathan et al. 2020; da Silva et al. 2021b; T.S. et al. 2024), other SARS-related CoVs with the capability to spread via the airborne route (Geng and Zhou 2021) could be potentially harbored by bats.

1.3.1 Presence of CoVs in European bats

Across Europe, there are approximately 55 species of bats (Russo 2023a) belonging to six families: *Emballonuridae*, *Molossidae*, *Pteropodidae*, *Rhinolophidae*, *Vespertilionidae* and *Miniopteridae*. In this continent, most bats are insectivorous consuming beetles, flies, moths, and other insects, although there are a couple of exceptions where *Rousettus aegyptiacus* feeds on fruit and *Nyctalus lasiopterus* also preys on small birds (Russo 2023b). In Europe, certain bat species are of particular interest with respect to CoVs. They play a crucial role in pest regulation, while some also function as pollinators and aid in the dispersal of seeds for numerous plants that hold significance for humans (Afelt et al. 2018; Lu et al. 2021). Some bats, such as the common pipistrelle and the greater horseshoe bat, and others display different ecological roles and behaviors that have ramifications for CoV ecology and evolution (Gonzalez and Banerjee 2022).

These differences in diet, ranging from insects to small mammals, and roosting behaviour, which can include solitary roosts or groups of hundreds to thousands of individuals, create unique environments conducive to CoV maintenance and transmission (Gannon and Bovard 2016; Gonzalez and Banerjee 2022). Some of these species, such as those that move across the continent such as the *Pipistrellus nathusii*, could move viruses over long distances (Shipley et al. 2019; Alcalde et al. 2021). Other species, like those that form dense colonies, such as the greater mouse-eared bat (*Myotis myotis*), might increase virus transmission events among and between species (Calisher et al. 2006b; Lazov et al. 2018; Ruiz-Aravena et al. 2022b). Despite significant advancements in understanding bat-associated CoVs, there are some research gaps regarding specific European bat species and their role in CoV transmission (Luis et al. 2013; Phelps et al. 2019; Olival et al. 2020).

Geographic gaps also exist, with certain regions in Europe lacking comprehensive surveys for bat-associated CoVs, potentially overlooking important reservoirs.

In recent years, both AlphaCoVs and BetaCoVs have been detected in bats of different species in several European countries. Vespertilionidae and Rhinolophidae are the most likely ancestral host families for AlphaCoVs in Europe, and BetaCoVs likely originated in Vespertilionidae (Russo 2023b). Figure 3 provides an overview of the bat genera reported to carry CoVs in Europe, highlighting the diversity of hosts involved in viral maintenance and transmission.

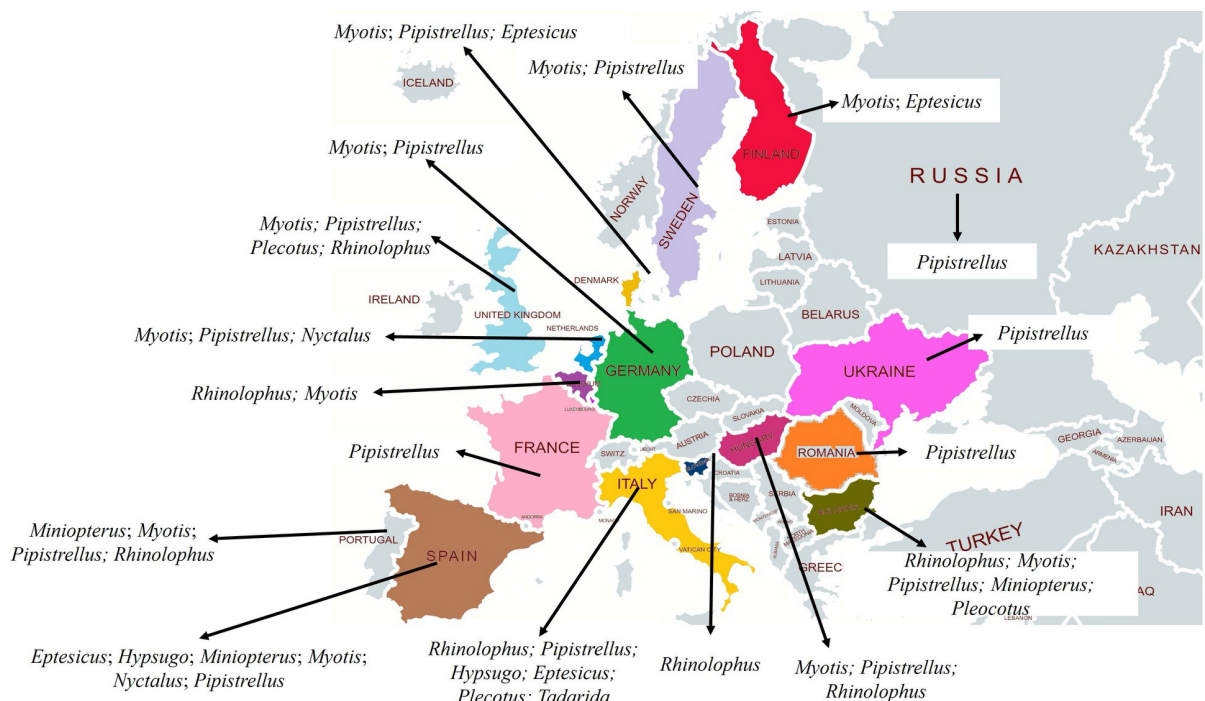


Figure 6: Bats genera that were found carrying CoVs in European countries (Hemnani et al. 2024a).

Geographically, 30 studies evaluated CoV presence in European bats, with significant contributions from Italy, Germany, and the UK, among others. CoVs were found in nine genera of bats across Rhinolophidae, Vespertilionidae, and Miniopteridae families, encompassing 26 species out of approximately 55 found in Europe. Samples collected included oral and anal swabs, faeces, guano, saliva, blood, and various organ tissues. One study (ours) attempted air sampling in bat environments but yielded negative results for CoVs. This systematic review revealed a striking genetic similarity between Portuguese CoVs and strains previously identified in Western Europe, with up to 98% nucleotide identity to UK and French strains. This suggests shared evolutionary histories and potential viral movement across European bat populations. The RdRp gene was the most targeted

genomic region, suitable for taxonomic classification at the subgenus level and phylogenetic comparisons (Hemnani et al. 2024a).

Notably, while some strains clustered by geographic region, others aligned more strongly with host species, irrespective of location. For example, AlphaCovs from *Pipistrellus pipistrellus* in Portugal exhibited close phylogenetic ties with strains from the same species in the UK and Italy, implying species-specific viral associations and possible co-evolutionary relationships. Simultaneously, reports of genetically similar CoVs from disparate host species within the same region suggest that co-roosting behaviours and ecological overlap may promote interspecies viral transmission.

To predict risks for CoV host transition and disease outbreaks, a deeper understanding of CoV hosts is essential, including the association of specific CoVs with bat species. Studying bats in various geographic locations and across different species can help identify host ranges and the potential role of these CoVs in causing new infectious diseases (Wood et al. 2012). These findings provide insight into bat CoV evolution and ecological transmission among bat taxa, guiding early warnings for bat-borne CoV zoonotic diseases. Bats, forming large colonies and flying long distances, can increase virus spread across geographic locations to other bats humans, and livestock or wild animals, often with minimal impact on their own immune systems (Calisher et al. 2006a; Breed et al. 2010; Moratelli and Calisher 2015; Hayman 2016). Given the vast genetic variation and wide range of locations where CoVs have been discovered in bats, there is a high probability of future disease outbreaks.

Despite these findings, significant gaps remain. Surveillance efforts are still geographically uneven, with some regions in Europe underrepresented in CoV studies. Additionally, the diversity of CoVs in European bats is likely underestimated due to limited sample sizes, one-time sampling, and insufficient integration of ecological metadata (e.g., reproductive cycles, colony structure, migratory patterns). These variables critically influence viral dynamics yet remain poorly characterized in many studies. Future studies could focus on longitudinal monitoring of CoV prevalence over time, investigating the impact of habitat changes on viral dynamics, and exploring interactions between different bat species and other wildlife. Such research could explain how CoV prevalence changes over time, identify seasonal patterns or trends, and determine factors influencing these changes. It could also help assess the impact of human activities and interventions, such as habitat destruction or conservation efforts, on CoV prevalence and transmission. Understanding colony size, density, composition, and reproductive cycles is also important,

as these factors could affect virus prevalence by changing transmission rates within and between roosts, and the prevalence and transmission of viruses in bat colonies by affecting patterns of behavior.

This systematic review highlighted the significant role of bats as reservoirs for a diverse array of CoVs, including those related to past outbreaks like SARS and MERS. SARS CoV-2, the causative agent of COVID-19. While our review focuses on bat populations, further comparative studies are needed to understand the prevalence of these pathogens in bats relative to other potential host reservoirs. This understanding is crucial for comprehensively assessing zoonotic risk and devising effective mitigation strategies. Our synthesis of 30 studies across multiple European countries illuminates the dynamics of CoV prevalence in bat populations. The identified genomic regions and the detection of Alpha and BetaCovs in nine bat genera accentuate the need for a nuanced understanding of the zoonotic potential residing within these species. The extensive distribution and variety of CoVs in bats can be attributed to genetic mutations and recombination, as observed in bat migration patterns, suggesting a long co-evolutionary relationship, along with their ability to host infections and co infections. The recurrent identification of CoVs in bat faeces serves as a reminder of the intricate ecological interactions driving viral transmission.

The primary challenges faced by European bats encompass the loss, degradation, and fragmentation of their habitats, overuse of pesticides, as well as societal intolerance, lack of awareness, developmental activities, and persecution (Ruiz-Aravena et al. 2022b). These factors result in the decline of bat colonies found in various locations such as buildings, trees, underground sites, and bridges, along with the depletion of areas abundant in insects for their feeding, disturbances, and direct persecution of bat populations. Understanding the interactions of these bats with humans and livestock, especially in regions where they frequently share habitats, is crucial for assessing zoonotic spillover risks.

References

- Afelt A, Frutos R, Devaux C (2018) Bats, coronaviruses, and deforestation: Toward the emergence of novel infectious diseases? *Front Microbiol* 9:1–5. <https://doi.org/10.3389/fmicb.2018.00702>
- Ahn M, Anderson DE, Zhang Q, et al (2019) Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nat Microbiol* 4:789–799. <https://doi.org/10.1038/S41564-019-0371-3>;
- Akem ES, Pemunta NV (2020) The bat meat chain and perceptions of the risk of contracting Ebola in the Mount Cameroon region. *BMC Public Health* 20:1–10. <https://doi.org/10.1186/S12889-020-08460-8/PEER-REVIEW>
- Alcalde JT, Jiménez M, Brila I, et al (2021) Transcontinental 2200 km migration of a Nathusius' pipistrelle (*Pipistrellus nathusii*) across Europe. *Mammalia* 85:161–163. <https://doi.org/10.1515/mammalia-2020-0069>
- Allen T, Murray KA, Zambrana-Torrel C, et al (2017) Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* 8:. <https://doi.org/10.1038/S41467-017-00923-8>;
- Alves RS, do Canto Olegário J, Weber MN, et al (2022) Detection of coronavirus in vampire bats (*Desmodus rotundus*) in southern Brazil. *Transbound Emerg Dis* 69:2384–2389. <https://doi.org/10.1111/tbed.14150>
- Amengual B, López-Roig M, Serra-Cobo J (2007) First record of seasonal over sea migration of *Miniopterus schreibersii* and *Myotis capaccinii* between Balearic Islands (Spain). *Acta Chiropt* 9:319–322. [https://doi.org/10.3161/1733-5329\(2007\)9\[319:FROSOS\]2.0.CO;2](https://doi.org/10.3161/1733-5329(2007)9[319:FROSOS]2.0.CO;2)
- Ancillotto L, Santini L, Ranc N, et al (2016) Extraordinary range expansion in a common bat: The potential roles of climate change and urbanisation. *Science of Nature* 103:. <https://doi.org/10.1007/S00114-016-1334-7>;
- Anderson SC, Ruxton GD (2020) The evolution of flight in bats: a novel hypothesis. *Mamm Rev* 50:426–439. <https://doi.org/10.1111/MAM.12211>;REQUESTEDJOURNAL:JOURNAL:13652907;PAGE:STRING:ARTICLE/CHAPTER
- Andrusier N, Mashiach E, Nussinov R, Wolfson HJ (2008) Principles of flexible protein-protein docking. *Proteins: Structure, Function and Genetics* 73:271–289. <https://doi.org/10.1002/prot.22170>

- Anthony SJ, Johnson CK, Greig DJ, et al (2017) Global patterns in coronavirus diversity. *Virus Evol* 3:1–15. <https://doi.org/10.1093/ve/vex012>
- Apoorva, Singh SK (2024) A tale of endurance: bats, viruses and immune dynamics. *Future Microbiol* 19:841–856. <https://doi.org/10.2217/fmb-2023-0233>
- Athukorala A, Helbig KJ, Mcsharry BP, et al (2022) Adenoviruses in Avian Hosts: Recent Discoveries Shed New Light on Adenovirus Diversity and Evolution. *Viruses* 14:1767. <https://doi.org/10.3390/v14081767>
- August TA, Mathews F, Nunn MA (2012) Alphacoronavirus detected in bats in the United Kingdom. *Vector-Borne and Zoonotic Diseases* 12:530–533. <https://doi.org/10.1089/vbz.2011.0829>
- Azhar EI, Hashem AM, El-Kafrawy SA, et al (2014) Detection of the Middle East respiratory syndrome coronavirus genome in an air sample originating from a camel barn owned by an infected patient. *mBio* 5:e01450-14. <https://doi.org/10.1128/mBio.01450-14>
- Bakkers MJG, Lang Y, Feitsma LJ, et al (2017) Betacoronavirus Adaptation to Humans Involved Progressive Loss of Hemagglutinin-Esterase Lectin Activity. *Cell Host Microbe* 21:356–366. <https://doi.org/10.1016/j.chom.2017.02.008>
- Banerjee A, Kulcsar K, Misra V, et al (2019) Bats and coronaviruses. *Viruses* 11:7–9. <https://doi.org/10.3390/v11010041>
- Batista T (2014) Paisagem e ecologia da região de Montemor-o-Novo Conversas à volta do espírito dos lugares
- Beere HM, Wolf BB, Cain K, et al (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2:469–475. <https://doi.org/10.1038/35019501;KWRD=LIFE+SCIENCES>
- Beniac DR, Andonov A, Grudeski E, Booth TF (2006) Architecture of the SARS coronavirus prefusion spike. *Nat Struct Mol Biol* 13:751–752. <https://doi.org/10.1038/NSMB1123;KWRD=LIFE+SCIENCES>
- Benkő M, Harrach B, Kremer EJ (2014) Do Nonhuman Primate or Bat Adenoviruses Pose a Risk for Human Health? *Future Microbiol* 9:269–272. <https://doi.org/10.2217/fmb.13.170>
- Bergner LM, Orton RJ, Benavides JA, et al (2020) Demographic and environmental drivers of metagenomic viral diversity in vampire bats. *Mol Ecol* 29:26–39. <https://doi.org/10.1111/MEC.15250>

- Berman HM, Westbrook J, Feng Z, et al (2000) The Protein Data Bank. *Nucleic Acids Res* 28:235–242. <https://doi.org/10.1093/nar/28.1.235>
- Biol Sci BJ, de Jesus Santos N, Santos da Paz E, et al (2019) Evaluation of bat-related knowledge, perceptions, and practices in an urban community: A strategy for Conservation Biology and health promotion. *Brazilian Journal of Biological Sciences* 6:e392–e392. <https://doi.org/10.21472/BJBS.061303>
- Bittar C, Machado RRG, Comelis MT, et al (2020) Alphacoronavirus Detection in Lungs, Liver, and Intestines of Bats from Brazil. *Microb Ecol* 79:203–212. <https://doi.org/10.1007/s00248-019-01391-x>
- Blatteis CM (2003) Fever: pathological or physiological, injurious or beneficial? *J Therm Biol* 28:1–13. [https://doi.org/10.1016/S0306-4565\(02\)00034-7](https://doi.org/10.1016/S0306-4565(02)00034-7)
- Boardman WSJ, Baker ML, Boyd V, et al (2020) Seroprevalence of three paramyxoviruses; Hendra virus, Tioman virus, Cedar virus and a rhabdovirus, Australian bat lyssavirus, in a range expanding fruit bat, the Grey-headed flying fox (*Pteropus poliocephalus*). *PloS One* 15:. <https://doi.org/10.1371/JOURNAL.PONE.0232339>;
- Bodewes R, Ruiz-Gonzalez A, Schapendonk CME, et al (2014) Viral metagenomic analysis of feces of wild small carnivores. *Virol J* 11:1–13. <https://doi.org/10.1186/1743-422X-11-89>
- Bokelmann M, Balkema-buschmann A (2021a) Coronaviruses in Bats. *Berl Munch Tierarztl Wochenschr* 134:1–16. <https://doi.org/10.2376/1439-0299-2020-44>
- Bokelmann M, Balkema-buschmann A (2021b) Coronaviruses in Bats. *Berl Munch Tierarztl Wochenschr* 134:1–16. <https://doi.org/10.2376/1439-0299-2020-44>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Bonny T, Driver J, Paisie T, et al (2017) Detection of Alphacoronavirus vRNA in the Feces of Brazilian Free-Tailed Bats (*Tadarida brasiliensis*) from a Colony in Florida, USA. *Diseases* 5:7. <https://doi.org/10.3390/diseases5010007>
- Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019a) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect* 8:1679–1687. <https://doi.org/10.1080/22221751.2019.1690953>
- Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019b) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect* 8:1679–1687. <https://doi.org/10.1080/22221751.2019.1690953>

- Botto Nuñez G, Becker DJ, Lawrence RL, Plowright RK (2020) Synergistic Effects of Grassland Fragmentation and Temperature on Bovine Rabies Emergence. *Ecohealth* 17:203–216. <https://doi.org/10.1007/S10393-020-01486-9>;
- Bredenbeek PJ, Pachuk CJ, Noten AFH, et al (1990) The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59; a highly conserved polymerase is expressed by an efficient ribosomal frameshifting mechanism. *Nucleic Acids Res* 18:1825–1832. <https://doi.org/10.1093/NAR/18.7.1825>
- Breed AC, Field HE, Smith CS, et al (2010) Bats without borders: Long-distance movements and implications for disease risk management. *Ecohealth* 7:204–212. <https://doi.org/10.1007/S10393-010-0332-Z/FIGURES/3>
- Breshears LE, Nguyen BT, Mata Robles S, et al (2022) Biosensor detection of airborne respiratory viruses such as SARS-CoV-2. *SLAS Technol* 27:4–17. <https://doi.org/10.1016/j.slant.2021.12.004>
- Brook CE, Dobson AP (2015) Bats as “special” reservoirs for emerging zoonotic pathogens. *Trends Microbiol* 23:. <https://doi.org/10.1016/j.tim.2014.12.004>
- Bryja J, Kaňuch P, Fornůsková A, et al (2009) Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe. *Biological Journal of the Linnean Society* 96:103–114. <https://doi.org/10.1111/j.1095-8312.2008.01093.x>
- Bueno LM, Rizotto LS, Viana A de O, et al (2022) High genetic diversity of alphacoronaviruses in bat species (Mammalia: Chiroptera) from the Atlantic Forest in Brazil. *Transbound Emerg Dis* 69:e2863–e2875. <https://doi.org/10.1111/tbed.14636>
- Burgin CJ, Colella JP, Kahn PL, Upham NS (2018) How many species of mammals are there? *J Mammal* 99:1–14. <https://doi.org/10.1093/JMAMMAL/GYX147>
- Cadet J, Richard Wagner J (2013) DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harb Perspect Biol* 5:. <https://doi.org/10.1101/CSHPERSPECT.A012559>,
- Calisher CH, Childs JE, Field HE, et al (2006a) Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19:531–545. <https://doi.org/10.1128/CMR.00017-06>
- Calisher CH, Childs JE, Field HE, et al (2006b) Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19:531–545. <https://doi.org/10.1128/CMR.00017-06>

- Cantlay JC, Ingram DJ, Meredith AL (2017) A Review of Zoonotic Infection Risks Associated with the Wild Meat Trade in Malaysia. *Ecohealth* 14:361–388. <https://doi.org/10.1007/S10393-017-1229-X>;
- Cao T, Jin JP (2020) Evolution of Flight Muscle Contractility and Energetic Efficiency. *Front Physiol* 11:565310. <https://doi.org/10.3389/FPHYS.2020.01038/BIBTEX>
- Cappelli F, Costantini V, Consoli D (2021) The trap of climate change-induced “natural” disasters and inequality. *Global Environmental Change* 70:. <https://doi.org/10.1016/J.GLOENVCHA.2021.102329>;
- Carlson CJ, Albery GF, Merow C, et al (2022) Climate change increases cross-species viral transmission risk. *Nature* 607:555–562. <https://doi.org/10.1038/S41586-022-04788-W>;
- Chang CK, Sue SC, Yu TH, et al (2006) Modular organization of SARS coronavirus nucleocapsid protein. *J Biomed Sci* 13:59–72. <https://doi.org/10.1007/S11373-005-9035-9/METRICS>
- Chaverri G, Ancillotto L, Russo D (2018) Social communication in bats. *Biological Reviews* 93:1938–1954. <https://doi.org/10.1111/brv.12427>
- CHEEVER FS, DANIELS JB (1949) A murine virus (JHM) causing disseminated encephalomyelitis with. *J Exp Med* 90:181–210. <https://doi.org/10.1084/JEM.90.3.181>,
- Chen Y, Liu Q, Guo D (2020) Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol* 92:418–423. <https://doi.org/10.1002/JMV.25681>
- Chua KB, Lek Koh C, Hooi PS, et al (2002) Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4:145–151. [https://doi.org/10.1016/S1286-4579\(01\)01522-2](https://doi.org/10.1016/S1286-4579(01)01522-2);
- Ciotti M, Ciccozzi M, Terrinoni A, et al (2020) The COVID-19 pandemic. *Crit Rev Clin Lab Sci* 0:365–388. <https://doi.org/10.1080/10408363.2020.1783198>
- Clark JS, Bell DM, Hersh MH, et al (2011) Individual-scale variation, species-scale differences: inference needed to understand diversity. *Ecol Lett* 14:1273–1287. <https://doi.org/10.1111/J.1461-0248.2011.01685.X>
- Cohen LE, Fagre AC, Chen B, et al (2023) Coronavirus sampling and surveillance in bats from 1996–2019: a systematic review and meta-analysis. *Nat Microbiol* 8:1176–1186. <https://doi.org/10.1038/s41564-023-01375-1>
- Collen A (2012) The evolution of echolocation in bats: a comparative approach. Doctoral thesis, UCL (University College London)

- Conceição-Neto N, Godinho R, Álvares F, et al (2017) Viral gut metagenomics of sympatric wild and domestic canids, and monitoring of viruses: Insights from an endangered wolf population. *Ecol Evol* 7:4135–4146. <https://doi.org/10.1002/ece3.2991>
- Conceição-Neto N, Zeller M, Lefrère H, et al (2015a) Modular approach to customise sample preparation procedures for viral metagenomics: A reproducible protocol for virome analysis. *Sci Rep* 5:1–14. <https://doi.org/10.1038/srep16532>
- Conceição-Neto N, Zeller M, Lefrère H, et al (2015b) Modular approach to customise sample preparation procedures for viral metagenomics: A reproducible protocol for virome analysis. *Sci Rep* 5:1–14. <https://doi.org/10.1038/srep16532>
- Conenna I, Muñoz-Garcia A, Korine C (2025) Physiological and Behavioural Strategies of Bats From Arid Environments. *Mamm Rev* e70005. <https://doi.org/10.1111/MAM.70005>
- Contini C, Di Nuzzo M, Barp N, et al (2020) The novel zoonotic COVID-19 pandemic: An expected global health concern. *J Infect Dev Ctries* 14:254–264. <https://doi.org/10.3855/jidc.12671>
- Cooper LN, Cretokos CJ, Sears KE (2012) The evolution and development of mammalian flight. *Wiley Interdiscip Rev Dev Biol* 1:773–779. <https://doi.org/10.1002/WDEV.50;CTYPE:STRING:JOURNAL>
- Corman VM, Kallies R, Philipps H, et al (2014a) Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *J Virol* 88:717–724. <https://doi.org/10.1128/JVI.01600-13>,
- Corman VM, Kallies R, Philipps H, et al (2014b) Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *J Virol* 88:717–724. <https://doi.org/10.1128/JVI.01600-13>,
- Cui X, Fan K, Liang X, et al (2023) Virus diversity, wildlife-domestic animal circulation and potential zoonotic viruses of small mammals, pangolins and zoo animals. *Nat Commun* 14:2488. <https://doi.org/10.1038/s41467-023-38202-4>
- da Silva PG, Gonçalves J, Nascimento MS, et al (2022) Detection of SARS-CoV-2 in the Indoor and Outdoor Areas of Urban Public Transport Systems of Three Major Cities of Portugal in 2021. *Int J Environ Res Public Health* 19
- da Silva PG, Mesquita JR, de São José Nascimento M, Ferreira VAM (2021a) Viral, host and environmental factors that favor anthroozoonotic spillover of coronaviruses: An opinionated review, focusing on SARS-CoV, MERS-CoV and SARS-CoV-2. *Science of the Total Environment* 750:141483. <https://doi.org/10.1016/j.scitotenv.2020.141483>

- da Silva PG, Nascimento MSJ, Soares RRG, et al (2021b) Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV. *Science of the Total Environment* 764:.. <https://doi.org/10.1016/j.scitotenv.2020.142802>
- da Silva PG, Nascimento MSJ, Soares RRG, et al (2021c) Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV. *Science of the Total Environment* 764:.. <https://doi.org/10.1016/j.scitotenv.2020.142802>
- De Benedictis P, Marciano S, Scaravelli D, et al (2014) Alpha and lineage C betaCoV infections in Italian bats. *Virus Genes* 48:366–371. <https://doi.org/10.1007/s11262-013-1008-x>
- De Coninck L, Faye L, Basler N, et al (2025) ViPER
- de Groot RJ, Baker SC, Baric RS, et al (2013) Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *J Virol* 87:7790–7792. <https://doi.org/10.1128/JVI.01244-13>
- De Oliveira HFM, Camargo NF, Hemprich-Bennett DR, et al (2020) Wing morphology predicts individual niche specialization in *Pteronotus mesoamericanus* (Mammalia: Chiroptera). *PloS One* 15:e0232601. <https://doi.org/10.1371/JOURNAL.PONE.0232601>
- DeDiego ML, Álvarez E, Almazán F, et al (2007) A Severe Acute Respiratory Syndrome Coronavirus That Lacks the E Gene Is Attenuated In Vitro and In Vivo. *J Virol* 81:1701–1713. <https://doi.org/10.1128/JVI.01467-06/ASSET/983812D0-AAA7-4C88-8679-A844299EF1C1/ASSETS/GRAPHIC/ZJV0040787220008.JPEG>
- Delaplace M, Huet H, Gambino A, Le Poder S (2021) Feline Coronavirus Antivirals: A Review. *Pathogens* 2021, Vol 10, Page 1150 10:1150. <https://doi.org/10.3390/PATHOGENS10091150>
- Delmas B, Gelfi J, L'Haridon R, et al (1992) Amino peptidase N is a major receptor for the enteropathogenic coronavirus TGEV. *Nature* 357:417–420. <https://doi.org/10.1038/357417A0>,
- Deng F, Ye G, Liu Q, et al (2016) Identification and Comparison of Receptor Binding Characteristics of the Spike Protein of Two Porcine Epidemic Diarrhea Virus Strains. *Viruses* 8:55

- Denzinger A, Schnitzler HU (2013) Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front Physiol* 4 JUL:48812. <https://doi.org/10.3389/FPHYS.2013.00164/BIBTEX>
- Diengdoh VL, Onde S, Hunt M, Brook BW (2022) Predicted impacts of climate change and extreme temperature events on the future distribution of fruit bat species in Australia. *Glob Ecol Conserv* 37:. <https://doi.org/10.1016/J.GECCO.2022.E02181>;
- Dietz M, Brombacher M, Erasmy M, et al (2018) Bat Community and Roost Site Selection of Tree-Dwelling Bats in a Well-Preserved European Lowland Forest. *Acta Chiropt* 20:117–127. <https://doi.org/10.3161/15081109ACC2018.20.1.008>
- Domańska-Blicharz K, Milek-Krupa J, Pikuła A (2021) Diversity of coronaviruses in wild representatives of the aves class in 47nthro. *Viruses* 13:1–19. <https://doi.org/10.3390/v13081497>
- Dong N, Fang L, Zeng S, et al (2015) Porcine Deltacoronavirus in mainland China. *Emerg Infect Dis* 21:2254–2255. <https://doi.org/10.3201/EID2112.150283>,
- Drexler JF, Corman VM, Drosten C (2014a) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Res* 101:45–56. <https://doi.org/10.1016/j.antiviral.2013.10.013>
- Drexler JF, Corman VM, Drosten C (2014b) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Res* 101:45–56. <https://doi.org/10.1016/j.antiviral.2013.10.013>
- Drexler JF, Corman VM, Müller MA, et al (2012) Bats host major mammalian paramyxoviruses. *Nat Commun* 3:. <https://doi.org/10.1038/ncomms1796>
- Drosten C, Günther S, Preiser W, et al (2003) Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1967–1976. <https://doi.org/10.1056/NEJMOA030747>,
- Drosten C, Meyer B, Müller MA, et al (2014) Transmission of MERS-Coronavirus in Household Contacts. *New England Journal of Medicine* 371:828–835. <https://doi.org/10.1056/NEJMOA1405858>,
- Drzewnioková P, Festa F, Panzarin V, et al (2021a) Best molecular tools to investigate coronavirus diversity in mammals: A comparison. *Viruses* 13:. <https://doi.org/10.3390/v13101975>
- Drzewnioková P, Festa F, Panzarin V, et al (2021b) Best molecular tools to investigate coronavirus diversity in mammals: A comparison. *Viruses* 13:. <https://doi.org/10.3390/v13101975>

- Du Y, Wang C, Zhang Y (2022) Viral Coinfections. *Viruses* 2022, Vol 14, Page 2645 14:2645. <https://doi.org/10.3390/V14122645>
- Edson D, Field H, McMichael L, et al (2015a) Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PloS One* 10:e0140670. <https://doi.org/10.1371/JOURNAL.PONE.0140670>
- Edson D, Field H, McMichael L, et al (2015b) Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PloS One* 10:e0140670. <https://doi.org/10.1371/JOURNAL.PONE.0140670>
- El-Sayed A, Kamel M (2021) Coronaviruses in humans and animals: the role of bats in viral evolution. *Environmental Science and Pollution Research* 2021 28:16 28:19589–19600. <https://doi.org/10.1007/S11356-021-12553-1>
- Farsani SMJ, Dijkman R, Jebbink MF, et al (2012) The first complete genome sequences of clinical isolates of human coronavirus 229E. *Virus Genes* 45:433–439. <https://doi.org/10.1007/S11262-012-0807-9>,
- Faust CL, McCallum HI, Bloomfield LSP, et al (2018) Pathogen spillover during land conversion. *Ecol Lett* 21:471–483. <https://doi.org/10.1111/ELE.12904>;
- Fehr AR, Perlman S (2015) Coronaviruses: An overview of their replication and pathogenesis. *Coronaviruses: Methods and Protocols* 1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1/TABLES/2
- Field H, Young P, Yob JM, et al (2001) The natural history of Hendra and Nipah viruses. *Microbes Infect* 3:307–314. [https://doi.org/10.1016/S1286-4579\(01\)01384-3](https://doi.org/10.1016/S1286-4579(01)01384-3)
- Figueiroa T, Galvão Bueno M, Bento Moura PE, et al (2025) Alpha and Betacoronavirus Detection in Neotropical Bats from Northeast Brazil Suggests Wide Geographical Distribution and Persistence in Natural Populations. *Animals* 2025, Vol 15, Page 332 15:332. <https://doi.org/10.3390/ANI15030332>
- Findley JS, Wilson DE (1982) Ecological Significance of Chiropteran Morphology. *Ecology of bats* 243–260. https://doi.org/10.1007/978-1-4613-3421-7_6
- Fitzgerald SD, Rautenschlein S, Mahsoub HM, et al (2020) Adenovirus Infections. In: *Diseases of Poultry*. Wiley, pp 321–363
- Frick WF, Kingston T, Flanders J (2020a) A review of the major threats and challenges to global bat conservation. *Ann N Y Acad Sci* 1469:5–25. <https://doi.org/10.1111/nyas.14045>

- Frick WF, Kingston T, Flanders J (2020b) A review of the major threats and challenges to global bat conservation. *Ann N Y Acad Sci* 1469:5–25. <https://doi.org/10.1111/nyas.14045>
- Fritz SA, Bininda-Emonds ORP, Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: Big is bad, but only in the tropics. *Ecol Lett* 12:538–549. <https://doi.org/10.1111/J.1461-0248.2009.01307.X>;
- Galindo-González J (2024) Avoiding novel, unwanted interactions among species to decrease risk of zoonoses. *Conservation Biology* 38:. <https://doi.org/10.1111/COBI.14232>;
- Galindo-González J (2022) Live animal markets: Identifying the origins of emerging infectious diseases. *Curr Opin Environ Sci Health* 25:. <https://doi.org/10.1016/J.COESH.2021.100310>;
- Gangopadhyaya A, Bhukya PL (2023) Factors Contributing to the Emergence of Viral Diseases. In: *Emerging Human Viral Diseases, Volume I*. Springer Nature Singapore, Singapore, pp 3–69
- Gannon MR, Bovard BN (2016) *The Value of Bats : Keystone*
- Ge XY, Li JL, Yang X Lou, et al (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503:535–538. <https://doi.org/10.1038/nature12711>
- Geng R, Zhou P (2021) Severe acute respiratory syndrome (SARS) related coronavirus in bats. *Animal Diseases* 1:1–8. <https://doi.org/10.1186/s44149-021-00004-w>
- Gerow CM, Rapin N, Voordouw MJ, et al (2019) Arousal from hibernation and reactivation of *Eptesicus fuscus* gammaherpesvirus (EfHV) in big brown bats. *Transbound Emerg Dis* 66:1054–1062. <https://doi.org/10.1111/TBED.13102>
- Ghosh P, Jayaram S, Patwardhan D, et al (2021) Diversity of coronavirus receptors. 6–8. <https://doi.org/10.20944/preprints202108.0071.v1>
- Gloza-Rausch F, Ipsen A, Seebens A, et al (2008a) Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14:626–631. <https://doi.org/10.3201/eid1404.071439>
- Gloza-Rausch F, Ipsen A, Seebens A, et al (2008b) Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14:626–631. <https://doi.org/10.3201/eid1404.071439>

- Goffard A, Demanche C, Arthur L, et al (2015) Alphacoronaviruses detected in 50nthro bats are phylogeographically linked to coronaviruses of 50nthropo bats. *Viruses* 7:6279–6290. <https://doi.org/10.3390/v7122937>
- Gonzalez V, Banerjee A (2022) Molecular, ecological, and behavioral drivers of the bat-virus relationship. *iScience* 25:104779. <https://doi.org/10.1016/j.isci.2022.104779>
- Grellet E, L'Hôte I, Goulet A, Imbert I (2022) Replication of the coronavirus genome: A paradox among positive-strand RNA viruses. *Journal of Biological Chemistry* 298:101923. <https://doi.org/10.1016/j.jbc.2022.101923>
- Gupta P, Singh MP, Goyal K, et al (2021a) Bats and viruses: a death-defying friendship. *Virusdisease* 32:467–479. <https://doi.org/10.1007/s13337-021-00716-0>
- Gupta P, Singh MP, Goyal K, et al (2021b) Bats and viruses: a death-defying friendship. *Virusdisease* 32:467–479. <https://doi.org/10.1007/s13337-021-00716-0>
- Gupta R (2005) Recent outbreak of rabies infections in Brazil transmitted by vampire bats. *Euro Surveill* 10:. <https://doi.org/10.2807/ESW.10.45.02831-EN>
- Guruprasad L (2021) Human coronavirus spike protein-host receptor recognition. *Prog Biophys Mol Biol* 161:39–53. <https://doi.org/10.1016/j.pbiomolbio.2020.10.006>
- Guthid S, Hanley KA, Althouse BM, Boots M (2020) Ecological processes underlying the emergence of novel enzootic cycles: Arboviruses in the neotropics as a case study. *PloS Negl Trop Dis* 14:1–22. <https://doi.org/10.1371/JOURNAL.PNTD.0008338>;
- Haagmans BL, Al Dhahiry SHS, Reusken CBEM, et al (2014) Middle East respiratory syndrome coronavirus in dromedary camels: An outbreak investigation. *Lancet Infect Dis* 14:140–145. [https://doi.org/10.1016/S1473-3099\(13\)70690-X](https://doi.org/10.1016/S1473-3099(13)70690-X)
- Han Y, Du J, Su H, et al (2019) Identification of diverse bat alphacoronaviruses and betacoronaviruses in china provides new insights into the evolution and origin of coronavirus-related diseases. *Front Microbiol* 10:454784. <https://doi.org/10.3389/FMICB.2019.01900/BIBTEX>
- Han Y, Xu P, Wang Y, et al (2023) Panoramic analysis of coronaviruses carried by representative bat species in Southern China to better understand the coronavirus sphere. *Nature Communications* 2023 14:1 14:1–20. <https://doi.org/10.1038/s41467-023-41264-z>
- Hanley DE, Bourassa MA, O'Brien JJ, et al (2003) A Quantitative Evaluation of ENSO Indices. *J Clim* 16:1249–1258. [https://doi.org/10.1175/1520-0442\(2003\)16<1249:AQEOEI>2.0.CO;2](https://doi.org/10.1175/1520-0442(2003)16<1249:AQEOEI>2.0.CO;2);

- Hao X, Qin LU, Zhao H (2024) A molecular phylogeny for all 21 families within Chiroptera (bats). *Integr Zool* 19:989–998. <https://doi.org/10.1111/1749-4877.12772>
- Hasday JD, Fairchild KD, Shanholtz C (2000) The role of fever in the infected host. *Microbes Infect* 2:1891–1904. [https://doi.org/10.1016/S1286-4579\(00\)01337-X](https://doi.org/10.1016/S1286-4579(00)01337-X)
- Hayman DTS (2016) Bats as Viral Reservoirs. *Annu Rev Virol* 3:77–99. <https://doi.org/10.1146/annurev-virology-110615-042203>
- Hemnani M, da Silva PG, Thompson G, et al (2024a) Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *Ecohealth*. <https://doi.org/10.1007/s10393-024-01688-5>
- Hemnani M, da Silva PG, Thompson G, et al (2024b) Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *Ecohealth*. <https://doi.org/10.1007/s10393-024-01688-5>
- Hemnani M, Silva PG da, Thompson G, et al (2023a) First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* 15:1–12. <https://doi.org/10.3390/v15071521>
- Hemnani M, Silva PG da, Thompson G, et al (2023b) First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* 15:1–12. <https://doi.org/10.3390/v15071521>
- Herrera JM, Costa P, Medinas D, et al (2015) Community composition and activity of insectivorous bats in Mediterranean olive farms. *Anim Conserv* 18:557–566. <https://doi.org/10.1111/ACV.12209/SUPPINFO>
- Hildebrandt E, Penzes JJ, Gifford RJ, et al (2020) Evolution of dependoparvoviruses across geological timescales—implications for design of AAV-based gene therapy vectors. *Virus Evol* 6:. <https://doi.org/10.1093/ve/veaa043>
- Hofmann H, Pyrc K, Van Der Hoek L, et al (2005) Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci U S A* 102:7988–7993. <https://doi.org/10.1073/PNAS.0409465102>,
- Honorato R V, Koukos PI, Jiménez-García B, et al (2021) Structural Biology in the Clouds: The WeNMR-EOSC Ecosystem. *Front Mol Biosci* 8
- Honorato R V, Trellet ME, Jiménez-García B, et al (2024) The HADDOCK2.4 web server for integrative 51nthropoc of biomolecular complexes. *Nat Protoc* 19:3219–3241. <https://doi.org/10.1038/s41596-024-01011-0>

- Huang YW, Dickerman AW, Piñeyro P, et al (2013) Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the united states. *mBio* 4:737–750. https://doi.org/10.1128/MBIO.00737-13/SUPPL_FILE/MBO005131645ST2.DOCX
- Hulva P, Horáček I, Strelkov PP, Benda P (2004) Molecular architecture of *Pipistrellus pipistrellus*/*Pipistrellus pygmaeus* complex (Chiroptera: Vespertilionidae): Further cryptic species and Mediterranean origin of the divergence. *Mol Phylogenet Evol* 32:1023–1035. <https://doi.org/10.1016/j.ympev.2004.04.007>
- Ingala M, Fernandes H, De Oliveira M, et al (2025) Bats in Habitats, Bats as Habitats: An integrative ecological framework for understanding synergistic interactions across levels of community organization. <https://doi.org/10.32942/X2RW59>
- Irving AT, Ahn M, Goh G, et al (2021a) Lessons from the host defences of bats, a unique viral reservoir. *Nature* 589:363–370. <https://doi.org/10.1038/s41586-020-03128-0>
- Irving AT, Ahn M, Goh G, et al (2021b) Lessons from the host defences of bats, a unique viral reservoir. *Nature* 589:363–370. <https://doi.org/10.1038/s41586-020-03128-0>
- Islam MS, Sazzad HMS, Satter SM, et al (2016) Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011–2014. *Emerg Infect Dis* 22:664. <https://doi.org/10.3201/EID2204.151747>
- Janetanakit T, Lumyai M, Bunpapong N, et al (2016) Porcine deltacoronavirus, Thailand, 2015. *Emerg Infect Dis* 22:757–759. <https://doi.org/10.3201/EID2204.151852>,
- Jebb D, Foley NM, Whelan C V., et al (2018) Population level mitogenomics of long-lived bats reveals dynamic heteroplasmy and challenges the Free Radical Theory of Ageing. *Scientific Reports* 2018 8:1 8:1–12. <https://doi.org/10.1038/s41598-018-31093-2>
- Ji W, Peng Q, Fang X, et al (2022) Structures of a deltacoronavirus spike protein bound to porcine and human receptors. *Nat Commun* 13:. <https://doi.org/10.1038/s41467-022-29062-5>
- Jones BD, Kaufman EJ, Peel AJ (2023) Viral Co-Infection in Bats: A Systematic Review. *Viruses* 15:. <https://doi.org/10.3390/v15091860>
- Jones DN, Ravelomanantsoa NAF, Yeoman CJ, et al (2022) Do gastrointestinal microbiomes play a role in bats' unique viral hosting capacity? *Trends Microbiol* 30:632–642. <https://doi.org/10.1016/J.TIM.2021.12.009/ASSET/BF65FB14-03F2-4E41-9A61-77CCA8033999/MAIN.ASSETS/GR2.JPG>
- Jones G, Jacobs D, Kunz T, et al (2009) Carpe noctem: the importance of bats as bioindicators. *Endanger Species Res* 8:93–115. <https://doi.org/10.3354/esr00182>

- Jones G, Teeling EC (2006) The evolution of echolocation in bats. *Trends Ecol Evol* 21:149–156. <https://doi.org/10.1016/J.TREE.2006.01.001/ATTACHMENT/C0C308DE-5941-4D66-AA93-3F77C85F48FF/MMC1.PDF>
- Kamins AO, Restif O, Ntiamoa-Baidu Y, et al (2011) Uncovering the fruit bat bushmeat commodity chain and the true extent of fruit bat hunting in Ghana, West Africa. *Biol Conserv* 144:3000–3008. <https://doi.org/10.1016/J.BIOCON.2011.09.003>
- Kasso M, Balakrishnan M (2013) Ecological and Economic Importance of Bats (Order Chiroptera). *ISRN Biodiversity* 2013:1–9. <https://doi.org/10.1155/2013/187415>
- Kim N, Ma J, Kim W, et al (2024) Genome-resolved metagenomics: a game changer for microbiome medicine. *Exp Mol Med* 56:1501–1512. <https://doi.org/10.1038/s12276-024-01262-7>
- Kim SH, Chang SY, Sung M, et al (2016) Extensive Viable Middle East Respiratory Syndrome (MERS) Coronavirus Contamination in Air and Surrounding Environment in MERS Isolation Wards. *Clinical Infectious Diseases* 63:363–369. <https://doi.org/10.1093/cid/ciw239>
- Korine C, Oelbaum PJ, Muñoz-Garcia A (2022) Stress Physiology, Foraging, and Ecophysiology of Bats in Urban Environments. 3–18. https://doi.org/10.1007/978-3-031-13173-8_1
- Kramer LD, Tavakoli NP (2021) VIRUSES OF TERRESTRIAL MAMMALS. In: *Studies in Viral Ecology*. Wiley, pp 541–583
- Ksiazek TG, Erdman D, Goldsmith CS, et al (2003a) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1953–1966. <https://doi.org/10.1056/NEJMOA030781>,
- Ksiazek TG, Erdman D, Goldsmith CS, et al (2003b) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1953–1966. <https://doi.org/10.1056/NEJMOA030781>,
- Kunz TH (1982a) Roosting ecology of bats. *Ecology of bats* 1–55. https://doi.org/10.1007/978-1-4613-3421-7_1
- Kunz TH (ed) (1982b) *Ecology of Bats*. <https://doi.org/10.1007/978-1-4613-3421-7>
- Kunz TH, de Torrez EB, Bauer D, et al (2011) Ecosystem services provided by bats. *Ann N Y Acad Sci* 1223:1–38. <https://doi.org/10.1111/j.1749-6632.2011.06004.x>
- Kunz TH., Fenton Mbrock (2003a) *Bat ecology*. 779
- Kunz TH., Fenton Mbrock (2003b) *Bat ecology*. 779

- Latinne A, Morand S (2022) Climate Anomalies and Spillover of Bat-Borne Viral Diseases in the Asia-Pacific Region and the Arabian Peninsula. *Viruses* 14:. <https://doi.org/10.3390/V14051100>;
- Lau SKP, Luk HKH, Wong ACP, et al (2019) Identification of a novel betacoronavirus (merbecovirus) in amur hedgehogs from China. *Viruses* 11:. <https://doi.org/10.3390/V11110980>,
- Lau SKP, Wong EYM, Tsang C-C, et al (2018a) Discovery and Sequence Analysis of Four Deltacoronaviruses from Birds in the Middle East Reveal Interspecies Jumping with Recombination as a Potential Mechanism for Avian-to-Avian and Avian-to-Mammalian Transmission. *J Virol* 92:. <https://doi.org/10.1128/JVI.00265-18>,
- Lau SKP, Woo PCY, Li KSM, et al (2005) Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 102:14040–14045. <https://doi.org/10.1073/PNAS.0506735102>,
- Lau SKP, Zhang L, Luk HKH, et al (2018b) Receptor usage of a novel bat lineage C betacoronavirus reveals evolution of middle east respiratory syndrome-related coronavirus spike proteins for human dipeptidyl peptidase 4 binding. *Journal of Infectious Diseases* 218:197–207. <https://doi.org/10.1093/INFDIS/JIY018>,
- Lazov CM, Chriél M, Baagøe HJ, et al (2018) Detection and characterization of distinct alphacoronaviruses in five different bat species in Denmark. *Viruses* 10:. <https://doi.org/10.3390/v10090486>
- Le VP, Song S, An BH, et al (2018) A novel strain of porcine deltacoronavirus in Vietnam. *Arch Virol* 163:203–207. <https://doi.org/10.1007/S00705-017-3594-8>,
- Lednicky J, Lauzard M, Fan ZH, et al (2020) Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *International Journal of Infectious Diseases* 100:476–482. <https://doi.org/10.1016/j.ijid.2020.09.025>
- Lednicky JA, Tagliamonte MS, White SK, et al (2021) Independent infections of porcine deltacoronavirus among Haitian children. *Nature* 600:133–137. <https://doi.org/10.1038/S41586-021-04111-Z>,
- Lee JH, Chung HC, Nguyen VG, et al (2016) Detection and Phylogenetic Analysis of Porcine Deltacoronavirus in Korean Swine Farms, 2015. *Transbound Emerg Dis* 63:248–252. <https://doi.org/10.1111/TBED.12490>,
- Lee YF, McCracken GF (2002) Foraging activity and food resource use of Brazilian free-tailed bats, *Tadarida brasiliensis* (Molossidae). *Ecoscience* 9:306–313.

- <https://doi.org/10.1080/11956860.2002.11682717>;WGROUP:STRING:PUBLICATION
- Lelli D, Papetti A, Sabelli C, et al (2013) Detection of coronaviruses in bats of various species in Italy. *Viruses* 5:2679–2689. <https://doi.org/10.3390/v5112679>
- Li F, Li W, Farzan M, Harrison SC (2005a) Structural biology: Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* (1979) 309:1864–1868. <https://doi.org/10.1126/SCIENCE.1116480>,
- Li W, Shi Z, Yu M, et al (2005b) Bats are natural reservoirs of SARS-like coronaviruses. *Science* (1979) 310:676–679. <https://doi.org/10.1126/SCIENCE.1118391>,
- Lindenmayer DB, Laurance WF (2017) The ecology, distribution, conservation and management of large old trees. *Biological Reviews* 92:1434–1458. <https://doi.org/10.1111/BRV.12290>;WGROUP:STRING:PUBLICATION
- Lu M, Wang X, Ye H, et al (2021) Does public fear that bats spread COVID-19 jeopardize bat conservation? *Biol Conserv* 254:108952. <https://doi.org/10.1016/j.biocon.2021.108952>
- Lu R, Zhao X, Li J, et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* 395:565–574. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8)
- Luby SP, Rahman M, Hossain MJ, et al (2006) Foodborne Transmission of Nipah Virus, Bangladesh. *Emerg Infect Dis* 12:1888. <https://doi.org/10.3201/EID1212.060732>
- Lucero Arteaga F, Miragaya M, Molina N, et al (2023) Identification of coronaviruses in bats and rodents in northern and central Argentina. *Arch Virol* 168:1–7. <https://doi.org/10.1007/s00705-023-05703-y>
- Luis AD, Hayman DTS, O’Shea TJ, et al (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? *Proceedings of the Royal Society B: Biological Sciences* 280:. <https://doi.org/10.1098/rspb.2012.2753>
- Lynteris C (2016) The Prophetic Faculty of Epidemic Photography: Chinese Wet Markets and the Imagination of the Next Pandemic. *Vis Anthropol* 29:118–132. <https://doi.org/10.1080/08949468.2016.1131484>;
- Madapong A, Saeng-chuto K, Lorsirigool A, et al (2016) Complete genome sequence of porcine deltacoronavirus isolated in Thailand in 2015. *Genome Announc* 4:. <https://doi.org/10.1128/GENOMEA.00408-16>,

- Maina JN (2000) What it Takes to Fly: The Structural and Functional Respiratory Refinements in Birds and Bats. *Journal of Experimental Biology* 203:3045–3064. <https://doi.org/10.1242/JEB.203.20.3045>
- Marinello MM, Bernard E (2014) Wing morphology of neotropical bats: A quantitative and qualitative analysis with implications for habitat use. *Can J Zool* 92:141–147. <https://doi.org/10.1139/CJZ-2013-0127;PAGE:STRING:ARTICLE/CHAPTER>
- Martínez-Abraín A, Jiménez J (2016) Anthropogenic 56nthr as incidental substitutes for original habitat. *Conservation Biology* 30:593–598. <https://doi.org/10.1111/COBI.12644;REQUESTEDJOURNAL:JOURNAL:15231739;WGROUPE:STRING:PUBLICATION>
- Mata VA, Da Silva LP, Issimo J Ver, et al (2021) Combining DNA metabarcoding and ecological networks to inform conservation biocontrol by small vertebrate predators. *Ecological Applications* 31:e02457. <https://doi.org/10.1002/EAP.2457>
- Mathias ML, Fonseca C, Rodrigues L, Grilo C, Lopes-Fernandes M, Palmeirim JM, Santos-Reis M, Alves PC, Cabral JÁ FM, Mira A, Eira C, Negrões N, Paupério J, Pita R, Rainho A, Rosalino LM TJ& VJ Livro vermelho dos mamíferos de Portugal continental, 2023rd edn. Fciências.ID, ICNF, Lisboa
- Mekibib B, Ariën KK (2016) Aerosol Transmission of Filoviruses. *Viruses* 2016, Vol 8, Page 148 8:148. <https://doi.org/10.3390/V8050148>
- Méndez-Rodríguez A, Horta P, Zarza H, et al (2024) Surveying Bat-Hosted Adenoviruses and Herpesviruses: A Comprehensive Analysis. *Diversity (Basel)* 16:46. <https://doi.org/10.3390/d16010046>
- Metzner W, Müller R (2016) Ultrasound Production, Emission, and Reception. 55–91. https://doi.org/10.1007/978-1-4939-3527-7_3
- Mihindukulasuriya KA, Wu G, St. Leger J, et al (2008a) Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J Virol* 82:5084–5088. <https://doi.org/10.1128/jvi.02722-07>
- Mihindukulasuriya KA, Wu G, St. Leger J, et al (2008b) Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J Virol* 82:5084–5088. <https://doi.org/10.1128/jvi.02722-07>
- Miller AK, Mifsud JCO, Costa VA, et al (2021) Slippery when wet: Cross-species transmission of divergent coronaviruses in bony and jawless fish and the evolutionary history of the Coronaviridae. *Virus Evol* 7:1–10. <https://doi.org/10.1093/ve/veab050>

- Mo M, Roache M, Davies J, et al (2021) Estimating flying-fox mortality associated with abandonments of pups and extreme heat events during the austral summer of 2019–20. *Pacific Conservation Biology* 28:124–139. <https://doi.org/10.1071/PC21003>
- Monto AS, Cowling BJ, Peiris JSM (2014) Coronaviruses. *Viral Infections of Humans: Epidemiology and Control* 199–223. https://doi.org/10.1007/978-1-4899-7448-8_10/TABLES/3
- Moratelli R, Calisher CH (2015) Bats and zoonotic viruses: Can we confidently link bats with emerging deadly viruses? *Mem Inst Oswaldo Cruz* 110:1–22. <https://doi.org/10.1590/0074-02760150048>
- Morawska L, Milton DK (2020) It is Time to Address Airborne Transmission of COVID-19. *Clinical Infectious Diseases*. <https://doi.org/10.1093/cid/ciaa939>
- Moreno A, Lelli D, De Sabato L, et al (2017) Detection and full genome characterization of two beta CoV viruses related to Middle East respiratory syndrome from bats in Italy. *Virol J* 14:1–11. <https://doi.org/10.1186/s12985-017-0907-1>
- Moussy C, Hosken DJ, Mathews F, et al (2013) Migration and dispersal patterns of bats and their influence on genetic structure. *Mamm Rev* 43:183–195. <https://doi.org/10.1111/J.1365-2907.2012.00218.X>
- Mühldorfer K, Speck S, Kurth A, et al (2011) Diseases and Causes of Death in European Bats: Dynamics in Disease Susceptibility and Infection Rates. *PloS One* 6:e29773. <https://doi.org/10.1371/JOURNAL.PONE.0029773>
- Naguib MM, Li R, Ling J, et al (2021) Live and Wet Markets: Food Access versus the Risk of Disease Emergence. *Trends Microbiol* 29:573–581. <https://doi.org/10.1016/J.TIM.2021.02.007/ASSET/25E2E3F1-49B1-4501-87B4-09B52F7908FC/MAIN.ASSETS/GR1.JPG>
- Nahar N, Asaduzzaman M, Mandal UK, et al (2020) Hunting Bats for Human Consumption in Bangladesh. *Ecohealth* 17:139–151. <https://doi.org/10.1007/S10393-020-01468-X/TABLES/8>
- Nakagawa S, Miyazawa T (2020) Genome evolution of SARS-CoV-2 and its virological characteristics. *Inflamm Regen* 40:0–6. <https://doi.org/10.1186/s41232-020-00126-7>
- Nal B, Chan C, Kien F, et al (2005) Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. *Journal of General Virology* 86:1423–1434. <https://doi.org/10.1099/VIR.0.80671-0/CITE/REFWORKS>

- Neuman BW, Kiss G, Kunding AH, et al (2011) A structural analysis of M protein in coronavirus assembly and morphology. *J Struct Biol* 174:11–22. <https://doi.org/10.1016/J.JSB.2010.11.021>
- Niederwerder MC, Hesse RA (2018) Swine enteric coronavirus disease: A review of 4 years with porcine epidemic diarrhoea virus and porcine deltacoronavirus in the United States and Canada. *Transbound Emerg Dis* 65:660–675. <https://doi.org/10.1111/TBED.12823>
- Nieto-Torres JL, DeDiego ML, Verdía-Báguena C, et al (2014) Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. *PloS Pathog* 10:e1004077. <https://doi.org/10.1371/JOURNAL.PPAT.1004077>
- Nojiri T, Wilson LAB, López-Aguirre C, et al (2021) Embryonic evidence uncovers convergent origins of laryngeal echolocation in bats. *Current Biology* 31:1353–1365.e3. <https://doi.org/10.1016/j.cub.2020.12.043>
- Nusová G, Uhrin M, Voigt CC, Kaňuch P (2020) Tracing the geographic origin of common pipistrelles (*Pipistrellus pipistrellus*) swarming at a mass hibernaculum. *Mammalian Biology* 100:601–610. <https://doi.org/10.1007/s42991-020-00057-3>
- Nyberg T, Ferguson NM, Nash SG, et al (2022) Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *The Lancet* 399:1303–1312. [https://doi.org/10.1016/S0140-6736\(22\)00462-7](https://doi.org/10.1016/S0140-6736(22)00462-7)
- Oedin M, Brescia F, Vidal E, Millon A (2022) Make flying-fox hunting sustainable again: Comparing expected demographic effectiveness and hunters' acceptance of more restrictive regulations. *Ambio* 51:1078–1089. <https://doi.org/10.1007/S13280-021-01630-X/FIGURES/6>
- Olival KJ, Cryan PM, Amman BR, et al (2020) Possibility for reverse zoonotic transmission of sars-cov-2 to free-ranging wildlife: A case study of bats. *PloS Pathog* 16:1–19. <https://doi.org/10.1371/journal.ppat.1008758>
- Olsen SJ, Chang HL, Cheung TYY, et al (2003) Transmission of the Severe Acute Respiratory Syndrome on Aircraft. *New England Journal of Medicine* 349:2416–2422. <https://doi.org/10.1056/NEJMoa031349>
- Openshaw JJ, Hegde S, Sazzad HMS, et al (2017a) Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. *Transbound Emerg Dis* 64:1287–1293. <https://doi.org/10.1111/tbed.12505>

- Openshaw JJ, Hegde S, Sazzad HMS, et al (2017b) Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. *Transbound Emerg Dis* 64:1287–1293. <https://doi.org/10.1111/TBED.12505>
- O’Shea TJ, Cryan PM, Cunningham AA, et al (2014) Bat flight and zoonotic viruses. *Emerg Infect Dis* 20:741–745. <https://doi.org/10.3201/eid2005.130539>
- Page RA, Bernal XE (2020) The challenge of detecting prey: Private and social information use in predatory bats. *Funct Ecol* 34:344–363. <https://doi.org/10.1111/1365-2435.13439/SUPPINFO>
- Papineau A, Berhane Y, Wylie TN, et al (2019) Genome Organization of Canada Goose Coronavirus, A Novel Species Identified in a Mass Die-off of Canada Geese. *Sci Rep* 9:. <https://doi.org/10.1038/S41598-019-42355-Y>,
- Peiris JSM, Guan Y, Yuen KY (2004) Severe acute respiratory syndrome. *Nat Med* 10:S88–S97. <https://doi.org/10.1038/nm1143>
- Pensaert MB, de Bouck P (1978) A new coronavirus-like particle associated with diarrhea in swine. *Arch Virol* 58:243–247. <https://doi.org/10.1007/BF01317606>,
- Perry RW (2013) A review of factors affecting cave climates for hibernating bats in temperate North America. *Environmental Reviews* 21:28–39. <https://doi.org/10.1139/ER-2012-0042;PAGEGROUP:STRING:PUBLICATION>
- Phelps KL, Hamel L, Alhmoud N, et al (2019) Bat research networks and viral surveillance: Gaps and opportunities in western Asia. *Viruses* 11:. <https://doi.org/10.3390/v11030240>
- Phillips AM, Gonzalez LO, Nekongo EE, et al (2017) Host proteostasis modulates influenza evolution. *Elife* 6:. <https://doi.org/10.7554/ELIFE.28652>
- Platto S, Zhou J, Wang Y, et al (2021a) Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease. *Biochem Biophys Res Commun* 538:2–13. <https://doi.org/10.1016/j.bbrc.2020.10.028>
- Platto S, Zhou J, Wang Y, et al (2021b) Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease. *Biochem Biophys Res Commun* 538:2–13. <https://doi.org/10.1016/j.bbrc.2020.10.028>
- Pratelli A, Tempesta M, Elia G, et al (2022) The knotty biology of canine coronavirus: A worrying model of coronaviruses’ danger. *Res Vet Sci* 144:190–195. <https://doi.org/10.1016/J.RVSC.2021.11.014>

- Prentice MB, Gilbertson MLJ, Storm DJ, et al (2024) Metagenomic sequencing sheds light on microbes putatively associated with pneumonia-related fatalities of white-tailed deer (*Odocoileus virginianus*). *Microb Genom* 10:. <https://doi.org/10.1099/mgen.0.001214>
- Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes De Novo Assembler. *Curr Protoc Bioinformatics* 70:1–29. <https://doi.org/10.1002/cpbi.102>
- Pujol FH, Branda F, Ceccarelli G, et al (2025) Nipah Virus: A Zoonotic Threat Re-Emerging in the Wake of Global Public Health Challenges. *Microorganisms* 2025, Vol 13, Page 124 13:124. <https://doi.org/10.3390/MICROORGANISMS13010124>
- Pyankov O V, Bodnev SA, Pyankova OG, Agranovski IE (2018a) Survival of aerosolized coronavirus in the ambient air. *J Aerosol Sci* 115:158–163. <https://doi.org/10.1016/j.jaerosci.2017.09.009>
- Pyankov O V, Bodnev SA, Pyankova OG, Agranovski IE (2018b) Survival of aerosolized coronavirus in the ambient air. *J Aerosol Sci* 115:158–163. <https://doi.org/10.1016/j.jaerosci.2017.09.009>
- Racey PA (2015) The Uniqueness of Bats. *Bats and Viruses: A New Frontier of Emerging Infectious Diseases* 1–22. <https://doi.org/10.1002/9781118818824.CH1>
- Rainho A Summer foraging habitats of bats in a Mediterranean region of the Iberian Peninsula. <https://doi.org/10.3161/150811007781694408>
- Ramanathan K, Antognini D, Combes A, et al (2020) Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. *Journal of Hospital Infection* 92:235–250
- Ramírez-Fráncel LA, García-Herrera LV, Losada-Prado S, et al (2022) Bats and their vital ecosystem services: a global review. *Integr Zool* 17:2–23. <https://doi.org/10.1111/1749-4877.12552>
- Ravi V, Saxena S, Panda PS (2022) Basic virology of SARS-CoV 2. *Indian J Med Microbiol* 40:182–186. <https://doi.org/10.1016/J.IJMMB.2022.02.005>
- Reguera J, Santiago C, Mudgal G, et al (2012) Structural Bases of Coronavirus Attachment to Host Aminopeptidase N and Its Inhibition by Neutralizing Antibodies. *PloS Pathog* 8:e1002859. <https://doi.org/10.1371/journal.ppat.1002859>
- Reusken CBEM, Lina PHC, Pielaat A, et al (2010) Circulation of group 2 coronaviruses in a bat species common to urban areas in Western Europe. *Vector Borne Zoonotic Dis* 10:785–791. <https://doi.org/10.1089/vbz.2009.0173>

- Reyes A, Semenkovich NP, Whiteson K, et al (2012) Going viral: Next-generation sequencing applied to phage populations in the human gut. *Nat Rev Microbiol* 10:607–617. <https://doi.org/10.1038/nrmicro2853>
- Reyes-del Valle J, Chávez-Salinas S, Medina F, del Angel RM (2005) Heat Shock Protein 90 and Heat Shock Protein 70 Are Components of Dengue Virus Receptor Complex in Human Cells. *J Virol* 79:4557–4567. <https://doi.org/10.1128/JVI.79.8.4557-4567.2005/ASSET/D00B8FCF-B4BA-4E36-84D5-BAC18F19286A/ASSETS/GRAPHIC/ZJV0080560520008.JPEG>
- Richard M, Kok A, de Meulder D, et al (2020) SARS-CoV-2 is transmitted via contact and via the air between ferrets. *Nat Commun* 11:. <https://doi.org/10.1038/S41467-020-17367-2>,
- Riskin DK, Racey PA (2010) How do sucker-footed bats hold on, and why do they roost head-up? *Biological Journal of the Linnean Society* 99:233–240. <https://doi.org/10.1111/J.1095-8312.2009.01362.X>
- Rizzo F, Edenborough KM, Toffoli R, et al (2017) Coronavirus and paramyxovirus in bats from Northwest Italy. *BMC Vet Res* 13:1–11. <https://doi.org/10.1186/s12917-017-1307-x>
- Rodrigues L, Zahn A, Rainho A, Palmeirim JM (2003) Contrasting the roosting behaviour and phenology of an insectivorous bat (*Myotis myotis*) in its southern and northern distribution ranges. *Mammalia* 67:321–335. <https://doi.org/10.1515/MAMM.2003.67.3.321/MACHINEREADABLECITATION/RIS>
- Roffler AA, Maurer DP, Lunn TJ, et al (2024) Bat humoral immunity and its role in viral pathogenesis, transmission, and zoonosis. *Front Immunol* 15:1–12. <https://doi.org/10.3389/fimmu.2024.1269760>
- Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun* 109:102433. <https://doi.org/https://doi.org/10.1016/j.jaut.2020.102433>
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022a) Ecology, evolution and spillover of coronaviruses from bats. Springer US
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022b) Ecology, evolution and spillover of coronaviruses from bats. Springer US
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022c) Ecology, evolution and spillover of coronaviruses from bats. Springer US

- Russo D (2023a) Chiroptera., 2023rd edn. Springer
- Russo D (2023b) Chiroptera., 2023rd edn. Springer
- Russo D, Cistrone L, Budinski I, et al (2017) Sociality influences thermoregulation and roost switching in a forest bat using ephemeral roosts. *Ecol Evol* 7:5310–5321. <https://doi.org/10.1002/ECE3.3111>
- Saeng-chuto K, Lorsirigool A, Temeeyasen G, et al (2017) Different Lineage of Porcine Deltacoronavirus in Thailand, Vietnam and Lao PDR in 2015. *Transbound Emerg Dis* 64:3–10. <https://doi.org/10.1111/TBED.12585>,
- Santana SE, Dial TO, Eiting TP, Alfaro ME (2011) Roosting Ecology and the Evolution of Pelage Markings in Bats. *PloS One* 6:e25845. <https://doi.org/10.1371/JOURNAL.PONE.0025845>
- Schnitzler HU, Denzinger A (2024) Foraging strategies of echolocating bats. *A Natural History of Bat Foraging: Evolution, Physiology, Ecology, Behavior, and Conservation* 83–107. <https://doi.org/10.1016/B978-0-323-91820-6.16003-6>
- Schnitzler HU, Moss CF, Denzinger A (2003) From spatial orientation to food acquisition in echolocating bats. *Trends Ecol Evol* 18:386–394. [https://doi.org/10.1016/S0169-5347\(03\)00185-X/ASSET/ECEFFF0C-8B89-45C9-A7C1-D3C758339FC8/MAIN.ASSETS/GR5.GIF](https://doi.org/10.1016/S0169-5347(03)00185-X/ASSET/ECEFFF0C-8B89-45C9-A7C1-D3C758339FC8/MAIN.ASSETS/GR5.GIF)
- Serra-Cobo J, López-Roig M (2017) Bats and emerging infections: An ecological and virological puzzle. *Adv Exp Med Biol* 972:35–48. https://doi.org/10.1007/5584_2016_131
- Shen YY, Liang L, Zhu ZH, et al (2010) Adaptive evolution of energy metabolism genes and the origin of flight in bats. *Proc Natl Acad Sci U S A* 107:8666–8671. https://doi.org/10.1073/PNAS.0912613107/SUPPL_FILE/STXT01.PDF
- Shipley R, Wright E, Selden D, et al (2019) Bats and viruses: Emergence of novel lyssaviruses and association of bats with viral zoonoses in the EU. *Trop Med Infect Dis* 4:1–22. <https://doi.org/10.3390/tropicalmed4010031>
- Silva PG, Branco PTBS, Soares RRG, et al (2022a) SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* 32:e13083. <https://doi.org/https://doi.org/10.1111/ina.13083>
- Silva PG, Branco PTBS, Soares RRG, et al (2022b) SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* 32:e13083. <https://doi.org/https://doi.org/10.1111/ina.13083>

- Simonin Y (2023) Emerging Diseases/Viruses Prevention, Control, Surveillance, and One Health. *Trop Med Infect Dis* 8:257. <https://doi.org/10.3390/tropicalmed8050257>
- Singh R, Kølvrå S, Bross P, et al (2006) Heat-shock protein 70 genes and human longevity: A view from Denmark. *Ann N Y Acad Sci* 1067:301–308. <https://doi.org/10.1196/ANNALS.1354.040;PAGE:STRING:ARTICLE/CHAPTER>
- Smith I, Wang LF (2013) Bats and their virome: An important source of emerging viruses capable of infecting humans. *Curr Opin Virol* 3:84–91. <https://doi.org/10.1016/j.coviro.2012.11.006>
- Smith SH, Somsen GA, Van Rijn C, et al (2020) Aerosol persistence in relation to possible transmission of SARS-CoV-2. *Physics of Fluids* 32:.. <https://doi.org/10.1063/5.0027844>,
- Soler-Tovar D, Escobar LE (2025) Rabies transmitted from vampires to cattle: An overview. *PloS One* 20:e0317214. <https://doi.org/10.1371/JOURNAL.PONE.0317214>
- Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. *Nat Rev Immunol* 2:185–194. <https://doi.org/10.1038/NRI749;KWRD=BIOMEDICINE>
- Stalin Raj V, Farag EABA, Reusken CBEM, et al (2014) Isolation of MERS coronavirus from dromedary camel, Qatar, 2014. *Emerg Infect Dis* 20:1339–1342. <https://doi.org/10.3201/EID2008.140663>,
- Stidsholt L (2025) Unlocking the mysteries of biosonar use and foraging behaviour in wild bats with on-board acoustic recording tags. *Journal of Experimental Biology* 228:.. <https://doi.org/10.1242/JEB.250777/368992>
- Subudhi S, Rapin N, Misra V (2019) Immune System Modulation and Viral Persistence in Bats: Understanding Viral Spillover. *Viruses* 2019, Vol 11, Page 192 11:192. <https://doi.org/10.3390/V11020192>
- Teeling EC, Jones G, Rossiter SJ (2016) Phylogeny, Genes, and Hearing: Implications for the Evolution of Echolocation in Bats. 25–54. https://doi.org/10.1007/978-1-4939-3527-7_2
- Tellier R, Li Y, Cowling BJ, Tang JW (2019) Recognition of aerosol transmission of infectious agents: A commentary. *BMC Infect Dis* 19:1–9. <https://doi.org/10.1186/s12879-019-3707-y>
- Thomas SP, Suthers RA (1972) The Physiology and Energetics of Bat Flight. *Journal of Experimental Biology* 57:317–335. <https://doi.org/10.1242/JEB.57.2.317>

- Tisza M, Javornik Cregeen S, Avadhanula V, et al (2023) Wastewater sequencing reveals community and variant dynamics of the collective human virome. *Nat Commun* 14:1–10. <https://doi.org/10.1038/s41467-023-42064-1>
- Tiwari R, Dhama K, Sharun K, et al (2020) COVID-19: animals, veterinary and zoonotic links. *Veterinary Quarterly* 40:169–182. <https://doi.org/10.1080/01652176.2020.1766725>
- Torquetti CG, Guimarães ATB, Soto-Blanco B (2021) Exposure to pesticides in bats. *Science of The Total Environment* 755:142509. <https://doi.org/10.1016/j.scitotenv.2020.142509>
- T.S. YI, Yuguo L, Wai WT, et al (2024) Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus. *New England Journal of Medicine* 350:1731–1739. <https://doi.org/10.1056/NEJMoa032867>
- Vakulenko Y, Deviatkin A, Drexler JF, Lukashev A (2021) Modular evolution of coronavirus genomes. *Viruses* 13:1270. <https://doi.org/10.3390/V13071270/S1>
- Verreault D, Moineau S, Duchaine C (2008) Methods for Sampling of Airborne Viruses. *Microbiology and Molecular Biology Reviews* 72:413–444. <https://doi.org/10.1128/mmbr.00002-08>
- Vlasova AN, Halpin R, Wang S, et al (2011) Molecular characterization of a new species in the genus Alphacoronavirus associated with mink epizootic catarrhal gastroenteritis. *Journal of General Virology* 92:1369–1379. <https://doi.org/10.1099/VIR.0.025353-0>,
- Voigt CC, Kingston T (2015) *Bats in the 64nthropocene: Conservation of bats in a changing world*. Springer International Publishing
- Wang B, Zhang A, Sun JL, et al (2005) Study of SARS transmission via liquid droplets in air. *J Biomech Eng* 127:32–38. <https://doi.org/10.1115/1.1835350>
- Wang L, Zhang Y, Byrum B (2014) Complete genome sequence of porcine coronavirus HKU15 strain IN2847 from the United States. *Genome Announc* 2:. <https://doi.org/10.1128/GENOMEA.00291-14>,
- Wang LF, Anderson DE (2019) Viruses in bats and potential spillover to animals and humans. *Curr Opin Virol* 34:79–89. <https://doi.org/10.1016/j.coviro.2018.12.007>
- Wang W, Lin XD, Guo WP, et al (2015) Discovery, diversity and evolution of novel coronaviruses sampled from rodents in China. *Virology* 474:19–27. <https://doi.org/10.1016/j.virol.2014.10.017>

- Wang Z, Huang G, Huang M, et al (2023) Global patterns of phylogenetic diversity and transmission of bat coronavirus. *Sci China Life Sci* 66:861–874. <https://doi.org/10.1007/s11427-022-2221-5>
- Waterhouse A, Bertoni M, Bienert S, et al (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46:W296–W303. <https://doi.org/10.1093/nar/gky427>
- Whittaker GR, André NM, Millet JK (2018) Improving Virus Taxonomy by Recontextualizing Sequence-Based Classification with Biologically Relevant Data: the Case of the Alphacoronavirus 1 Species . *mSphere* 3:. <https://doi.org/10.1128/MSPHEREDIRECT.00463-17>,
- WHO (2021) Coronavirus disease (COVID-19): How is it transmitted?
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2021a) Analysis of partial sequences of the RNA-dependent RNA polymerase gene as a tool for genus and subgenus classification of coronaviruses. *Journal of General Virology* 101:1261–1269. <https://doi.org/10.1099/jgv.0.001494>
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2020) Partial RdRp sequences offer a robust method for Coronavirus subgenus classification. *bioRxiv* 2020.03.02.974311. <https://doi.org/10.1101/2020.03.02.974311>
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2021b) Analysis of partial sequences of the RNA-dependent RNA polymerase gene as a tool for genus and subgenus classification of coronaviruses. *Journal of General Virology* 101:1261–1269. <https://doi.org/10.1099/JGV.0.001494/CITE/REFWORKS>
- Wong ACP, Lau SKP, Woo PCY (2021) Interspecies jumping of bat coronaviruses. *Viruses* 13:. <https://doi.org/10.3390/V13112188>,
- Wong S, Lau S, Woo P, Yuen KY (2007a) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91. <https://doi.org/10.1002/rmv.520>
- Wong S, Lau S, Woo P, Yuen KY (2007b) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91. <https://doi.org/10.1002/rmv.520>
- Woo PCY, De Groot RJ, Haagmans B, et al (2023a) ICTV Virus Taxonomy Profile: Coronaviridae 2023. *Journal of General Virology* 104:. <https://doi.org/10.1099/JGV.0.001843>
- Woo PCY, Groot RJ De, Haagmans B, et al (2023b) Family : Coronaviridae. 1–27
- Woo PCY, Lau SKP (2019) Viruses and bats. *Viruses* 11:2–5. <https://doi.org/10.3390/v11100884>

- Woo PCY, Lau SKP, Chu C, et al (2005) Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. *J Virol* 79:884–895. <https://doi.org/10.1128/JVI.79.2.884-895.2005>,
- Woo PCY, Lau SKP, Huang Y, Yuen KY (2009a) Coronavirus diversity, phylogeny and interspecies jumping. *Exp Biol Med* 234:1117–1127. <https://doi.org/10.3181/0903-MR-94>
- Woo PCY, Lau SKP, Lam CSF, et al (2012a) Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavi. *J Virol* 86:3995–4008. <https://doi.org/10.1128/jvi.06540-11>
- Woo PCY, Lau SKP, Lam CSF, et al (2014a) Discovery of a Novel Bottlenose Dolphin Coronavirus Reveals a Distinct Species of Marine Mammal Coronavirus in Gammacoronavirus. *J Virol* 88:1318–1331. <https://doi.org/10.1128/jvi.02351-13>
- Woo PCY, Lau SKP, Lam CSF, et al (2009b) Comparative Analysis of Complete Genome Sequences of Three Avian Coronaviruses Reveals a Novel Group 3c Coronavirus. *J Virol* 83:908–917. <https://doi.org/10.1128/JVI.01977-08>,
- Woo PCY, Lau SKP, Lam CSF, et al (2012b) Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavirus. *J Virol* 86:3995–4008. <https://doi.org/10.1128/JVI.06540-11>,
- Woo PCY, Lau SKP, Teng JLL, et al (2014b) New hepatitis E virus genotype in camels, the Middle East. *Emerg Infect Dis* 20:1044–1048. <https://doi.org/10.3201/eid2006.140140>
- Woo PCY, Lau SKP, Wernery U, et al (2014c) Novel betacoronavirus in dromedaries of the Middle East, 2013. *Emerg Infect Dis* 20:560–572. <https://doi.org/10.3201/EID2004.131769>,
- Woo PCY, Wang M, Lau SKP, et al (2007) Comparative Analysis of Twelve Genomes of Three Novel Group 2c and Group 2d Coronaviruses Reveals Unique Group and Subgroup Features. *J Virol* 81:1574–1585. <https://doi.org/10.1128/JVI.02182-06>,
- Wood JLN, Leach M, Waldman L, et al (2012) A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:2881–2892. <https://doi.org/10.1098/RSTB.2012.0228>

- Wu Z, Lu L, Du J, et al (2018) Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. *Microbiome* 6:. <https://doi.org/10.1186/S40168-018-0554-9>,
- Xiao W, Wang M, Wei W, et al (2004) [Detection of SARS-CoV and RNA on aerosol samples from SARS-patients admitted to hospital]. *Zhonghua Liu Xing Bing Xue Za Zhi* 25:882–885
- Xu Q, Wang F, Jiao W, et al (2023) Virtual Screening-Based Peptides Targeting Spike Protein to Inhibit Porcine Epidemic Diarrhea Virus (PEDV) Infection. *Viruses* 15:381. <https://doi.org/10.3390/v15020381>
- Yang D, Leibowitz JL (2015) The structure and functions of coronavirus genomic 3' and 5' ends. *Virus Res* 206:120–133. <https://doi.org/10.1016/J.VIRUSRES.2015.02.025>
- Yen HL, Sit THC, Brackman CJ, et al (2022) Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. *The Lancet* 399:1070–1078. [https://doi.org/10.1016/S0140-6736\(22\)00326-9](https://doi.org/10.1016/S0140-6736(22)00326-9)
- Yinda CK, Zell R, Deboutte W, et al (2017) Highly diverse population of Picornaviridae and other members of the Picornavirales, in Cameroonian fruit bats. *BMC Genomics* 18:249. <https://doi.org/10.1186/s12864-017-3632-7>
- Yuan Y, Cao D, Zhang Y, et al (2017) Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nature Communications* 2017 8:1 8:1–9. <https://doi.org/10.1038/ncomms15092>
- Zagmajster M (2019) Bats. *Encyclopedia of Caves*, Third Edition 94–101. <https://doi.org/10.1016/B978-0-12-814124-3.00013-3>
- Zaki AM, van Boheemen S, Bestebroer TM, et al (2012) Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New England Journal of Medicine* 367:1814–1820. <https://doi.org/10.1056/NEJMOA1211721>,
- Zhao B, Chen C, Yang X, Li Y (2011) Role of two-way airflow owing to temperature difference in severe acute respiratory syndrome transmission: Revisiting the largest nosocomial severe acute respiratory syndrome outbreak in Hong Kong. *J R Soc Interface* 8:699–710. <https://doi.org/10.1098/rsif.2010.0486>
- Zhou P, Fan H, Lan T, et al (2018) Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* 556:255–259. <https://doi.org/10.1038/s41586-018-0010-9>

- Zhou P, Yang X Lou, Wang XG, et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579:270–273.
<https://doi.org/10.1038/s41586-020-2012-7>
- Zhu N, Zhang D, Wang W, et al (2020a) A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 382:727–733.
<https://doi.org/10.1056/NEJMOA2001017>,
- Zhu N, Zhang D, Wang W, et al (2020b) A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 382:727–733.
<https://doi.org/10.1056/NEJMOA2001017>,
- Zukal J, Pikula J, Bandouchova H (2015) Bats as bioindicators of heavy metal pollution: history and prospect. *Mammalian Biology* 80:220–227.
<https://doi.org/10.1016/j.mambio.2015.01.001>
- Zumla A, Hui DS, Perlman S (2015) Middle East respiratory syndrome. *The Lancet* 386:995–1007. [https://doi.org/10.1016/S0140-6736\(15\)60454-8](https://doi.org/10.1016/S0140-6736(15)60454-8)

Scientific aims

Main goal

The general objective of this PhD thesis is to investigate the presence, diversity, and distribution of CoVs in bat populations and their environments in Portugal, contributing to a better understanding of the mechanisms of viral maintenance, transmission, and potential spillover at the human–animal–environment interface. This research aligns with the second objective of the **Call for PhD Research Studentships Maria de Sousa – *SEAGULL* – Seeking Epidemiological and Virological Answers** Studentship Call, that funded the current PhD thesis: Human-animal interaction, mechanisms of resistance and interspecies transmission of viruses, and the risk of pandemics, by addressing critical gaps in our knowledge of bat-borne CoVs in Portugal and the ecological and environmental factors that may influence their transmission dynamics.

Specific Objectives

To achieve this, the following specific objectives were established:

1. To carry out field sampling of bats in cave and building roosts across central and southern Portugal, collecting biological samples (oral and rectal swabs, feces, and blood) to detect and characterize coronaviruses, and to perform environmental air sampling to assess the potential for airborne virus presence.
2. To investigate the prevalence and genetic characteristics of coronaviruses in crevice- and tree-dwelling bats in Portugal by screening biological samples, and to analyze phylogenetic relationships to better understand habitat-specific differences in viral transmission and the potential risk of spillover events.
3. To conduct metagenomic next-generation sequencing to explore viral diversity and reconstruct coronavirus genomes, to identify other viral families, and to perform in silico protein docking with APN receptors of bats, pigs, and humans to assess potential cross-species transmission and zoonotic risk.

Chapter 2- First Report of *Alphacoronavirus* Circulating in Cavernicolous Bats from Portugal

Contents from this Chapter were adapted from the following review papers:

Hemnani, M., Silva, P. G. D., Thompson, G., Poeta, P., Rebelo, H., & Mesquita, J. R. (2023). First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses*, 15(7), 1521. <https://doi.org/10.3390/v15071521>

First report of Alphacoronavirus circulating in cavernicolous bats from Portugal

Mahima Hemnani ¹, Priscilla Gomes da Silva ^{1,2,3,4,5}, Gertrude Thompson ^{1,6}, Patricia Poeta ^{8,9,10,11}, Hugo Rebelo ^{6,7}, João R. Mesquita ^{1,2*}

¹ School of Medicine and Biomedical Sciences, Porto University, 4050-313 Porto, Portugal; up202110040@edu.icbas.up.pt (M.H)

² Epidemiology Research Unit (EPIunit), Institute of Public Health, University of Porto, Porto, Portugal; up202002072@edu.icbas.up.pt (PGdS)

³ Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal

⁴ LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

⁵ ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

⁶ Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, 4485-661 Vairão, Portugal

⁷ ESS, Instituto Politécnico de Setúbal, 2910-761 Setúbal, Portugal

⁸ Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os Montes e Alto Douro, 5000-801 Vila Real, Portugal

⁹ Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, 1099-085 Caparica, Portugal

¹⁰ Veterinary and Animal Research Centre (CECAV), University of Trás-os Montes e Alto Douro, 5000-801 Vila Real, Portugal

¹¹ Veterinary and Animal Research Centre, Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), Vila Real, Portugal

* Correspondence: jrmesquita@icbas.up.pt

Abstract: The emergence of novel coronaviruses (CoVs) has emphasized the need to understand their diversity and distribution in animal populations. Bats have been identified as crucial reservoirs for CoVs, and they are found in various bat species worldwide. In this study, we investigated the presence of CoVs of four cavernicolous bats in six locations in the center and south of Portugal. We collected faeces, anal, and buccal swab samples, as well as air samples from the locations using a Coriolis air sampler. Our results indicate that CoVs were more readily detected in faecal samples compared to anal and buccal swab samples. No CoVs were detected in the air samples. Phylogenetic analysis showed that the detected viruses belong to the Alphacoronavirus genus. This study represents the first report of Alphacoronaviruses circulating in bats in Portugal and highlights the importance of continuous surveillance for novel CoVs in bat populations globally. Ongoing surveillance for CoVs in bat populations is essential as they are a vital source of these viruses. It is crucial to understand the ecological relationships between animals, humans, and the environment to prevent and control the emergence and transmission of infectious diseases. Further ecological studies are needed to investigate the factors contributing to the emergence and transmission of zoonotic viruses.

Keywords: Coronavirus; cavernicolous bats; Alphacoronavirus; Portugal

Resumo com as principais conclusões

Este capítulo relata a circulação de AlphaCoVs em morcegos cavernícolas de Portugal, permitiu a documentação desses vírus pela primeira vez no país, expandindo assim a distribuição geográfica conhecida dessas linhagens. A predominância de detecções em amostras fecais, em comparação com zaragatoas anais e orais, indica que o trato gastrointestinal representa o principal local de replicação e eliminação viral, sustentando uma rota de transmissão entérica–oral consistente com relatos anteriores de disseminação ambiental via excretas de morcegos. Além disso, os AlphaCoVs foram identificados em várias espécies de morcegos, incluindo *Myotis myotis*, *Miniopterus schreibersii*, *Rhinolophus mehelyi* e *R. ferrumequinum*, evidenciando um amplo espectro de hospedeiros dentro da ordem Chiroptera e sugerindo dinâmicas ativas de transmissão interespecífica.

A análise filogenética revelou ainda um alto grau de similaridade genética (93–100% de identidade) entre as estirpes portuguesas e aquelas previamente descritas em morcegos da Bulgária, Itália e Espanha, destacando uma conectividade viral regional e potenciais vias de disseminação transfronteiriça. Apesar do uso de um amostrador de ar Coriolis, nenhum RNA viral foi detectado em amostras de ar, limitação provavelmente associada a fatores metodológicos, como tempo de amostragem sub ótimas ou características intrínsecas do dispositivo baseado em ciclone, apontando para a necessidade de abordagens aprimoradas em futuras vigilâncias virais aéreas.

Em conjunto, esses achados reforçam o papel ecológico dos morcegos como reservatórios de coronavírus, moldado por suas adaptações fisiológicas únicas, estruturas sociais e tamanhos de colônia elevados, que favorecem a manutenção e propagação viral. Além disso, enfatizam a importância da vigilância virológica contínua para uma melhor compreensão da evolução, transmissão e risco zoonótico dos CoVs.

1. Introduction

Coronaviruses (CoVs) are viruses belonging to the order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae. They are positive sense RNA viruses, with one of the largest genomes within RNA viruses [1]. They have an envelope with structures protruding from the surface called “spikes” [2]. CoVs have diverse animal hosts ranging from mammals to bird species, mainly causing enteric and respiratory diseases of varying severity [3] and are classified into four genera [4]. Alpha (α) and Beta (β) CoVs, which commonly cause disease in mammals, are considered pathogenic viruses. On the other hand, Gamma (γ) and Delta (δ) CoVs, also known as avian CoVs [5], evolved from CoVs originating from birds, mostly causing disease in avian species [6–8]. Due to their long genomes of around 30kb, high recombination frequency and high mutation rates [9,10], CoVs have the potential to adapt to new host species with altered pathogenicity, without sacrificing important elements to continue viable, causing a broad spectrum of diseases [11].

Furthermore, The most iconic examples of viral spillover to humans occurred in 2002/2003, when a highly pathogenic human CoV causing severe acute respiratory syndrome emerged in China (SARS-CoV), causing outbreaks worldwide [12], and in 2012, when another CoV emerged in the Arabian Peninsula, the Middle East Respiratory Syndrome (MERS-CoV), also causing severe acute respiratory syndrome [13]. At the end of 2019, another CoV named SARS-CoV-2 emerged in the city of Wuhan, in China, and has since then been the causative agent of the COVID-19 pandemic [14].

Many studies have repeatedly pointed to bats as the natural and primary reservoirs of various viruses that are closely related to other mammalian coronaviruses (CoVs), shedding insight on the critical role that bats play in CoV transmission and evolution, and also highlighting these animals as a significant source of viral diversity and potential spillover events to other mammals, including humans [11]. Bats are found to be hosts of at least 30 different CoVs with complete genome sequences available, and many more considering those without whole-genome sequences available [14]. In this way, they are considered the mammals hosting the highest number of CoVs [1] and the evolutionary source for several human CoVs [15].

Regardless, studies have shown that bats have special traits that allow them to replicate and excrete viruses that are lethal to other mammals without displaying severe clinical indications of disease [4]. They have genetic changes in their immune system, which can protect them from the toxic development of infectious pathologies or prevent manifestation of clinical signs after an infection [1]. Moreover, they can display a decrease of the body

temperature [16], which is a strategy for reduced viral replication and pathogenesis [17], and their ability to coexist with pathogens [18]. Understanding how bats maintain a virus within a population is important for predicting spillover transmission events [4].

Additionally, other ecological characteristics may facilitate viral spread: bats have commensal relationships with viruses, and the bat virome is even associated with enhanced immunity[19]. Social organization in bats also contribute for the maintenance of virus in the population [14]. Several species of bats form large colonies with many individuals, thus facilitating the spread of viruses in bat populations [20].

Characterizing the transmission of pathogens from wildlife to humans is an ongoing and critical scientific challenge. However, this endeavor is often impeded by various limitations, particularly in detecting and studying elusive wild species [21]. Understanding the dynamics of pathogen spillover events and their implications for public health requires overcoming these obstacles to gain a comprehensive understanding of zoonotic diseases [22]. This way, we can better mitigate the risks, improve early detection, and implement effective strategies for preventing and managing potential outbreaks.

Manual handling of bats to collect samples has some technical difficulties, since some roosts are physically inaccessible, and others are toxic or unsafe for humans to explore [23]. Moreover, studies that involve manual capturing these bats or accessing roosts are also disturbing for the bats [23]and they might end up changing roots because of this disturbance, which would be costly for the colony [24]. In one study, a decrease in bat population density was attributed to drastically reduced adult female survival rates, which was a direct result of human disturbance to the bat colony. This low survival or permanent emigration of adult females may be the primary reason for the decline of certain colonies experiencing disturbances, and it can have a significant impact on colony persistence [25].

Additionally, procedures that require accessing caves can be particularly harmful to bats that are hibernating because they can awake them and use up their fat reserves unnecessarily [26]. To overcome these difficulties, a non-invasive sampling technique that does not need direct contact with the bats could be used. Considering that SARS-CoV and MERS-CoV have been reported to be detected in air samples [27], and all the evidence supporting the airborne transmission of SARS-CoV-2 as one of the main drivers of the COVID-19 pandemic [28], studying bat CoVs presence in air might be an alternative non-invasive sampling technique to study CoVs among bat populations, as the viruses carried by them might be present in the air of these animals habitats.

In Europe, to date, there are 25 studies that have evaluated the presence of CoVs in bats: six studies in Italy, four in Germany, two in Holland, two in Ukraine and one in Belgium, Bulgaria, Denmark, Finland, France, Hungary, Luxembourg, Romania, Slovenia, Spain, Sweden, Poland and the United Kingdom . So far, there are no studies on the presence of CoVs in bats and in their environment in Portugal. Hence, the primary objective of this study is to investigate the presence and genetic features of CoVs in various types of bat cavernicolous roosts across Portugal. The study aims to comprehensively examine the occurrence and diversity of CoVs in different bat habitats, ranging from natural cave systems to large buildings.

To achieve this, a prospective sampling and testing was conducted, targeting a diverse array of bat roosts distributed throughout the country. Moreover, considering the potential for airborne transmission of known coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2, we also performed air sampling in closed habitat environments to assess the potential presence of bat CoVs in air, as this could pose as an alternative non-invasive method for monitoring bat CoVs that does not involve capturing of the animal to collect clinical samples. This information will contribute to the broader understanding of CoV diversity, their circulation patterns, and the potential for spillover events. The findings of this study can also have significant implications for both bat conservation and public health.

2. Materials and Methods

Sampling location

Air and bat sampling were carried out during July 2022, at six locations in the center and south of Portugal, namely two large historical buildings and four caves in the municipalities of Montemor-o-Velho, Pombal, Tomar and Moura (Figure 1).

Air sampling was performed in each of these locations using a Coriolis Compact® air sampler. The sampler was placed in the middle of each cave, at approximately 1.3m height. Each sampling was performed for 60min with a 50L/min airflow rate. After sampling, 4mL of PBS was added to the sampling cones and the samples were immediately stored at 4°C for transport to the laboratory until further processing.

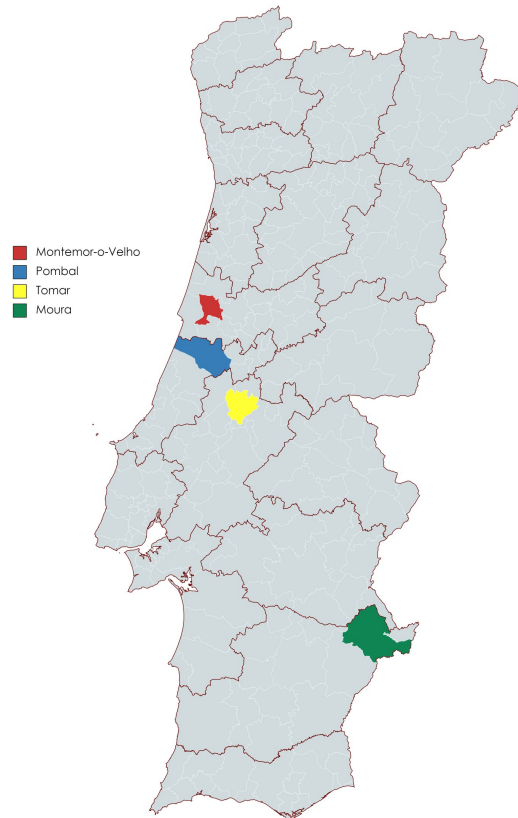


Figure 1. Selected sampling locations in Portugal used in this study.

Bats were captured using hand nets to collect specimens for study. We captured a total of 42 bats, belonging to three different genera and four different species: *Myotis myotis*, *Miniopterus schreibersii*, *Rhinolophus mehelyi*, and *Rhinolophus ferrumequinum*. The captured bats were handled carefully to ensure their well-being throughout the process and morphological identification was done by expert Hugo Rebelo

During the handling procedure, anal swabs were taken from each bat, resulting in a total of 42 anal swab samples. Additionally, buccal swabs were also collected from all 42 bats. Furthermore, whenever feces were shed during the collection procedure, they were collected as well, resulting in a total of 14 stool samples. Overall, we obtained a comprehensive set of 98 samples, consisting of anal swabs, buccal swabs, and stool samples from the captured bats. Details of the samples collected can be found in table 1.

Table 1: Details of the species found according to each location, number of individuals found in each location, matrices collected and quantity of each matrices collected.

Location	Species	Number of individuals found in each location	Matrices collected		
			Stool samples	Anal swab	Buccal swab
Pombal	<i>Myotis myotis</i>	1	0	1	1
Tomar	<i>Miniopterus schreibersii</i>	7	1	7	7
	<i>Rhinolophus mehelyi</i>	2	1	2	2
	<i>Myotis myotis</i>	5	3	5	5
Moura	<i>Miniopterus schreibersii</i>	7	2	7	7
	<i>Rhinolophus mehelyi</i>	6	1	6	6
	<i>Myotis myotis</i>	9	4	9	9
	<i>Rhinolophus ferrumequinum</i>	5	2	5	5
Total		42	14	42	42

After the collection, the bats were promptly released back into their natural habitat. This is important to minimize any disturbance to their normal behavior and preserve the integrity of the study population. All procedures related to bat capture and handling were carried out in strict compliance with the permits issued by the Instituto da Conservação da Natureza e Florestas, ensuring adherence to the regulations and guidelines set forth by the conservation authority.

Screening for coronaviruses

The samples were stored at -20°C until further processing. Anal and buccal swabs were homogenized by vortexing in 500 µL of PBS pH 7.2. RNA was extracted from the faecal suspension using the QIAamp viral mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions using 140 µl of the cleared supernatants (after 1400g for 2 minutes). The eluted RNA was then kept at -80°C until further processing.

The extracted RNA was tested for CoVs using a broad-spectrum pan-CoV nested RT-PCR assay targeting the conserved region of RNA-dependent RNA polymerase (RdRp) with a final product size of 440 bp [10]. The sensitivity of the nested pan-CoV primers has been evaluated by comparing them with various protocols. This evaluation involved combining primers from different studies to achieve optimal performance. The aim was to enhance the chances of detecting both known and unknown coronaviruses from diverse sample sources [10]. It has been reported that utilizing a small partial region of the RdRp (RNA-dependent

RNA polymerase) of coronaviruses is adequate for determining subgenus-level taxonomic classifications. This classification accuracy is comparable to that achieved using complete genome sequences [29]. For the first round of PCR, we used the One-Step RT-PCR kit (GRiSP®, Porto, Portugal). Amplification reactions with positive and negative controls were performed in Veriti 96 Well Thermal with the following conditions: initial cycle of 3 min at 95°C (enzymatic activation, denaturation of the DNA template), followed by 40 cycles at 95°C for 15 s, 50°C for 15 s, and 72°C for 2 s, with a final elongation at 72 °C for 10 min. For the second run, 2µL of the first run products were used as templates in the Xpert Fast Hotstart Mastermix (2x) with dye (GriSP®, Porto, Portugal). PCR was performed in a final volume of 25µL. The amplification reactions with positive and negative controls were carried out in the same thermocycler with the following conditions: initial cycle of 3 min at 95°C (enzymatic activation, denaturation of the DNA template), followed by 40 cycles at 95°C for 15 s, 52°C for 15 s and 72°C for 2 s, with a final elongation at 72 °C for 10 min. PCR amplification products were subjected to electrophoresis at 120V for 30 min on a 1% agarose gel stained with Xpert Green Safe DNA gel stain (Grisp, Porto, Portugal) and then irradiated with UV light to identify target DNA fragments. A DNA weight comparison was used for measurements (100 bp DNA ladder; Grisp, Porto, Portugal).

Sanger sequencing and phylogenetic analysis

Positive amplicons were then purified with the GRS PCR Purification Kit (Grisp, Porto, Portugal) and, using Sanger sequencing, bidirectional sequencing was performed with the specific primers of the target gene. The sequences were then aligned with the software package BioEdit Sequence Alignment Editor v7.1.9, version 2.1 (Ibis Biosciences, Carlsbad, CA, USA) and compared with the sequences available in the NCBI nucleotide database (GenBank, Carlsbad, CA, USA) (<http://blast.ncbi.nlm.nih.gov/Blast>). The sequences obtained were included for phylogenetic analysis and submitted to GenBank under the accession numbers (OQ613363- OQ613369).

These sequences, together with 41 reference strains from the 4 CoV genera (Alpha-, Beta-, Gamma-, and Deltacoronavirus) obtained from GenBank, were aligned using MEGA 11 software [30]. Models function on MEGA 11 was used to opt for the model with the smallest Bayesian information criterion (BIC) score [31] using the maximum likelihood method, based on the general time reversible model using a discrete Gamma distribution and assuming evolutionarily invariable sites, 1000 bootstraps replicated, followed by editing with the Interactive Tree of Life (iTOL) platform [32].

3. Results

In the study, a total of six air samples were collected and none tested positive for CoVs. However, out of the 98 samples obtained from bats, seven samples (8.87%) exhibited amplicons of the expected size. These seven samples were further analyzed through bidirectional sequencing and nucleotide BLAST analysis. The results revealed that all seven samples were characterized as *Alphacoronavirus*.

Interestingly, although a smaller number of stool samples were analyzed compared to anal and buccal swabs, the stool matrix yielded the highest number of positive results with six samples testing positive. In contrast, only one anal swab and no buccal swabs showed positive results. It is worth noting that the anal swab that exhibited a positive result also corresponded to a positive stool sample, both obtained from a *Miniopterus schreibersii* (AN25 and F25).

Overall, the identified CoVs were found in different bat species. Two samples were from *Myotis myotis*, three from *Miniopterus schreibersii*, one from *Rhinolophus mehelyi*, and one from *Rhinolophus ferrumequinum*. For further information and specific sample details, refer to Table 1 of the study.

Table 2. Details of the positive samples from this study, where F represents feces samples, and AN represents anal swabs.

Collection site	Sample ID	Host species	Acession number	Shared identity
Tomar	F5	<i>Myotis myotis</i>	OQ613369	<i>Miniopterus schreibersii</i> -Bulgaria-GU190240.1
	F8	<i>Miniopterus schreibersii</i>	OQ613367	<i>Miniopterus schreibersii</i> -Italy-ON834690.1
	F11	<i>Rhinolophus mehelyi</i>	OQ613367	<i>Miniopterus schreibersii</i> -Bulgaria-GU190240.1

Moura	F15	<i>Myotis. Myotis</i>	OQ613368	<i>Miniopterus schreibersii</i> - Bulgaria- GU190240.1
	F25	<i>Miniopterus schreibersii</i>	OQ613365	<i>Hypsugo savii</i> - Spain- HQ184061.1
	AN25	<i>Miniopterus schreibersii</i>	OQ613364	<i>Miniopterus schreibersii</i> - Bulgaria- GU190240.1
	F30	<i>Rhinolophus ferrumequinum</i>	OQ613363	<i>Miniopterus schreibersii</i> - Bulgaria- GU190240.1

Sequence analysis conducted on the acquired CoV sequences revealed significant similarities to sequences obtained from bats discovered in Bulgaria, Italy, and Spain. The identities ranged from 93% to 100%, indicating a close relationship between the CoV strains circulating in European bats. Further characterization through BLAST analysis indicated that the sequences exhibited the strongest matches with CoVs identified in *Miniopterus schreibersii* (n=6) and *Hypsugo savii* (n=1) from Bulgaria/Italy and Spain, respectively. To confirm the classification, a phylogenetic analysis was performed using the seven obtained CoV sequences along with 41 reference strains. The analysis affirmed their placement within the *Alphacoronavirus* genus, as depicted in Figure 2.

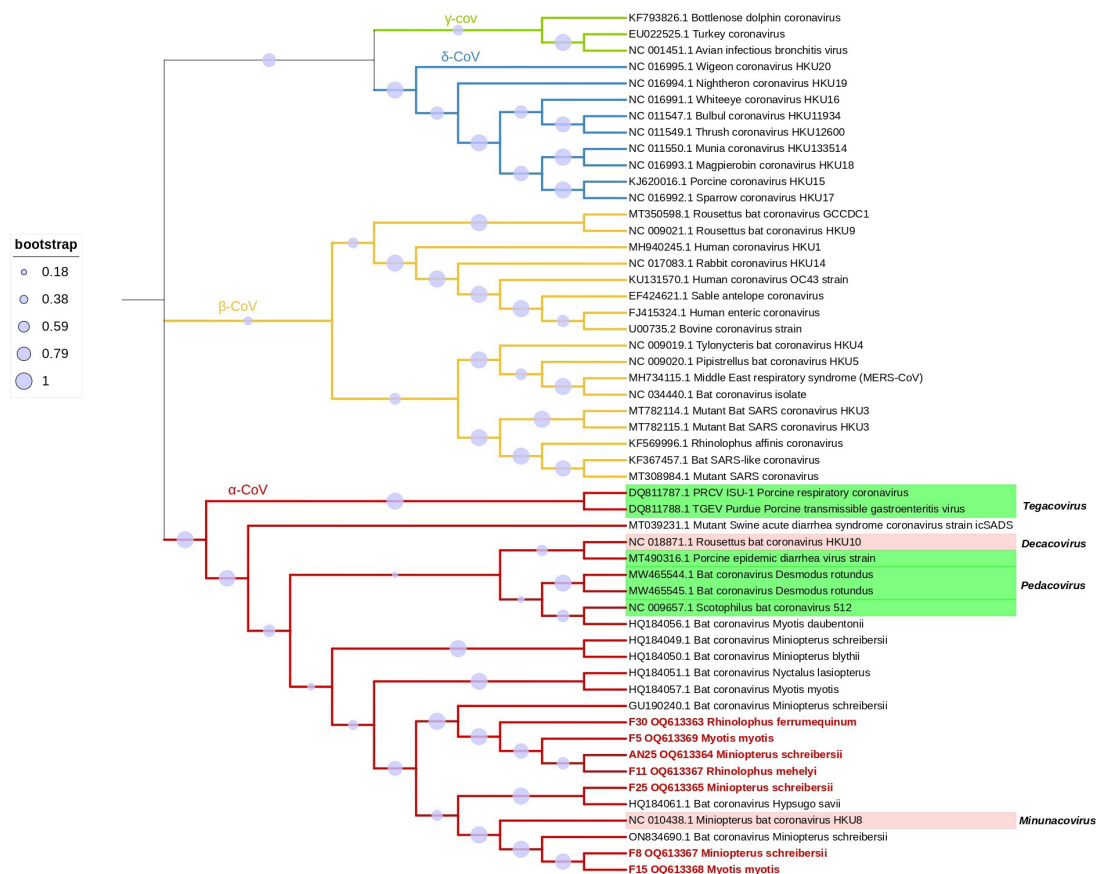


Figure 1. Phylogenetic tree constructed for the alpha, beta, gamma, deltacoronavirus and the alphacoronaviruses subgenus, using 46 reference strains and 7 strains identified in this study. Phylogenetic analysis was based on a 406 nt partial region of the RdRp. The tree was constructed using MEGA 11 and using the maximum likelihood based on the GTR + G + I model, and 1000 bootstraps were replicated. Samples from this study are indicated in red with the description of sample number, GenBank accession number and host bat species.

4. Discussion

In this study we aimed to investigate the circulation of CoVs in two distinct epidemiological aspects: airborne CoVs at bat roosts and CoVs found specifically in cavernicolous bats in Portugal. This study represents the first-ever description of CoVs in bats in the country, providing crucial insights into the viral ecology and diversity of these animals. In total, 42 individuals were screened for CoVs by nested RT-PCR followed by sequencing. In this study, CoVs were not detected in the air. However, the detection was primarily observed in feces samples (n=6), suggesting that virus replication occurs in the gastrointestinal tract, highlighting the potential for faecal-oral transmission routes. The CoV strains found in the

bat populations in our study are closely related to *Alphacoronavirus* strains retrieved from the bat species *M. schreibersii* from Bulgaria and Italy and *Hypsugo savii* from Spain. In the phylogenetic tree based on partial RdRp gene, the sequences in our study clustered with other members of the genus *Alphacoronavirus*, supported by 90% bootstrap value. Our sequences clustered with the same reference strains as indicated in table 2.

The bat species sampled in this study do not migrate over long distances [33], hence no long distance transmission has likely occurred and viruses are probably circulating solely in the studied region. The identified bat CoVs clustered together but not according to the species. The sequences exhibited the strongest matches with CoVs identified in *Miniopterus schreibersii* and *Hypsugo savii* bat species, from Bulgaria/Italy and Spain, respectively, which suggests a potential lack of association between the bat species and the CoV strains under investigation. These findings point towards a broad CoV host range within the Chiroptera order, but further studies characterizing the CoVs full length genomes are necessary in order to make more definitive conclusions. As such, these viruses seem to not evolve within a certain bat species, but instead geographical location appears to have had a greater influence on the evolution and spread.

Our approach for detection and characterization of CoVs has resourced to a partial RdRp region with primers described by [10], because according to the authors, this region was sufficiently informative to allow classification within known CoV genera. The RdRp exhibits a certain degree of sequence conservation across different CoVs subgenus. Focusing on this specific region, we can obtain valuable taxonomic information without the need for analyzing the entire viral genome and is highly effective in determining taxonomic classifications, reaching the subgenus-level [29].

To date, both Alpha and Beta CoVs have been found in bats [14]. Bat CoVs are known to be excreted at higher viral loads in stools, making the enteric route a major environmental source for the CoV spillover events [34]. The CoVs detected in this present study were detected mostly in feces, confirming the enteric route of transmission as the most significant, being consistent with other studies with bats [35,36]. The faecal-oral route has also been described in CoVs from other animals such as with feline coronavirus (FCoV), canine coronavirus (CoV) and swine coronavirus (SADS-CoV) – all of them classified as Alpha-CoV. The replication of CoVs in the enteric location suggests an adaptation of the virus to the bat host's gastrointestinal environment. Further investigations into the mechanisms underlying this viral replication in the gastrointestinal tract may help unravel the unique interplay between the CoVs and the bats' immune system. However, it is worth noting that

the detection of CoVs exclusively in feces samples in this study does not completely rule out the possibility of other modes of transmission, such as respiratory or direct contact routes. Previously, it has been reported the presence of SARS-CoV [37,38] and MERS-CoV [39–41] in air samples, with the discussion of the airborne route of transmission for these human CoVs gaining notoriety during the SARS-CoV-2 pandemic, with many reports of SARS-CoV-2 presence in indoor and outdoor samples throughout the world [27,42–44] and the World Health Organization acknowledging the airborne route as a transmission route for SARS-CoV-2 [45]. In response to the aforementioned findings, we conducted air sampling within bat caves and buildings to investigate the potential presence of CoVs in the air within these environments. Despite our efforts, we were unable to detect any CoVs from the collected air samples. Several factors could have contributed to this lack of detection, and we hypothesize that sampling conditions played a significant role. One potential factor that might have influenced our results is the sampling duration. The duration of air sampling plays a crucial role in capturing an adequate number of airborne particles, including viral particles. If the sampling duration was insufficient, it could have led to a lower likelihood of capturing CoVs present in the air. Little is known on the airborne route of bat CoVs and the possibility of low viral copy excretion, generating aerosols with undetectable loads, could be also the case in bats. Therefore, it is possible that the duration of our air sampling was not optimized for the detection of CoVs, resulting in negative findings.

Additionally, the type of sampler used in this study was cyclone-based. While cyclone-based samplers are commonly employed for air sampling, they might not be the most effective option for capturing CoVs. Notwithstanding, both the choice of air sampler and duration of air sampling has been successfully applied in detecting SARS-CoV-2 , an airborne CoV [27,46,47]. All in all, it is important to acknowledge these limitations and consider alternative sampling strategies for future investigations such as alternative sampling durations and utilizing samplers specifically designed for capturing viral particles that could enhance the sensitivity of CoV detection in the air [48].

When analyzing these results it is also important to emphasize that virus concentrations in air can be low at the place and time of collection, which might yield negative results that do not necessarily mean that there was no virus present in the air at the moment of collection [46]. Further attempts of air sampling in bats environments should be performed with adjustments to the protocol such as longer sampling times, and use of different air samplers that might be more suitable for sampling airborne pathogens, such as samplers using the impinger, filter and water-based condensation methods. Also, our air sampling was done

during a dry summer and there may be other times of the year that could host more favourable climatic conditions for the virus persistence in the air.

Prior to this study, nothing was known about the diversity of CoVs in bats or in the air where bats are found in mainland Portugal where 27 species are acknowledged to occur, and now our results show that there are Alphacoronaviruses circulating in cavernicolous bats from Portugal, and they are closely related to bat species from Bulgaria, Italy and Spain. Anthropogenic changes such as deforestation, habitat fragmentation, land-use, agriculture and urbanization can promote the transmission of infectious diseases by increase the chances of human contact with bats [14]. Since bats are likely to harbour more than one species of viruses at the same time, which might allow the CoVs to incorporate genes from other viral families through recombination events [49], it is important to continue monitoring CoVs in bats and draw the baseline for future surveillance [35] where faecal sampling can play a relevant role, as demonstrated in this study. This may help to better understand the evolution and ecology of this group of viruses, their epidemiology, and the transmission of viruses between bats and between bats and humans and other animals.

5. Conclusions

This manuscript presents significant insights into the prevalence and genetic diversity of coronaviruses (CoVs) in bats and the air of bat roosts in Portugal. These findings are valuable for comprehending the epidemiology and ecology of CoVs, which are critical for effective public health interventions in controlling viral spillover events and spread. Our study identified CoVs in faecal samples from bats, indicating that gastrointestinal transmission is a likely route. This aligns with prior research demonstrating that bat feces play a pivotal role in the environmental shedding of CoVs. The detection of CoVs in multiple bat species suggests that the virus can circulate between different bat populations. It is important to acknowledge the limitations of the sampling conditions and techniques employed as the study did not detect CoVs in air samples from bat roosts. Further investigation is needed to explore the airborne transmission of CoVs and optimize sampling strategies to capture airborne viruses in bat habitats. The manuscript underscores bats' role as natural reservoirs of CoVs, showcasing their ability to replicate and excrete viruses without displaying severe clinical symptoms. Bats possess unique genetic and physiological adaptations that enable them to coexist with pathogens, reducing viral replication and pathogenesis. Additionally, their social organization and large colony sizes facilitate viral spread within bat populations. These findings significantly contribute to the global understanding of CoV ecology and

transmission dynamics. They highlight the importance of ongoing research on bat viromes and the surveillance of CoVs in bats and their environments. Detecting and characterizing transmission events from wildlife to humans remains a substantial scientific challenge, but it is vital for improving public health efforts and mitigating the risk of future viral spillover events. These findings underscore the need for continuous research and surveillance to mitigate the dangers associated with zoonotic diseases, thus expanding our knowledge of CoV ecology and transmission dynamics.

Author Contributions: Conceptualization, Mahima Hemnani, Hugo Rebelo, João Mesquita.; methodology, Mahima Hemnani, Priscilla Gomes da Silva and Hugo Rebelo; formal analysis, Mahima Hemnani, Hugo Rebelo and João Mesquita; investigation, Mahima Hemnani and Priscilla Gomes da Silva; resources, Hugo Rebelo and João Mesquita.; sample collection, Mahima Hemnani, Priscilla Gomes da Silva and Hugo Rebelo; writing—original draft preparation, Mahima Hemnani; writing—review and editing, Mahima Hemnani, Priscilla Gomes da Silva, Hugo Rebelo, Patricia Poeta, Gertrude Thompson and João Mesquita; supervision, Hugo Rebelo and João Mesquita; project administration, Hugo Rebelo, Patricia Poeta, Gertrude Thompson and João Mesquita; funding acquisition, Mahima Hemnani, Hugo Rebelo and João Mesquita. All authors have read and agreed to the published version of the manuscript.

Funding: Mahima Hemnani thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of her PhD work under the Maria de Souza scholarship contract number 2021.09380.BD. This work was also funded by FCT, under the projects UIDB/04750/2020, LA/P/0064/2020, UIDP/00772/2020 and LA/P/0059/2020.

Institutional Review Board Statement: License to capture was obtained from Instituto da Conservação da Natureza e das Florestas (ICNB), Portugal

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bokelmann, M.; Balkema-buschmann, A. Coronaviruses in Bats. *Berl. Munch. Tierarztl. Wochenschr.* **2021**, *134*, 1–16, doi:10.2376/1439-0299-2020-44.
2. Domańska-Blicharz, K.; Miłek-Krupa, J.; Piłkuła, A. Diversity of Coronaviruses in Wild Representatives of the Aves Class in Poland. *Viruses* **2021**, *13*, 1–19, doi:10.3390/v13081497.
3. Balboni, A.; Palladini, A.; Bogliani, G.; Battilani, M. Detection of a Virus Related to Betacoronaviruses in Italian Greater Horseshoe Bats. *Epidemiol. Infect.* **2011**, *139*, 216–219, doi:10.1017/S0950268810001147.
4. Banerjee, A.; Kulcsar, K.; Misra, V.; Frieman, M.; Mossman, K. Bats and Coronaviruses. *Viruses* **2019**, *11*, 7–9, doi:10.3390/v11010041.
5. Gloza-Rausch, F.; Ipsen, A.; Seebens, A.; Götttsche, M.; Panning, M.; Drexler, J.F.; Petersen, N.; Annan, A.; Grywna, K.; Müller, M.; et al. Detection and Prevalence Patterns of Group I Coronaviruses in Bats, Northern Germany. *Emerg. Infect. Dis.* **2008**, *14*, 626–631, doi:10.3201/eid1404.071439.
6. Mihindukulasuriya, K.A.; Wu, G.; St. Leger, J.; Nordhausen, R.W.; Wang, D. Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J. Virol.* **2008**, *82*, 5084–5088, doi:10.1128/jvi.02722-07.
7. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Lau, C.C.Y.; Tsang, A.K.L.; Lau, J.H.N.; Bai, R.; Teng, J.L.L.; Tsang, C.C.C.; Wang, M.; et al. Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavi. *J. Virol.* **2012**, *86*, 3995–4008, doi:10.1128/jvi.06540-11.
8. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Tsang, A.K.L.; Hui, S.-W.; Fan, R.Y.Y.; Martelli, P.; Yuen, K.-Y. Discovery of a Novel Bottlenose Dolphin Coronavirus Reveals a Distinct Species of Marine Mammal Coronavirus in Gammacoronavirus. *J. Virol.* **2014**, *88*, 1318–1331, doi:10.1128/jvi.02351-13.
9. Grellet, E.; L'Hôte, I.; Goulet, A.; Imbert, I. Replication of the Coronavirus Genome: A Paradox among Positive-Strand RNA Viruses. *J. Biol. Chem.* **2022**, *298*, 101923, doi:10.1016/j.jbc.2022.101923.
10. Drzewnioková, P.; Festa, F.; Panzarin, V.; Lelli, D.; Moreno, A.; Zecchin, B.; De Benedictis, P.; Leopardi, S. Best Molecular Tools to Investigate Coronavirus Diversity in Mammals: A Comparison. *Viruses* **2021**, *13*, doi:10.3390/v13101975.

11. Woo, P.C.Y.; Lau, S.K.P.; Huang, Y.; Yuen, K.Y. Coronavirus Diversity, Phylogeny and Interspecies Jumping. *Exp. Biol. Med.* **2009**, *234*, 1117–1127, doi:10.3181/0903-MR-94.
12. Peiris, J.S.M.; Guan, Y.; Yuen, K.Y. Severe Acute Respiratory Syndrome. *Nat. Med.* **2004**, *10*, S88–S97, doi:10.1038/nm1143.
13. Zumla, A.; Hui, D.S.; Perlman, S. Middle East Respiratory Syndrome. *Lancet* **2015**, *386*, 995–1007, doi:10.1016/S0140-6736(15)60454-8.
14. Platto, S.; Zhou, J.; Wang, Y.; Wang, H.; Carafoli, E. Biodiversity Loss and COVID-19 Pandemic: The Role of Bats in the Origin and the Spreading of the Disease. *Biochem. Biophys. Res. Commun.* **2021**, *538*, 2–13, doi:10.1016/j.bbrc.2020.10.028.
15. Ruiz-Aravena, M.; McKee, C.; Gamble, A.; Lunn, T.; Morris, A.; Snedden, C.E.; Yinda, C.K.; Port, J.R.; Buchholz, D.W.; Yeo, Y.Y.; et al. Ecology, Evolution and Spillover of Coronaviruses from Bats; Springer US, 2022; Vol. 20; ISBN 4157902100652.
16. Voigt, C.C.; Kingston, T. Bats in the Anthropocene : Conservation of Bats in a Changing World. Springer Cham, London/NY. Pp. 600.; 2016; ISBN 9783319252186.
17. O'Shea, T.J.; Cryan, P.M.; Cunningham, A.A.; Fooks, A.R.; Hayman, D.T.S.; Luis, A.D.; Peel, A.J.; Plowright, R.K.; Wood, J.L.N. Bat Flight and Zoonotic Viruses. *Emerg. Infect. Dis.* **2014**, *20*, 741–745, doi:10.3201/eid2005.130539.
18. Ahn, M.; Cui, J.; Irving, A.T.; Wang, L.F. Unique Loss of the PYHIN Gene Family in Bats amongst Mammals: Implications for Inflammasome Sensing. *Sci. Rep.* **2016**, *6*, 1–7, doi:10.1038/srep21722.
19. Gupta, P.; Singh, M.P.; Goyal, K.; Tripti, P.; Ansari, M.I.; Obli Rajendran, V.; Dhama, K.; Malik, Y.S. Bats and Viruses: A Death-Defying Friendship. *VirusDisease* **2021**, *32*, 467–479, doi:10.1007/s13337-021-00716-0.
20. Kerth, G. Animal Sociality: Bat Colonies Are Founded by Relatives. *Curr. Biol.* **2008**, *18*, 740–742, doi:10.1016/j.cub.2008.07.058.
21. van Dam, A.; van Engelen, W.; Müller-Mahn, D.; Agha, S.; Junglen, S.; Borgemeister, C.; Bollig, M. Complexities of Multispecies Coexistence: Animal Diseases and Diverging Modes of Ordering at the Wildlife–Livestock Interface in Southern Africa. *Environ. Plan. E Nat. Sp.* **2023**, doi:10.1177/25148486231160637.
22. Johnson, C.K.; Hitchens, P.L.; Pandit, P.S.; Rushmore, J.; Evans, T.S.; Young, C.C.W.; Doyle, M.M. Global Shifts in Mammalian Population Trends Reveal Key Predictors of Virus Spillover Risk. *Proc. R. Soc. B Biol. Sci.* **2020**, *287*, doi:10.1098/rspb.2019.2736.

23. Garrett, N.R.; Watkins, J.; Francis, C.; Simmons, N.B.; Ivanova, N. V; Naaum, A.; Briscoe, A.; Drinkwater, R.; Clare, E.L. Out of Thin Air: Surveying Tropical Bat Roosts through Air Sampling of EDNA. *bioRxiv* **2022**, 2022.11.07.515479, doi:10.7717/peerj.14772.
24. Cardiff, S.G.; Ratrimomanarivo, F.H.; Rembert, G.; Goodman, S.M. Hunting, Disturbance and Roost Persistence of Bats in Caves at Ankarana, Northern Madagascar. *Afr. J. Ecol.* **2009**, 47, 640–649, doi:10.1111/j.1365-2028.2008.01015.x.
25. López-Roig, M.; Serra-Cobo, J. Impact of Human Disturbance, Density, and Environmental Conditions on the Survival Probabilities of Pipistrelle Bat (*Pipistrellus Pipistrellus*). *Popul. Ecol.* **2014**, 56, 471–480, doi:10.1007/s10144-014-0437-2.
26. Society, B.E.; Ecology, A. Effects of Disturbance on the Energy Expenditure of Hibernating Bats Author (s): J . R . Speakman , P . I . Webb and P . A . Racey Published by : British Ecological Society Stable URL : <https://www.jstor.org/stable/2404227>. **1991**, 28, 1087–1104.
27. da Silva, P.G.; Nascimento, M.S.J.; Soares, R.R.G.; Sousa, S.I.V.; Mesquita, J.R. Airborne Spread of Infectious SARS-CoV-2: Moving Forward Using Lessons from SARS-CoV and MERS-CoV. *Sci. Total Environ.* **2021**, 764, doi:10.1016/j.scitotenv.2020.142802.
28. Zhang, R.; Li, Y.; Zhang, A.L.; Wang, Y.; Molina, M.J. Identifying Airborne Transmission as the Dominant Route for the Spread of COVID-19. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, 117, 14857–14863, doi:10.1073/pnas.2009637117.
29. Wilkinson, D.A.; Joffrin, L.; Lebarbenchon, C.; Mavingui, P. Analysis of Partial Sequences of the RNA-Dependent RNA Polymerase Gene as a Tool for Genus and Subgenus Classification of Coronaviruses. *J. Gen. Virol.* **2021**, 101, 1261–1269, doi:10.1099/jgv.0.001494.
30. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, 38, 3022–3027, doi:10.1093/molbev/msab120.
31. Zhang, D.; Kan, X.; Huss, S.E.; Jiang, L.; Chen, L.Q.; Hu, Y. Using Phylogenetic Analysis to Investigate Eukaryotic Gene Origin. *J. Vis. Exp.* **2018**, 2018, doi:10.3791/56684.
32. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v4: Recent Updates and New Developments. *Nucleic Acids Res.* **2019**, 47, 256–259, doi:10.1093/nar/gkz239.
33. Amengual, B.; López-Roig, M.; Serra-Cobo, J. First Record of Seasonal over Sea Migration of *Miniopterus Schreibersii* and *Myotis Capaccinii* between Balearic Islands (Spain). *Acta Chiropterologica* **2007**, 9, 319–322, doi:10.3161/1733-5329(2007)9[319:FROSOS]2.0.CO;2.

34. Wong, S.; Lau, S.; Woo, P.; Yuen, K.Y. Bats as a Continuing Source of Emerging Infections in Humans. *Rev. Med. Virol.* **2007**, *17*, 67–91, doi:10.1002/rmv.520.
35. Alves, R.S.; do Canto Olegário, J.; Weber, M.N.; da Silva, M.S.; Canova, R.; Sauthier, J.T.; Baumbach, L.F.; Witt, A.A.; Varela, A.P.M.; Mayer, F.Q.; et al. Detection of Coronavirus in Vampire Bats (*Desmodus Rotundus*) in Southern Brazil. *Transbound. Emerg. Dis.* **2022**, *69*, 2384–2389, doi:10.1111/tbed.14150.
36. Bonny, T.; Driver, J.; Paisie, T.; Salemi, M.; Morris, J.; Shender, L.; Smith, L.; Enloe, C.; Oxenrider, K.; Gore, J.; et al. Detection of Alphacoronavirus VRNA in the Feces of Brazilian Free-Tailed Bats (*Tadarida Brasiliensis*) from a Colony in Florida, USA. *Diseases* **2017**, *5*, 7, doi:10.3390/diseases5010007.
37. Wang, B.; Zhang, A.; Sun, J.L.; Liu, H.; Hu, J.; Xu, L.X. Study of SARS Transmission via Liquid Droplets in Air. *J. Biomech. Eng.* **2005**, *127*, 32–38, doi:10.1115/1.1835350.
38. Xiao, W.; Wang, M.; Wei, W.; Wang, J.; Zhao, J.; Yi, B.; Li, J. [Detection of SARS-CoV and RNA on aerosol samples from SARS-patients admitted to hospital]. *Zhonghua Liu Xing Bing Xue Za Zhi* **2004**, *25*, 882–885.
39. Pyankov, O. V.; Bodnev, S.A.; Pyankova, O.G.; Agranovski, I.E. Survival of Aerosolized Coronavirus in the Ambient Air. *J. Aerosol Sci.* **2018**, *115*, 158–163, doi:10.1016/j.jaerosci.2017.09.009.
40. Kim, S.H.; Chang, S.Y.; Sung, M.; Park, J.H.; Kim, H. Bin; Lee, H.; Choi, J.P.; Choi, W.S.; Min, J.Y. Extensive Viable Middle East Respiratory Syndrome (MERS) Coronavirus Contamination in Air and Surrounding Environment in MERS Isolation Wards. *Clin. Infect. Dis.* **2016**, *63*, 363–369, doi:10.1093/cid/ciw239.
41. Azhar, E.I.; Hashem, A.M.; El-Kafrawy, S.A.; Sohrab, S.S.; Aburizaiza, A.S.; Farraj, S.A.; Hassan, A.M.; Al-Saeed, M.S.; Jamjoom, G.A.; Madani, T.A. Detection of the Middle East Respiratory Syndrome Coronavirus Genome in an Air Sample Originating from a Camel Barn Owned by an Infected Patient. *MBio* **2014**, *5*, e01450-14, doi:10.1128/mBio.01450-14.
42. da Silva, P.G.; Gonçalves, J.; Nascimento, M.S.; Sousa, S.I. V; Mesquita, J.R. Detection of SARS-CoV-2 in the Indoor and Outdoor Areas of Urban Public Transport Systems of Three Major Cities of Portugal in 2021. *Int. J. Environ. Res. Public Health* **2022**, *19*.
43. Lednický, J.; Lauzard, M.; Fan, Z.H.; Jutla, A.; Tilly, T.; Gangwar, M.; Usmani, M.; Shankar, S.N.; Mohamed, K.; Eiguren-Fernandez, A.; et al. Viable SARS-CoV-2 in the Air

- of a Hospital Room with COVID-19 Patients. *Int. J. Infect. Dis.* **2020**, *100*, 476–482, doi:10.1016/j.ijid.2020.09.025.
44. Morawska, L.; Milton, D.K. It Is Time to Address Airborne Transmission of COVID-19. *Clin. Infect. Dis.* **2020**, doi:10.1093/cid/ciaa939.
 45. WHO Coronavirus Disease (COVID-19): How Is It Transmitted?
 46. Silva, P.G.; Branco, P.T.B.S.; Soares, R.R.G.; Mesquita, J.R.; Sousa, S.I. V SARS-CoV-2 Air Sampling: A Systematic Review on the Methodologies for Detection and Infectivity. *Indoor Air* **2022**, *32*, e13083, doi:https://doi.org/10.1111/ina.13083.
 47. da Silva, P.G.; Mesquita, J.R.; de São José Nascimento, M.; Ferreira, V.A.M. Viral, Host and Environmental Factors That Favor Anthroponotic Spillover of Coronaviruses: An Opinionated Review, Focusing on SARS-CoV, MERS-CoV and SARS-CoV-2. *Sci. Total Environ.* **2021**, *750*, 141483, doi:10.1016/j.scitotenv.2020.141483.
 48. Breshears, L.E.; Nguyen, B.T.; Mata Robles, S.; Wu, L.; Yoon, J.Y. Biosensor Detection of Airborne Respiratory Viruses Such as SARS-CoV-2. *SLAS Technol.* **2022**, *27*, 4–17, doi:10.1016/j.slant.2021.12.004.
 49. Zhou, P.; Fan, H.; Lan, T.; Yang, X. Lou; Shi, W.F.; Zhang, W.; Zhu, Y.; Zhang, Y.W.; Xie, Q.M.; Mani, S.; et al. Fatal Swine Acute Diarrhoea Syndrome Caused by an HKU2-Related Coronavirus of Bat Origin. *Nature* **2018**, *556*, 255–259, doi:10.1038/s41586-018-0010-9.

Chapter 3- Presence of *Alphacoronavirus* in Tree- and Crevice- Dwelling Bats from Portugal

Contents from this Chapter were adapted from the following review papers:

Hemnani, M., da Silva, P. G., Thompson, G., Poeta, P., Rebelo, H., & Mesquita, J. R. (2024). Presence of Alphacoronavirus in Tree-and Crevice-Dwelling Bats from Portugal. *Viruses*, 16(3), 434. <https://doi.org/10.3390/v16030434>

Presence of *Alphacoronavirus* in tree and crevice-dwelling bats from Portugal

Mahima Hemnani¹, **Priscilla Gomes da Silva**^{1,2,3,4,5}, **Gertrude Thompson**^{1,6}, **Patrícia Poeta**^{7,8,9,10}, **Hugo Rebelo**^{6,11} and **João R. Mesquita**^{1,2,3*}

¹ School of Medicine and Biomedical Sciences, Porto University, 4050-313 Porto, Portugal; up202110040@edu.icbas.up.pt (M.H.); up202002072@edu.icbas.up.pt (P.G.d.S.); gathompson@icbas.up.pt (G.T.)

² Epidemiology Research Unit (EPIunit), Institute of Public Health, University of Porto, 4099-002 Porto, Portugal

³ Laboratório Para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), 4050-313 Porto, Portugal

⁴ LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, 4099-002 Porto, Portugal

⁵ ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, 4099-002 Porto, Portugal

⁶ Biopolis-CIBIO/InBIO Laboratório Associado, Campus de Vairão, 4485-661 Vairão, Portugal; hugo.rebelo@cibio.up.pt

⁷ Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os Montes e Alto Douro, 5000-801 Vila Real, Portugal; ppoeta@utad.pt

⁸ Associated Laboratory for Green Chemistry (LAQV), Chemistry Department, Faculty of Science and Technology, University NOVA of Lisbon, 2829-516 Caparica, Portugal;

⁹ Associate Laboratory for Animal and Veterinary Science (AL4Animals), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

¹⁰ Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal.

¹¹ cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, 1749-016 Lisbon, Portugal; herebelo@fc.ul.pt

* Correspondence: jrmesquita@icbas.up.pt

Abstract: Coronaviruses (CoVs) are RNA viruses capable of infecting a wide range of hosts, including mammals and birds, and have caused significant epidemics and the ongoing COVID-19 pandemic. Bats, the second most diverse mammalian order, are hosts for various CoVs due to their unique immune responses and ecological traits. This study investigates CoV prevalence in crevice and tree-dwelling bats in Portugal, a country with limited prior research on bat CoVs. Using nested RT-PCR and sequencing, we screened 87 stool samples from bats, identifying one sample (1.15%) positive for *Alphacoronavirus*, belonging to *Pipistrellus pipistrellus*. Phylogenetic analysis revealed close genetic relationships with *Alphacoronavirus* strains from the same bat species in Europe. The low prevalence suggests habitat-specific differences in viral transmission, with cave-dwelling bats exhibiting higher CoV prevalence due to population density and behavior. These findings underscore the necessity for sustained surveillance efforts aimed at comprehending CoV dynamics within bat populations, especially concerning the risk of spillover events and viral evolution. Vital to this understanding is the monitoring of bat migration patterns, which serves as a crucial tool for elucidating CoV ecology and epidemiology. Such efforts are essential for ongoing research endeavors aimed at mitigating the potential for future zoonotic disease outbreaks.

Keywords: Coronaviruses; *Alphacoronavirus*; *Pipistrellus pipistrellus*

Resumo com as principais conclusões

Este capítulo relata a detecção de AlphaCoVs em morcegos arborícolas e fissurícolas de Portugal, e ampliou o nicho ecológico conhecido da circulação de CoVs na região. Embora a prevalência detectada (1,15%) tenha sido consideravelmente menor do que a observada em morcegos cavernícolas (8,87%), esse achado demonstra que a presença de CoVs não se limita a espécies associadas a cavernas. O estudo reforça a existência de um clado monofilético de CoVs circulando especificamente entre espécies do gênero *Pipistrellus* em toda a Europa, com a espécie de *P. pipistrellus* apresentando estreita relação genética com *Pedacovirus*, um subgênero com potencial patogênico documentado em diversos hospedeiros animais, sugerindo, portanto, um certo grau de especificidade de hospedeiro dentro do gênero. A análise filogenética também revelou uma clara divergência genética entre os CoVs associados a *Pipistrellus* e aqueles previamente detectados em morcegos cavernícolas em Portugal, indicando uma partição tanto ecológica quanto evolutiva entre as comunidades de morcegos e seus papéis na manutenção e transmissão dos CoVs. A estreita relação das estirpes detectadas com *Pedacovirus* patogênicos ressalta sua relevância zoonótica e destaca a necessidade de vigilância contínua de morcegos arborícolas e fissurícolas como parte dos esforços mais amplos de monitoramento de CoVs. Por fim, o intercâmbio genético observado dentro das colônias de *P. pipistrellus* enfatiza o papel da migração dos morcegos na ecologia viral, sugerindo que o deslocamento entre colônias pode facilitar a disseminação de CoVs entre regiões geograficamente distantes, com implicações significativas para a compreensão da evolução, ecologia e epidemiologia viral.

1. Introduction

Coronavirus (CoVs) are members of the Coronaviridae family, which form a monophyletic group within the Nidovirales order, and are considered the largest known RNA viruses [1]. They possess an enveloped structure, enabling them to infect a broad spectrum of hosts, such as mammals, birds, amphibians, and teleost fish [2]. This versatility leads to a variety of clinical presentations, ranging from asymptomatic infections to severe, often fatal diseases. [3]. The Coronaviridae family comprises three known subfamilies: Letovirinae, which infects frogs; Pitovirinae, which primarily infects fish; and Orthocoronavirinae, responsible for infecting mammals (such as bats) and birds [4,5]. Members of the Orthocoronavirinae subfamily were implicated in the SARS and MERS epidemics, as well as the COVID-19 pandemic, and other several pathogens of clinical, veterinary, and economic importance [6–8].

The Orthocoronavirinae primarily target epithelial cells, so are associated with gastrointestinal infections (faecal-oral routes), and respiratory (through the contaminated surfaces or objects, and respiratory aerosols/droplets) [9]. Orthocoronaviruses are divided into four genera: Alpha-, Beta- (which infects mammals), and Gamma-, and Delta-Covs (which mainly infects birds) [10–12].

Bats (Chiroptera order) are taxonomically diverse, and the second most diverse group among mammals, representing approximately 20% of the mammal diversity [13]. They are distributed globally, using a vast array of roosts like rock crevices, caves, trees, and in urban areas they are frequently found in barns, houses, cellars and bridges [14]. These roosting sites play pivotal roles in various aspects of bat life. They serve as essential locations for mating rituals, providing safe environments for hibernation during colder seasons, and acting as nurseries for rearing young bats [15,16]. Moreover, these roosts facilitate social interactions among bat populations, allowing for communication and behavioral exchanges critical for their survival [17]. Furthermore, these roosts offer vital protection from harsh weather conditions, such as extreme temperatures and precipitation, as well as from potential predators that pose threats to bat populations [16].

Bats have gained attention for their remarkable propensity to host a vast array of viral species, making them as one of the mammalian groups hosts for the highest viral diversities [8]. This intriguing phenomenon is thought to be intricately linked to a combination of ecological and evolutionary traits unique to bats. [13,18]. Understanding the interplay between these ecological and evolutionary factors is essential for unravelling the complex

dynamics of viral diversity in bat populations [19]. This underscores the importance of conducting viral research in bats, as it has significant implications not only for public health but also for wildlife conservation and our deeper comprehension of viral evolution [19].

Viral diversity in bats has been explained by a number of factors including bats' altered immune function that regulates inflammatory genes [20], which can protect them from the development of infectious pathologies [21]. The process of mounting an immune response characterized by excessive inflammation can have deleterious consequences for vertebrates. Such overactivation of the immune system not only compromises the individual's health but also imposes a substantial energy cost. While inflammation is a critical component of the immune response, its excessive and prolonged activation can impose significant physiological costs on vertebrates, potentially compromising vital functions such as growth, reproduction, and maintenance of homeostasis [19]. Therefore, it has been hypothesized that through a series of mechanisms associated to bats' flying abilities they may be able to tolerate viral infection and replication without the excessive inflammation [22,23]. This flight might produce a fever-like response characterized by elevated metabolism and core body temperature ($>38^{\circ}\text{C}$), making them survive viral infections [13]. It is proposed that this tolerance makes them less virulent to their natural hosts than to novel viruses [24]. Their ability to fly also gives them more mobility than the majority of other mammals, which also enhances the potential for viruses to spread rapidly and widely between bat populations. This, associated with their social organizations forming large colonies with many individuals in caves, for example, also contributes to the maintenance of viruses in the population [25]. Bats are known to harbour a wide diversity of Alpha and Beta-CoVs. Bats are found to be hosts of at least 30 different CoVs with complete genome sequences available, and many more considering those without whole-genome sequences available [25]. In Europe, to date, there are 26 studies that have evaluated the presence of CoVs in bats: six studies in Italy, four in Germany, two in Holland, two in Ukraine and one in Belgium, Bulgaria, Denmark, Finland, France, Hungary, Luxembourg, Romania, Slovenia, Spain, Sweden, Poland, United Kingdom and Portugal [26–48].

In Portugal, research into the presence of CoVs in bats remains limited, with only one study conducted [49]. This investigation targeted a diverse array of bat roosts such as caves and large buildings across the country, providing an understanding of the distribution and prevalence of CoVs among cave-dwelling bats in Portugal. This approach allowed to explore the potential reservoirs of CoVs in different ecological settings, shedding light on the complex interactions between bats and their environments. The present study aims to shed

light on potential disparities in viral occurrence and diversity among bat populations inhabiting low density bat populations, namely fissure-dwelling settings such as crevices and trees.

2. Materials and Methods

Sampling location

Bat sampling was carried out during June 2023, at three protected landscape areas in the centre of Portugal, in the municipalities of Serra da Estrela (n=2) and Serra do Açor (n=1) (Figure 1). Captures were carried out from dusk till dawn for a total of 3 nights, using steel-framed mist nets.

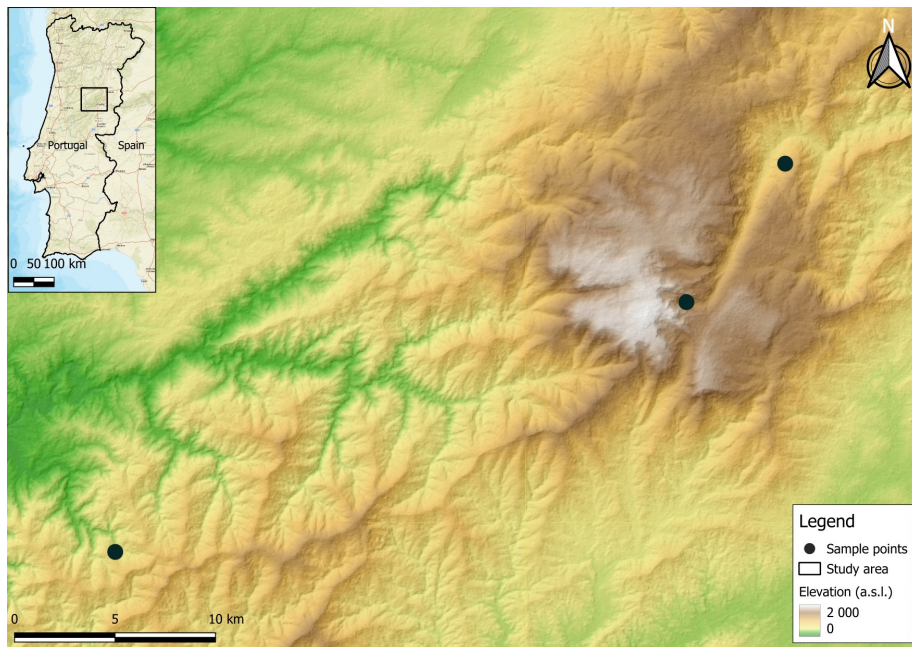


Figure 7: Selected bat sampling locations in Portugal used in this study

Captured bats were kept in individual cotton bags until they were processed, and their gender, age and weight were assessed. The captured bats were handled with meticulous care to prioritize their welfare throughout every stage of the procedure. Special attention was given to ensure their safety and minimize any potential stress or discomfort. Additionally, morphological identification was conducted using widely recognized taxonomic keys and guides commonly utilized in bat research. This approach helped to accurately classify the captured individuals based on their physical characteristics, ensuring precise identification and reliable data collection for subsequent analysis. By adhering to established protocols and standards for bat handling and identification, we aimed to uphold the highest standards of

animal welfare and scientific rigor in our study. [50]. The faeces that were shed during the collection procedure were collected, resulting in a total of 87 stool samples from bats belonging to seven genera and 13 species. Details of the samples collected can be found in table 1.

Table 2: Bat species, number of individuals and number of stool samples according to collection site.

Location	Species	Number of Individuals Found in Each Location	Stool samples collected from each species
Serra da Estrela day one	<i>Pipistrellus pipistrellus</i>	11	10
	<i>Eptesicus serotinus</i>	2	2
	<i>Myotis emarginatus</i>	2	2
	<i>Nyctalus leisleri</i>	5	4
	<i>Barbastella barbastellus</i>	1	0
	<i>Nyctalus lasiopterus</i>	1	0
	<i>Eptesicus serotinus/isabellinus</i>	2	2
	<i>Myotis daubentonii</i>	1	1
Serra da Estrela day two	<i>Plecotus austriacus</i>	1	1
	<i>Plecotus auritus</i>	6	6
	<i>Nyctalus leisleri</i>	1	1
	<i>Hypsugo savii</i>	4	3
	<i>Pipistrellus pipistrellus</i>	11	11
	<i>Myotis mystacinus</i>	3	3
	<i>Eptesicus serotinus</i>	1	0
	<i>Barbastella barbastellus</i>	1	1
Serra da Acor	<i>Nyctalus leisleri</i>	13	12
	<i>Nyctalus lasiopterus</i>	10	10
	<i>Pipistrellus pipistrellus</i>	2	2
	<i>Plecotus auritus</i>	15	13
	<i>Eptesicus serotinus</i>	1	1
	<i>Myotis bechsteinii</i>	1	1

After the capture sessions concluded, great care was taken to swiftly release the bats back into their native environment. This critical step is essential to minimize any potential disruption to their natural behaviours and to ensure the integrity of the research population. By returning the bats to their familiar surroundings without delay, we aim to mitigate stress and disturbance, allowing them to resume their normal activities without undue interference. This approach not only safeguards the welfare of the bats but also maintains the ecological balance of their habitat, fostering conditions conducive to accurate and reliable research outcomes. All actions pertaining to the capture and handling of bats were executed in strict

accordance with the permits provided by the Instituto da Conservação da Natureza e Florestas, guaranteeing full adherence to the rules and recommendations established by the conservation authority (licence number: 1 274/2023/CAPT).

Screening for coronaviruses

The samples were preserved at a temperature of -20°C until they were ready for further handling. To prepare them, they were mixed thoroughly by vortexing in 500 µL of PBS with a pH of 7.2. The RNA was extracted from the faecal mixture using the QIAamp viral mini kit (Qiagen, Hilden, Germany, reference number: 133226410), following the manufacturer's instructions and utilizing 140 µl of the clarified supernatants obtained after centrifugation at 1400g for 2 minutes. The resulting RNA was subsequently stored at -80°C for future processing.

The extracted RNA was tested for CoVs by employing a broad-spectrum pan-CoV nested RT-PCR assay, which focused on the conserved section of the RNA-dependent RNA polymerase (RdRp) and resulted in a final product measuring 440bp [51]. The first and second round of the PCRs, as well as electrophoresis were done as described in [49,52].

Our approach for the detection and characterization of CoVs has resourced to a partial RdRp region with primers described by [53]. The sensitivity of the nested pan-CoV primers has been previously assessed by comparing them with other pan-CoV approaches [53]. This thorough assessment determined the efficacy of the primers in the broad spectrum detection of CoVs and ultimately reached the conclusion of increasing the chances of detecting both recognized and unidentified CoVs in a wide range of sample sources [51]. It has been documented that utilizing a small segment of the RdRp from CoVs is sufficient for determining taxonomic classifications even at the subgenus level [54]. This accuracy in classification is similar to what can be achieved using complete genome sequences [54].

Sanger Sequencing and Phylogenetic Analysis

The next step involved isolating the positive amplicon using the GRS PCR Purification Kit (Grisp, Porto, Portugal, reference number: GK64.0100). Subsequently, bidirectional sequencing using the specific primers [53] for the target gene was carried out through Sanger sequencing. The sequence was then aligned using alignment clustal with the software package BioEdit Sequence Alignment Editor v7.1.9, version 2.1 (Ibis Biosciences, Carlsbad, CA, USA) and compared with the sequences available in the NCBI nucleotide database (GenBank, Carlsbad, CA, USA) (<http://blast.ncbi.nlm.nih.gov/Blast>). The obtained sequence was included for phylogenetic analysis and submitted to GenBank under the accession number OR625571.

This sequence was aligned with other 55 reference strains representing the four CoV genera (Alpha-, Beta-, Gamma-, and Deltacoronavirus), all sourced from GenBank. The alignment process was conducted using MEGA X software (Kumar et al. 2018). The MEGA X software's model selection function was employed to choose the model with the lowest Bayesian information criterion (BIC) score [55]. This selection was based on the maximum likelihood method and utilized the general time reversible model with a discrete Gamma distribution, assuming evolutionarily invariable sites. This was followed by 1000 bootstraps replicates. The final step involved editing the alignment with the Interactive Tree of Life (iTOL) platform [56].

3. Results

In this study, from the collection of 87 stool samples from bats from 3 sites, only one sample (1.15%; 95% confidence interval [CI]: 0.03-6.24) exhibited amplicons of the expected size for CoV using the pan-CoV nested RT-PCR. It was then further analyzed through bidirectional sequencing and nucleotide BLAST analysis. The bat testing positive for CoV belonged to the species *Pipistrellus pipistrellus* and was collected during the first day at Serra da Estrela.

The retrieved CoV sequence was assigned the accession number OR625571. BLAST analyses provided highest hits to sequences from *Alphacoronavirus*. Additional characterization through BLAST showed that the obtained sequence displayed highest similarities to CoVs retrieved from other *Pipistrellus pipistrellus*. Highest identities were revealed to previously identified *Alphacoronavirus* in the United Kingdom (West Sussex) under the accession number OQ401253, with percentage identity of 98.04%, followed by an *Alphacoronavirus* sequence previously identified in France under the accession number KT345296, with percentage identity of 96.93% and another three *Alphacoronavirus* sequences previously identified in Italy under the accession numbers KY780386, OQ134959 and OQ134958, with percentage identities of 96.39-96.67%. Phylogenetic analysis was subsequently conducted using the acquired CoV sequence alongside 57 reference strains. The analysis confirmed its placement within the *Alphacoronavirus* genus, subgenus *Pedacovirus*, as illustrated in Figure 2.



Figure 8: Phylogenetic tree constructed for the Gamma-, Delta-, Beta- and Alphacoronavirus, using 57 reference strains and the one strain identified in this study. Phylogenetic analysis was based on a 406 nt partial region of the RdRp. The tree was constructed using MEGA X and using the maximum likelihood based on the GTR + G model, and 1000 bootstraps were replicated. The sample from this study is indicated in red, with the description of sample number, GenBank accession number and host bat species.

4. Discussion

In this research, our primary objective focused on the investigation of the presence and characterization of CoVs specifically within crevice and tree-dwelling bat populations inhabiting Portugal. This study stands as a milestone, marking the second recorded instance of CoVs detected in bats in Portugal. Notably, it represents the pioneering documentation of CoVs in non-cave-dwelling bat species within the region. By delving into the viral ecology

and diversity inherent to these bat populations, our research provides crucial insights into the intricate dynamics of CoVs transmission and the potential reservoirs of these viruses within bat communities. Through our findings, we aim to contribute significantly to the collective understanding of zoonotic disease dynamics, ultimately aiding in the formulation of effective strategies for disease surveillance, prevention, and management.

We conducted screening on a total of 87 stool samples using nested RT-PCR followed by sequencing. From this screening only one sample showed to be positive for Alpha-CoV (1.15%; 95% confidence interval [CI]: 0.03-6.24), which is low compared to other studies that reported prevalence rates usually ranging from 3% to 17% [26,41,57,58]. Unexpectedly, our prior investigation into CoV excretion in Portugal revealed an occurrence of 8.87%, indicating a higher prevalence of CoVs in cave-dwelling bats than crevice and tree-dwelling bats [49]. This could have happened because of differences habitat characteristics, variations in bat species behaviour, proximity to potential virus reservoirs, and fluctuations in bat population density.

These factors can potentially influence the likelihood of detecting CoV in bat populations. Even if the study had opted for a country-wide and multi-year surveillance, the prevalence rate might not necessarily increase. This is because the effectiveness of surveillance efforts depends on various factors beyond the scope of the study design, such as the choice of sampling locations. Conducting surveillance in different habitats, such as caves or other bat habitats, may yield different results due to variations in environmental conditions and bat species composition, and more importantly animal densities. The CoV strain identified in the bat in our study exhibited close genetic relationships with *Alphacoronavirus* strains previously isolated from the same bat species, *P. pipistrellus*, from the United Kingdom (West Sussex) [40], more specifically from the *Pedacovirus* subgenus, and it also clustered with other strains previously isolated also from *P. pipistrellus*, from France and Italy [42,43]. The phylogenetic tree based on the partial RdRp gene, confirmed that our sequence clustered with other members of the *Alphacoronavirus* genus retrieved from *P. pipistrellus*, with strong support, as indicated by > 90% bootstrap value. Moreover, the retrieved *Pedacovirus* sequence also clustered with sequences found in other *Pipistrellus* spp. throughout Europe, namely in *P. nathusii* and *P. pygmaeus*, suggesting host specificity and raising important questions on viral circulation.

Our phylogenetic tree analysed several bat Alpha-CoV sequences from GenBank and the Alpha-CoV found in our study. Surprisingly, all strains were retrieved from the same bat genus (*Pipistrellus*), and in most cases, from the same species (*Pipistrellus pipistrellus*),

forming the same monophyletic branch. This clustering strongly implies that closely related viruses are prevalent within the same host species, even when geographically separated. This phenomenon has been corroborated by previous research findings, indicating a compelling pattern of viral association with specific bat hosts irrespective of geographical location [57,59].

In contrast, the research involving cave-dwelling bats revealed that the clustering of bat CoVs was not primarily determined by the species of bats. Instead, it appeared that geographical location played a more significant role in influencing the evolution and dissemination of these viruses [49]. This suggests that environmental factors, such as habitat characteristics and local ecological dynamics, may exert a more significant influence on the genetic diversity and distribution of coronaviruses in cave-dwelling bat populations.

P. pipistrellus distribution encompasses a substantial portion of Europe, with confirmed sightings extending as far north as Norway and southern Finland. To the south, its presence has been established in Greece, Cyprus, and Turkey [60]. Beyond the borders of Europe, it has been observed in northwest Africa, Asia Minor, and across the Middle East to Iran and Afghanistan [60]. In Portugal, this species is widely distributed across the entire national territory being considered one of the most abundant bat species in the country [50]. These bats, characterized by their preference for dwelling in crevices, commonly seek refuge in various human-made structures, leading them to roost in buildings, warehouses, and architectural relics such as churches and castles, among others. Their adaptation to utilizing human infrastructures for roosting highlights their status as synanthropic species, demonstrating their ability to thrive in close proximity to human populations [61]. This close association with human habitats not only shows the remarkable adaptability of these bats but also the importance of understanding their ecological dynamics in urban environments. However, due to its high plasticity, it can also utilize crevices in rocks, cliffs, tree cavities, occasionally underground shelters during the hibernation season, additionally, also seek shelter beneath tree bark [62].

P. pipistrellus are bats that are not very known for flying long distances, but recent findings regarding their genetic information indicates a significant degree of genetic exchange within summer colonies in central Europe [63]. This observation implies two distinct possibilities: the occurrence migrations motivated by the search for suitable mates in distant habitats, and also propelled by factors such as competition for resources or territory, or the need to establish new breeding grounds [64]. Although, this bat does not have a strong migratory character, since this species is usually known to remain confined to their own colonies even

if another colony is located 10km away [43]. This also indicate that these viruses may not primarily evolve within a specific bat species, instead, their evolution and spread appear to be significantly influenced by geographical location. This suggests factors such as environmental conditions, host availability, and interactions with other wildlife species could play pivotal roles in shaping the evolutionary dynamics and spread of these viruses across different regions. Another possible interpretation is that the presence of similar strains in geographically separated areas could be attributed to transmission occurring during the migration of bats. Considering the genetic divergence observed between the sampled strain and others, it is likely that direct transmission has not occurred, but rather, it would likely entail the involvement of intermediate strains that have not been included in the sampled data [57].

All these findings combined to our results suggest the existence of *Pedacovirus*-like Alpha-CoV strains specific to bats species (*P. pipistrellus*), although with low occurrence, underscores the significance of understanding viral diversity within bat populations. While the occurrence of these strains may be relatively low, their presence highlights the potential for novel viral variants to emerge and circulate among bat species[65]. Moreover, the observation that this pattern may extend to synanthropic bat species, which inhabit a wide range of roosting habitats including natural environments and human-built structures like roofs and windows, is of considerable importance. This suggests that human activities and urbanization may play a role in shaping the viral ecology of bats, potentially facilitating spillover events and the transmission of bat-borne viruses to other wildlife and human populations [18]. These findings emphasize the need for continued surveillance and research efforts to monitor viral diversity in bat populations and assess the associated risks to public health and wildlife conservation.

Bats play crucial roles as ecosystem service providers, making significant contributions to biodiversity conservation and ecosystem health. Their diverse dietary preferences and foraging behaviours make them effective suppressors of agro-forestry pests and disease vectors. By preying on insects such as moths, beetles, and mosquitoes, bats help regulate insect populations, thereby reducing agricultural damage and the spread of vector-borne diseases [66]. Additionally, bats' role as pollinators for various plant species contributes to the maintenance of plant diversity and ecosystem stability. Thus, recognizing the ecological importance of bats highlights the need for their conservation and the preservation of their habitats to ensure the continued provision of these valuable ecosystem services. [67].

Human-induced alterations, such as deforestation, habitat fragmentation, and land conversion for agriculture and urban development, can exert profoundly adverse effects on the transmission of infectious diseases from wild animals to both livestock and humans [25] and favouring the emergence of infectious diseases. The faecal-oral route has also been described in CoVs from other animals such as with feline CoVs (FCoV), canine CoVs, and swine CoVs (SADS-CoV) – all of them classified as Alpha-CoV, highlighting the potential for similar transmission mechanisms across diverse animal taxa [49] Both Alpha and Beta-CoVs have high detection rates in bat stool samples, suggesting gastrointestinal replication and that these animals' excretions are a major environmental source for the shedding of CoVs in spillover events [68,69]. A previous study also confirmed the gastrointestinal replication by not finding evidence of CoVs in the air caves where bats and bat's excretion in found [49]. Understanding the faecal-oral route in these CoVs not only provides insights into their pathogenesis but also underscores the importance of comprehensive surveillance and control measures to mitigate the risk of interspecies transmission and potential outbreaks.

5. Conclusions

This study provides unique results for the prevalence and dynamics of CoVs in Portuguese bats. Before this study, our understanding of bat CoVs in Portugal was limited to studies with cave-dwelling species. Our findings now reveal that CoVs are also present, although in much lower frequency, in crevice and tree-dwelling bats. Data here presented also strongly reinforces a monophyletic clade within CoVs circulating in *Pipistrellus* spp. bats of Europe. This clade exhibits close genetic relatedness to other Pedacoviruses, known to cause pathogenic effects in various animal species, suggesting a potential role of bats when considering CoVs species barrier crossing. This suggests that these bats may serve as important reservoirs or vectors for coronaviruses, thereby contributing to the emergence and dissemination of novel viral strains with zoonotic potential. Additionally, the genetic disparity observed between *Pipistrellus*-associated CoVs and other Alpha-CoVs found in bats within Portugal shows a significant phylogenetic distinction. This genetic differentiation strongly implies a physical segregation between *Pipistrellus* spp. bats and cave-dwelling bat species. Such genetic and ecological partitioning highlights the complex dynamics of bat communities and their respective roles in harboring and transmitting coronaviruses, emphasizing the need for further research to elucidate the mechanisms driving these patterns of viral diversity and distribution. It is of paramount importance to maintain ongoing monitoring of CoVs in bats to establish a foundational dataset for future surveillance.

Furthermore, monitoring the migratory behaviours of bats is essential in enhancing our comprehension of CoV evolution, ecology, and other bat-associated viruses and their epidemiology.

Author Contributions: Conceptualization, Mahima Hemnani, Hugo Rebelo, João Mesquita.; methodology, Mahima Hemnani, Priscilla Gomes da Silva and Hugo Rebelo; formal analysis, Mahima Hemnani, Hugo Rebelo and João Mesquita; investigation, Mahima Hemnani and Priscilla Gomes da Silva; re-sources, Hugo Rebelo and João Mesquita.; sample collection, Mahima Hemnani, Priscilla Gomes da Silva and Hugo Rebelo; writing—original draft preparation, Mahima Hemnani; writing—review and editing, Mahima Hemnani, Priscilla Gomes da Silva, Hugo Rebelo, Patricia Poeta, Gertrude Thompson and João Mesquita; supervision, Hugo Rebelo and João Mesquita; project administration, Hugo Rebelo, Patricia Poeta, Gertrude Thompson and João Mesquita; funding acquisition, Mahima Hemnani, Hugo Rebelo and and João Mesquita. All authors have read and agreed to the published version of the manuscript.

Funding: Mahima Hemnani thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of her PhD work under the Maria de Souza scholarship contract number 2021.09380.BD. This work was also funded by FCT, under the projects UIDB/04750/2020, LA/P/0064/2020, UIDP/00772/2020 (Doi:10.54499/UIDB/00772/2020) and LA/P/0059/2020.

Institutional Review Board Statement: License to capture was obtained from Instituto da Conservação da Natureza e das Florestas (ICNB), Portugal

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gorbalenya, A.E.; Enjuanes, L.; Ziebuhr, J.; Snijder, E.J. Nidovirales: Evolving the Largest RNA Virus Genome. *Virus Res.* **2006**, *117*, 17–37, doi:10.1016/j.virusres.2006.01.017.
2. Miller, A.K.; Mifsud, J.C.O.; Costa, V.A.; Grimwood, R.M.; Kitson, J.; Baker, C.; Brosnahan, C.L.; Pande, A.; Holmes, E.C.; Gemmell, N.J.; et al. Slippery When Wet: Cross-Species Transmission of Divergent Coronaviruses in Bony and Jawless Fish and the Evolutionary History of the Coronaviridae. *Virus Evol.* **2021**, *7*, 1–10, doi:10.1093/ve/veab050.
3. Woo, P.C.Y.; Groot, R.J. De; Haagmans, B.; Lau, S.K.P.; Benjamin, W. Family : Coronaviridae. **2023**, 1–27.
4. Bukhari, K.; Mulley, G.; Gulyaeva, A.A.; Zhao, L.; Shu, G.; Jiang, J.; Neuman, B.W. Description and Initial Characterization of Metatranscriptomic Nidovirus-like Genomes from the Proposed New Family Abysoviridae, and from a Sister Group to the Coronavirinae, the Proposed Genus Alphaletovirus. *Virology* **2018**, *524*, 160–171, doi:10.1016/j.virol.2018.08.010.
5. Mordecai, G.J.; Miller, K.M.; Di Cicco, E.; Schulze, A.D.; Kaukinen, K.H.; Ming, T.J.; Li, S.; Tabata, A.; Teffer, A.; Patterson, D.A.; et al. Endangered Wild Salmon Infected by Newly Discovered Viruses. *Elife* **2019**, *8*, 1–18, doi:10.7554/eLife.47615.
6. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Lai, K.K.Y.; Huang, Y.; Lee, P.; Luk, G.S.M.; Dyrting, K.C.; Chan, K.-H.; Yuen, K.-Y. Comparative Analysis of Complete Genome Sequences of Three Avian Coronaviruses Reveals a Novel Group 3c Coronavirus. *J. Virol.* **2009**, *83*, 908–917, doi:10.1128/jvi.01977-08.
7. Jacob Machado, D.; Scott, R.; Guirales, S.; Janies, D.A. Fundamental Evolution of All Orthocoronavirinae Including Three Deadly Lineages Descendent from Chiroptera-Hosted Coronaviruses: SARS-CoV, MERS-CoV and SARS-CoV-2. *Cladistics* **2021**, *37*, 461–488, doi:10.1111/cla.12454.
8. Letko, M.; Seifert, S.N.; Olival, K.J.; Plowright, R.K.; Munster, V.J. Bat-Borne Virus Diversity, Spillover and Emergence. *Nat. Rev. Microbiol.* **2020**, *18*, 461–471, doi:10.1038/s41579-020-0394-z.
9. A.A. Joseph; A.H. Fagbami Coronaviruses : A Review of Their Properties and Diversity. *African J. Clin. Exp. Microbiol.* **2020**, *21*, 258–271.
10. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Lau, C.C.Y.; Tsang, A.K.L.; Lau, J.H.N.; Bai, R.; Teng, J.L.L.; Tsang, C.C.C.; Wang, M.; et al. Discovery of Seven Novel Mammalian

and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavi. *J. Virol.* **2012**, *86*, 3995–4008, doi:10.1128/jvi.06540-11.

11. Cui, J.; Li, F.; Shi, Z.L. Origin and Evolution of Pathogenic Coronaviruses. *Nat. Rev. Microbiol.* **2019**, *17*, 181–192, doi:10.1038/s41579-018-0118-9.
12. Han, Y.; Du, J.; Su, H.; Zhang, J.; Zhu, G.; Zhang, S.; Wu, Z.; Jin, Q. Identification of Diverse Bat Alphacoronaviruses and Betacoronaviruses in China Provides New Insights into the Evolution and Origin of Coronavirus-Related Diseases. *Front. Microbiol.* **2019**, *10*, doi:10.3389/fmicb.2019.01900.
13. Hayman, D.T.S. Bats as Viral Reservoirs. *Annu. Rev. Virol.* **2016**, *3*, 77–99, doi:10.1146/annurev-virology-110615-042203.
14. Senthilkumar, K.; Marimuthu, G. Tree Roosting Fruit Bats (Chiroptera: Pteropodidae) in Southern Tamil Nadu. *Int. J. Appl. BioResearch* **2012**, *14*, 4–10.
15. Banerjee, A.; Kulcsar, K.; Misra, V.; Frieman, M.; Mossman, K. Bats and Coronaviruses. *Viruses* **2019**, *11*, 7–9, doi:10.3390/v11010041.
16. Kunz, T.H. Roosting Ecology of Bats. *Ecol. bats* **1982**, 1–55, doi:10.1007/978-1-4613-3421-7_1.
17. Chaverri, G.; Ancillotto, L.; Russo, D. Social Communication in Bats. *Biol. Rev.* **2018**, *93*, 1938–1954, doi:10.1111/brv.12427.
18. Ruiz-Aravena, M.; McKee, C.; Gamble, A.; Lunn, T.; Morris, A.; Snedden, C.E.; Yinda, C.K.; Port, J.R.; Buchholz, D.W.; Yeo, Y.Y.; et al. *Ecology, Evolution and Spillover of Coronaviruses from Bats*; Springer US, 2022; Vol. 20; ISBN 4157902100652.
19. Jones, B.D.; Kaufman, E.J.; Peel, A.J. Viral Co-Infection in Bats: A Systematic Review. *Viruses* **2023**, *15*, doi:10.3390/v15091860.
20. Subudhi, S.; Rapin, N.; Misra, V. Immune System Modulation and Viral Persistence in Bats: Understanding Viral Spillover. *Viruses* **2019**, *11*, doi:10.3390/v11020192.
21. Bokelmann, M.; Balkema-buschmann, A. Coronaviruses in Bats. *Berl. Munch. Tierarztl. Wochenschr.* **2021**, *134*, 1–16, doi:10.2376/1439-0299-2020-44.
22. Mandl, J.N.; Schneider, C.; Schneider, D.S.; Baker, M.L. Going to Bat(s) for Studies of Disease Tolerance. *Front. Immunol.* **2018**, *9*, 1–13, doi:10.3389/fimmu.2018.02112.
23. Gorbunova, V.; Seluanov, A.; Kennedy, B.K. The World Goes Bats: Living Longer and Tolerating Viruses. *Cell Metab.* **2020**, *32*, 31–43, doi:10.1016/j.cmet.2020.06.013.

24. O'Shea, T.J.; Cryan, P.M.; Cunningham, A.A.; Fooks, A.R.; Hayman, D.T.S.; Luis, A.D.; Peel, A.J.; Plowright, R.K.; Wood, J.L.N. Bat Flight and Zoonotic Viruses. *Emerg. Infect. Dis.* **2014**, *20*, 741–745, doi:10.3201/eid2005.130539.
25. Platto, S.; Zhou, J.; Wang, Y.; Wang, H.; Carafoli, E. Biodiversity Loss and COVID-19 Pandemic: The Role of Bats in the Origin and the Spreading of the Disease. *Biochem. Biophys. Res. Commun.* **2021**, *538*, 2–13, doi:10.1016/j.bbrc.2020.10.028.
26. Gloza-Rausch, F.; Ipsen, A.; Seebens, A.; Göttische, M.; Panning, M.; Drexler, J.F.; Petersen, N.; Annan, A.; Grywna, K.; Müller, M.; et al. Detection and Prevalence Patterns of Group I Coronaviruses in Bats, Northern Germany. *Emerg. Infect. Dis.* **2008**, *14*, 626–631, doi:10.3201/eid1404.071439.
27. Annan, A.; Baldwin, H.J.; Corman, V.M.; Klose, S.M.; Owusu, M.; Nkrumah, E.E.; Badu, E.K.; Anti, P.; Agbenyega, O.; Meyer, B.; et al. Human Betacoronavirus 2c EMC/2012-Related Viruses in Bats, Ghana and Europe. *Emerg. Infect. Dis.* **2013**, *19*, 456–459, doi:10.3201/eid1903.121503.
28. Rihtarič, D.; Hostnik, P.; Steyer, A.; Grom, J.; Toplak, I. Identification of SARS-like Coronaviruses in Horseshoe Bats (*Rhinolophus Hipposideros*) in Slovenia. *Arch. Virol.* **2010**, *155*, 507–514, doi:10.1007/s00705-010-0612-5.
29. Lazov, C.M.; Chriél, M.; Baagøe, H.J.; Fjederholt, E.; Deng, Y.; Kooi, E.A.; Belsham, G.J.; Bøtner, A.; Rasmussen, T.B. Detection and Characterization of Distinct Alphacoronaviruses in Five Different Bat Species in Denmark. *Viruses* **2018**, *10*, doi:10.3390/v10090486.
30. Lecis, R.; Mucedda, M.; Pidinchedda, E.; Pittau, M.; Alberti, A. Molecular Identification of Betacoronavirus in Bats from Sardinia (Italy): First Detection and Phylogeny. *Virus Genes* **2019**, *55*, 60–67, doi:10.1007/s11262-018-1614-8.
31. De Sabato, L.; Lelli, D.; Faccin, F.; Canziani, S.; Di Bartolo, I.; Vaccari, G.; Moreno, A. Full Genome Characterization of Two Novel Alpha-Coronavirus Species from Italian Bats. *Virus Res.* **2019**, *260*, 60–66, doi:10.1016/j.virusres.2018.11.007.
32. Kivistö, I.; Tidenberg, E.M.; Lilley, T.; Suominen, K.; Forbes, K.M.; Vapalahti, O.; Huovilainen, A.; Sironen, T. First Report of Coronaviruses in Northern European Bats. *Vector-Borne Zoonotic Dis.* **2020**, *20*, 155–158, doi:10.1089/vbz.2018.2367.
33. Lwande, O.W.; Thalín, T.; de Jong, J.; Sjödin, A.; Näslund, J.; Evander, M.; Ecker, F. Alphacoronavirus in a Daubenton's Myotis Bat (*Myotis Daubentonii*) in Sweden. *Viruses* **2022**, *14*, 1–10, doi:10.3390/v14030556.

34. Reusken, C.B.E.M.; Lina, P.H.C.; Pielaat, A.; de Vries, A.; Dam-Deisz, C.; Adema, J.; Drexler, J.F.; Drosten, C.; Kooi, E.A. Circulation of Group 2 Coronaviruses in a Bat Species Common to Urban Areas in Western Europe. *Vector Borne Zoonotic Dis.* **2010**, *10*, 785–791, doi:10.1089/vbz.2009.0173.
35. Drexler, J.F.; Gloza-Rausch, F.; Glende, J.; Corman, V.M.; Muth, D.; Goettsche, M.; Seebens, A.; Niedrig, M.; Pfefferle, S.; Yordanov, S.; et al. Genomic Characterization of Severe Acute Respiratory Syndrome-Related Coronavirus in European Bats and Classification of Coronaviruses Based on Partial RNA-Dependent RNA Polymerase Gene Sequences. *J. Virol.* **2010**, *84*, 11336–11349, doi:10.1128/jvi.00650-10.
36. Balboni, A.; Palladini, A.; Bogliani, G.; Battilani, M. Detection of a Virus Related to Betacoronaviruses in Italian Greater Horseshoe Bats. *Epidemiol. Infect.* **2011**, *139*, 216–219, doi:10.1017/S0950268810001147.
37. Drexler, J.F.; Corman, V.M.; Wegner, T.; Tateno, A.F.; Zerbinati, R.M.; Gloza-Rausch, F.; Seebens, A.; Müller, M.A.; Drosten, C. Amplification of Emerging Viruses in a Bat Colony. *Emerg. Infect. Dis.* **2011**, *17*, 449–456, doi:10.3201/eid1703.100526.
38. Kemenesi, G.; Dallos, B.; Görföl, T.; Boldogh, S.; Estók, P.; Kurucz, K.; Kutas, A.; Földes, F.; Oldal, M.; Németh, V.; et al. Molecular Survey of RNA Viruses in Hungarian Bats: Discovering Novel Astroviruses, Coronaviruses, and Caliciviruses. *Vector-Borne Zoonotic Dis.* **2014**, *14*, 846–855, doi:10.1089/vbz.2014.1637.
39. Falcón, A.; Vázquez-Morón, S.; Casas, I.; Aznar, C.; Ruiz, G.; Pozo, F.; Perez-Breña, P.; Juste, J.; Ibáñez, C.; Garin, I.; et al. Detection of Alpha and Betacoronaviruses in Multiple Iberian Bat Species. *Arch. Virol.* **2011**, *156*, 1883–1890, doi:10.1007/s00705-011-1057-1.
40. August, T.A.; Mathews, F.; Nunn, M.A. Alphacoronavirus Detected in Bats in the United Kingdom. *Vector-Borne Zoonotic Dis.* **2012**, *12*, 530–533, doi:10.1089/vbz.2011.0829.
41. Lelli, D.; Papetti, A.; Sabelli, C.; Rosti, E.; Moreno, A.; Boniotti, M.B. Detection of Coronaviruses in Bats of Various Species in Italy. *Viruses* **2013**, *5*, 2679–2689, doi:10.3390/v5112679.
42. De Benedictis, P.; Marciano, S.; Scaravelli, D.; Priori, P.; Zecchin, B.; Capua, I.; Monne, I.; Cattoli, G. Alpha and Lineage C BetaCoV Infections in Italian Bats. *Virus Genes* **2014**, *48*, 366–371, doi:10.1007/s11262-013-1008-x.
43. Goffard, A.; Demanche, C.; Arthur, L.; Pinçon, C.; Michaux, J.; Dubuisson, J. Alphacoronaviruses Detected in French Bats Are Phylogeographically Linked to Coronaviruses of European Bats. *Viruses* **2015**, *7*, 6279–6290, doi:10.3390/v7122937.

44. Fischer, K.; Zeus, V.; Kwasnitschka, L.; Kerth, G.; Haase, M.; Groschup, M.H.; Balkema-Buschmann, A. Insectivorous Bats Carry Host Specific Astroviruses and Coronaviruses across Different Regions in Germany. *Infect. Genet. Evol.* **2016**, *37*, 108–116, doi:10.1016/j.meegid.2015.11.010.
45. Monchatre-Leroy, E.; Boué, F.; Boucher, J.M.; Renault, C.; Moutou, F.; Gouilh, M.A.; Umhang, G. Identification of Alpha and Beta Coronavirus in Wildlife Species in France: Bats, Rodents, Rabbits, and Hedgehogs. *Viruses* **2017**, *9*, doi:10.3390/v9120364.
46. Yurchenko, O.; Dubina, D.; Sokolovskyi, D.; Gaidash, O. Possible Zoonotic Viral Threats Associated with Bats in Southern Ukraine. *Proc. Theriol. Sch.* **2017**, *2017*, 150–153, doi:10.15407/ptt2017.15.150.
47. Pauly, M.; Pir, J.B.; Loesch, C.; Sausy, A.; Snoeck, C.J.; Hübschen, J.M.; Muller, C.P. Novel Alphacoronaviruses and Paramyxoviruses Cocirculate with Type 1 and Severe Acute Respiratory System (SARS)-Related Betacoronaviruses in Synanthropic Bats of Luxembourg. *Appl. Environ. Microbiol.* **2017**, *83*, doi:10.1128/AEM.01326-17.
48. Moreno, A.; Lelli, D.; De Sabato, L.; Zaccaria, G.; Boni, A.; Sozzi, E.; Prosperi, A.; Lavazza, A.; Cella, E.; Castrucci, M.R.; et al. Detection and Full Genome Characterization of Two Beta CoV Viruses Related to Middle East Respiratory Syndrome from Bats in Italy. *Virol. J.* **2017**, *14*, 1–11, doi:10.1186/s12985-017-0907-1.
49. Hemnani, M.; Silva, P.G. da; Thompson, G.; Poeta, P.; Rebelo, H.; Mesquita, J.R. First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* **2023**, *15*, 1–12, doi:10.3390/v15071521.
50. Mathias ML, Fonseca C, Rodrigues L, Grilo C, Lopes-Fernandes M, Palmeirim JM, Santos-Reis M, Alves PC, Cabral JA, F.M.; Mira A, Eira C, Negrões N, Paupério J, Pita R, Rainho A, Rosalino LM, T.J.& V.J. *Livro Vermelho Dos Mamíferos de Portugal Continental*; 2023rd ed.; FCIências.ID, ICNF, Lisboa;
51. Drzewnioková, P.; Festa, F.; Panzarin, V.; Lelli, D.; Moreno, A.; Zecchin, B.; De Benedictis, P.; Leopardi, S. Best Molecular Tools to Investigate Coronavirus Diversity in Mammals: A Comparison. *Viruses* **2021**, *13*, doi:10.3390/v13101975.
52. Hemnani, M.; Rodrigues, D.; Santos, N.; Santos-Silva, S.; Figueiredo, M.E.; Henriques, P.; Ferreira-e-Silva, J.; Rebelo, H.; Poeta, P.; Thompson, G.; et al. Molecular Detection and Characterization of Coronaviruses in Migratory Ducks from Portugal Show the Circulation of Gammacoronavirus and Deltacoronavirus. *Animals* **2022**, *12*, doi:10.3390/ani12233283.

53. Drzewnioková, P.; Festa, F.; Panzarin, V.; Lelli, D.; Moreno, A.; Zecchin, B.; De Benedictis, P.; Leopardi, S. Best Molecular Tools to Investigate Coronavirus Diversity in Mammals: A Comparison. *Viruses* **2021**, *13*, 1–16, doi:10.3390/v13101975.
54. Wilkinson, D.A.; Joffrin, L.; Lebarbenchon, C.; Mavingui, P. Analysis of Partial Sequences of the RNA-Dependent RNA Polymerase Gene as a Tool for Genus and Subgenus Classification of Coronaviruses. *J. Gen. Virol.* **2021**, *101*, 1261–1269, doi:10.1099/jgv.0.001494.
55. Zhang, D.; Kan, X.; Huss, S.E.; Jiang, L.; Chen, L.Q.; Hu, Y. Using Phylogenetic Analysis to Investigate Eukaryotic Gene Origin. *J. Vis. Exp.* **2018**, *2018*, doi:10.3791/56684.
56. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v4: Recent Updates and New Developments. *Nucleic Acids Res.* **2019**, *47*, 256–259, doi:10.1093/nar/gkz239.
57. Bittar, C.; Machado, R.R.G.; Comelis, M.T.; Bueno, L.M.; Beguelini, M.R.; Morielle-Versute, E.; Nogueira, M.L.; Rahal, P. Alphacoronavirus Detection in Lungs, Liver, and Intestines of Bats from Brazil. *Microb. Ecol.* **2020**, *79*, 203–212, doi:10.1007/s00248-019-01391-x.
58. Bueno, L.M.; Rizotto, L.S.; Viana, A. de O.; Silva, L.M.N.; de Moraes, M.V. dos S.; Benassi, J.C.; Scagion, G.P.; Dorlass, E.G.; Lopes, B.L.T.; Cunha, I.N.; et al. High Genetic Diversity of Alphacoronaviruses in Bat Species (Mammalia: Chiroptera) from the Atlantic Forest in Brazil. *Transbound. Emerg. Dis.* **2022**, *69*, e2863–e2875, doi:10.1111/tbed.14636.
59. Rizzo, F.; Edenborough, K.M.; Toffoli, R.; Culasso, P.; Zoppi, S.; Dondo, A.; Robetto, S.; Rosati, S.; Lander, A.; Kurth, A.; et al. Coronavirus and Paramyxovirus in Bats from Northwest Italy. *BMC Vet. Res.* **2017**, *13*, 1–11, doi:10.1186/s12917-017-1307-x.
60. Hulva, P.; Horáček, I.; Strelkov, P.P.; Benda, P. Molecular Architecture of *Pipistrellus* *Pipistrellus*/*Pipistrellus* *Pygmaeus* Complex (Chiroptera: Vespertilionidae): Further Cryptic Species and Mediterranean Origin of the Divergence. *Mol. Phylogenet. Evol.* **2004**, *32*, 1023–1035, doi:10.1016/j.ympev.2004.04.007.
61. Frick, W.F.; Kingston, T.; Flanders, J. A Review of the Major Threats and Challenges to Global Bat Conservation. *Ann. N. Y. Acad. Sci.* **2020**, *1469*, 5–25, doi:10.1111/nyas.14045.
62. Dietz, M.; Brombacher, M.; Erasmy, M.; Fenchuk, V.; Simon, O. Bat Community and Roost Site Selection of Tree-Dwelling Bats in a Well-Preserved European Lowland Forest. *Acta Chiropterologica* **2018**, *20*, 117–127, doi:10.3161/15081109ACC2018.20.1.008.

63. Bryja, J.; Kaňuch, P.; Fornůšková, A.; Bartonička, T.; Řehák, Z. Low Population Genetic Structuring of Two Cryptic Bat Species Suggests Their Migratory Behaviour in Continental Europe. *Biol. J. Linn. Soc.* **2009**, *96*, 103–114, doi:10.1111/j.1095-8312.2008.01093.x.
64. Nusová, G.; Uhrin, M.; Voigt, C.C.; Kaňuch, P. Tracing the Geographic Origin of Common Pipistrelles (*Pipistrellus Pipistrellus*) Swarming at a Mass Hibernaculum. *Mamm. Biol.* **2020**, *100*, 601–610, doi:10.1007/s42991-020-00057-3.
65. Brook, C.E.; Dobson, A.P. Bats as “special” Reservoirs for Emerging Zoonotic Pathogens. *Trends Microbiol.* **2015**, *23*, doi:10.1016/j.tim.2014.12.004.
66. Kasso, M.; Balakrishnan, M. Ecological and Economic Importance of Bats (Order Chiroptera). *ISRN Biodivers.* **2013**, *2013*, 1–9, doi:10.1155/2013/187415.
67. Moussy, C.; Hosken, D.J.; Mathews, F.; Smith, G.C.; Aegerter, J.N.; Bearhop, S. Migration and Dispersal Patterns of Bats and Their Influence on Genetic Structure. *Mamm. Rev.* **2013**, *43*, 183–195, doi:10.1111/j.1365-2907.2012.00218.x.
68. Wong, S.; Lau, S.; Woo, P.; Yuen, K.Y. Bats as a Continuing Source of Emerging Infections in Humans. *Rev. Med. Virol.* **2007**, *17*, 67–91, doi:10.1002/rmv.520.
69. Watanabe, S.; Masangkay, J.S.; Nagata, N.; Morikawa, S.; Mizutani, T.; Fukushi, S.; Alviola, P.; Omatsu, T.; Ueda, N.; Iha, K.; et al. Bat Coronaviruses and Experimental Infection of Bats, the Philippines. *Emerg. Infect. Dis.* **2010**, *16*, 1217–1223, doi:10.3201/eid1608.100208.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Chapter 4- Metagenomic Analysis of Viral Diversity in Portuguese Bats

Contents from this Chapter were adapted from the following review papers:

Hemnani, M., Karatas, M., Cruz, A. V., da Silva, P. G., Thompson, G., Poeta, P., ... & Mesquita, J. R. (2025). Metagenomic analysis of viral diversity in Portuguese bats. *Veterinary Research Communications*, 49(6), 319. <http://dx.doi.org/10.1007/s11259-025-10888-5>

Metagenomic Analysis of Viral Diversity in Portuguese Bats

Mahima Hemnani¹, Mustafa Karatas², Andreia V. S. Cruz¹, Priscilla Gomes da Silva³, Gertrude Thompson^{1,4}, Patrícia Poeta^{5,6,7,8}, Hugo Rebelo⁹, Jelle Matthijnsens², João R. Mesquita^{1,11,12*}

¹School of Medicine and Biomedical Sciences, Porto University, 4050-313 Porto, Portugal; up202110040@edu.icbas.up.pt (M.H.); up201303679@edu.icbas.up.pt (A.V.S.C); gathompson@icbas.up.pt (G.T.)

²KU Leuven, Department of Microbiology, Immunology, and Transplantation, Rega Institute, Laboratory of Viral Metagenomics, Leuven, Belgium; mustafa.karatas@kuleuven.be (M.K.); jelle.matthijnsens@kuleuven.be (J.M.)

³Department of Protein Science, Affinity Proteomics Division, Science for Life Laboratory (SciLifeLab), KTH Royal Institute of Technology; priscilla.gomes@scilifelab.se (P.G.d.S)

⁴CIBIO-InBIO, Research Centre in Biodiversity and Genetic Resources, University of Porto, Campus de Vairão, 4485-661 Vairão, Portugal

⁵Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal; ppoeta@utad.pt (P.P.)

⁶Associated Laboratory for Green Chemistry (LAQV), Chemistry Department, Faculty of Science and Technology, University NOVA of Lisbon, 2829-516 Caparica, Portugal

⁷Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

⁸Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

⁹cE3c—Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; herebelo@ciencias.ulisboa.pt (H.R.)

¹¹Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente (ICETA), Universidade do Porto (UP), Rua D. Manuel II, Apartado 55142, 4051-401 Porto, Portugal

¹²Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), 1300-477 Lisboa, Portugal

*corresponding author: jrmesquita@icbas.up.pt

ABSTRACT

Bats are highly diverse mammals and known reservoirs of numerous zoonotic viruses. Their role in the ecology of emerging infectious diseases continues to be of significant interest. This study aimed to evaluate the occurrence of coronaviruses (CoVs) in Portuguese bats and predict the affinity of their spike proteins with the aminopeptidase N (APN) receptor of several host species. The study also explored the viral diversity in bat samples using metagenomic sequencing. Ten bats (five *Myotis myotis* and five *Miniopterus schreibersii*) were captured at an underground roost in 2022 (Central Portugal), and faecal samples, oral, and anal swabs were collected (n=27). A Pan-CoV nested RT-PCR was used for initial screening, followed by viral metagenomic sequencing of all faecal samples and one CoV-positive buccal swab. In silico protein docking studies were performed between a Portuguese bat CoV spike protein and APNs of bats, pigs, and humans. Pan-CoV nested RT-PCR identified three positive samples: two faecal samples and one buccal sample. Metagenomic sequencing allowed us to determine two near complete CoV genomes. Protein docking predicted strong binding of this spike protein to bat, porcine, and human APN receptors. Metagenomics also identified picornaviruses, adenovirus, and dependoparvovirus in faecal samples. This study reports the first near complete genome sequences of two members of the *Alphacoronavirus* genus from a Portuguese bat. The identification of other viral families highlights the diverse virome of these cave-dwelling bat species. Protein docking studies suggest a potential for cross-species transmission of this bat CoV between bats, porcines and humans, though further research is needed to confirm these interactions.

Keywords: Bat, Coronavirus, Picornavirus, Adenovirus, Dependoparvovirus, Metagenomics

Resumo com as principais conclusões

Este último capítulo identificou e sequenciou com sucesso dois genomas quase completos de AlphaCoV provenientes de morcegos de Portugal, representando a primeira caracterização genómica abrangente de AlphaCoVs nessas espécies em Portugal e fornecendo informações essenciais sobre sua composição genética e relações evolutivas. A análise metagenómica de amostras fecais revelou ainda um viroma diversificado, incluindo vírus das famílias *Picornaviridae*, *Adenoviridae* (*Mastadenovirus*) e *Parvoviridae* (*Dependoparvoviridae*), evidenciando a rica diversidade viral abrigada por morcegos cavernícolas e reforçando seu papel como reservatórios de múltiplos táxons virais. Notavelmente, uma amostra (F45) que inicialmente havia testado negativo para CoV por RT-PCR convencional foi posteriormente identificada como positiva por meio de sequenciamento metagenómico (discrepância atribuída a incompatibilidades de primers) ilustrando a maior sensibilidade e natureza não enviesada das abordagens metagenómicas na detecção de estirpes virais novas ou divergentes. As análises *in silico* de acoplamento proteico previram fortes afinidades de ligação entre a proteína spike do novo CoV identificado e os receptores de aminopeptidase N (APN) de morcegos, suínos e humanos, indicando um potencial de transmissão interespecífica e destacando um risco zoonótico que merece validação experimental adicional. Em conjunto, esses achados ressaltam a importância de uma abordagem *One Health* na vigilância viral, demonstrando que o monitoramento contínuo das populações de morcegos é essencial para compreender a ecologia viral, antecipar possíveis eventos de transbordamento e orientar estratégias proativas de saúde pública voltadas à mitigação do surgimento de novas doenças infecciosas.

INTRODUCTION

Bats, belonging to the order Chiroptera, are a highly diverse taxa with over 1,400 species identified worldwide (Bokelmann and Balkema-buschmann 2021b; Wang et al. 2023), and of great relevance in a One Health context (Figueiroa et al. 2025). They are the only mammals capable of sustained flight and have successfully adapted to a wide range of ecological niches, enabling them to inhabit a vast array of habitats and roosts (Irving et al. 2021b; Soler-Tovar and Escobar 2025) .

These animals represent a unique group of mammals that have evolved to coexist and be a reservoir for a vast diversity of viruses (Tiwari et al. 2020; Gupta et al. 2021a), such as *Coronaviridae*, *Adenoviridae*, *Parvoviridae*, *Picornaviridae*, *Rhabdoviridae*, *Herpesviridae*, *Filoviridae*, *Paramyxoviridae*, *Astroviridae* (Calisher et al. 2006a; Drexler et al. 2012; Smith and Wang 2013; Moreno et al. 2017; Wang and Anderson 2019; Gupta et al. 2021b) and members of a total of 66 genera from 15 families of RNA and five families of DNA viruses have been detected from 75 bat genera. Over millions of years, these animals have developed a specialized immune system that allows them to harbor these viruses (Apoorva and Singh 2024), several of which have zoonotic potential (Irving et al. 2021b; Soler-Tovar and Escobar 2025; Pujol et al. 2025).

They are also natural reservoirs for coronaviruses (CoVs) related to those that have caused outbreaks in humans, such as SARS, MERS and SARS-CoV-2 (El-Sayed and Kamel 2021), even though these specific human pathogens have not been directly detected from bats. On the other hand, their unique ability to fly enables them to migrate over long distances, significantly increasing the potential for both inter- and intra-species transmission (Drexler et al. 2014b; Gupta et al. 2021a). A recent systematic review reported that, to date, there are 30 studies in Europe that have examined the presence of CoVs in bats, covering countries such as Italy, Germany, the Netherlands, Ukraine, Belgium, Bulgaria, Denmark, Finland, France, Hungary, Luxembourg, Romania, Slovenia, Spain, Sweden, Switzerland, Poland, the United Kingdom, and Portugal (Hemnani et al. 2024). Bats are known to host at least 30 different CoVs with complete genome sequences available, representing different species or lineages, and many more when including those lacking full genomes (Platto et al. 2021a).

CoVs possess a non-segmented, positive-stranded RNA genome, with a typical size of 27 - 32 kb, making it the largest among all RNA viruses (Nakagawa and Miyazawa 2020). Their virions are enveloped, having structures protruding from the surface called “spikes”

(Domańska-Blicharz et al. 2021). Their lengthy genomes, high rates of recombination, and frequent mutations (Woo et al. 2009a; Grellet et al. 2022) can facilitate the infection of a wide range of hosts, including mammals, birds, amphibians, and teleost fish (Miller et al. 2021), and adapt to new host species with modified pathogenicity (Drzewnioková et al. 2021a). This adaptability results in diverse clinical outcomes, from asymptomatic cases to severe and sometimes fatal diseases (Woo et al. 2023b). CoVs are classified into four genera: *Alphacoronavirus* (AlphaCoV) and *Betacoronavirus* (BetaCoV), which primarily cause diseases in mammals and are considered pathogenic, and *Gammacoronavirus* (GammaCoV) and *Deltacoronavirus* (DeltaCoV), which evolved from CoVs originally found in birds. While most GammaCoV and DeltaCoV strains cause diseases in birds, this is not exclusively the case (Mihindukulasuriya et al. 2008a; Woo et al. 2014a).

Recent efforts have increasingly focused on using metagenomic techniques (Kim et al. 2024) to explore the vast array of pathogens present in wildlife species (Prentice et al. 2024). Considering the extensive diversity of viruses and the rising frequency of direct and indirect interactions at the human-domestic and animal-wildlife interface (Cui et al. 2023), it is likely that many viruses remain unidentified, along with numerous unrecognized instances of cross-species transmission. Advancing our knowledge of the viruses involved in spillover events and successful host transitions is essential for deepening our understanding of the factors driving the emergence of new diseases (Ruiz-Aravena et al. 2022b; Gangopadhyaya and Bhukya 2023).

Usually, studying viromes can be challenging because most of the genetic material in a sample is of non-viral origin (Reyes et al. 2012). Next-generation sequencing has transformed the discovery of new viruses in humans and animals across diverse ecosystems (Bodewes et al. 2014), especially with new approaches that include enrichment for virus-like-particles (VLPs) from a sample and the performance of random amplification, if the starting material needs to be increased before NGS library preparation (Reyes et al. 2012; Conceição-Neto et al. 2015a).

Molecular docking is a widely used tool to simulate the 3D structure of protein-ligand or protein-protein complexes, examining their interactions, and predicting binding affinities (Andrusier et al. 2008). The CoV spike protein is a transmembrane homotrimeric protein required for attachment to the host cell-specific receptors and subsequent catalysis of the virus-host cell membrane fusion (Guruprasad 2021). In CoVs, it extends from the viral surface, giving them their characteristic crown-like appearance (Ghosh et al. 2021). One such receptor is aminopeptidase N (APN), a membrane-bound metalloprotease involved in

peptide digestion, which also functions as a viral receptor for certain coronaviruses, facilitating entry into host cells (Reguera et al. 2012).

Despite increasing awareness of bats as natural reservoirs for diverse viruses, including coronaviruses (CoVs), there is still a lack of data on CoV circulation in European bat populations, particularly in regions like Portugal where surveillance has been limited. Moreover, the potential for cross-species transmission through molecular mechanisms such as receptor binding remains poorly explored in these populations.

Therefore, the aim of this study is to investigate the occurrence of CoVs in bats from an underground roost in Portugal, while also utilizing viral metagenomics to explore the CoV genome as well as other potential viral families co-circulating within these hosts. Computational protein docking studies were also employed to predict the binding affinity between the spike protein of bat CoV and the APN receptor of mammal species like bats, porcines and humans to predict infection. This research underscores the importance of a One Health approach to understanding the interconnectedness of human, animal, and environmental health, which is crucial for preventing zoonotic outbreaks and promoting public health resilience.

MATERIAL AND METHODS

Bat sampling

Bats were captured in July 2022 at an Montemor I underground roost (municipality of Montemor-o-Novo, Central Portugal), a shelter for the conservation of species such as the greater mouse-eared bat (*Myotis myotis*) and the common bent-wing bat (*Miniopterus schreibersii*)

We used hand nets for bat capture, resulting in a total of ten individuals from two species: *M. myotis* (n=5) and *M. schreibersii* (n=5). Each bat was carefully handled to prioritize its well-being, with species identification conducted using standard morphological techniques. After capture, the bats were placed in individual cotton bags for 5 to 20 minutes, during which time three types of matrices were collected namely fresh faecal samples (n=7), anal (n=10) and buccal swabs (n=10). Each swabbed matrix was placed in an individual sterile Eppendorf tube and stored dry at -20°C until further processing, without the addition of buffer. To minimize stress and disruption, after processing, the bats were safely released and promptly returned to their natural environment, enabling them to quickly resume normal behavior with minimal interference. This approach not only ensured the bats' well-being but also preserved the ecological integrity of their habitat. All capture and handling procedures were carried out in full compliance with permits issued by the Instituto da Conservação da Natureza e das Florestas (ICNF), adhering strictly to the regulations and guidelines set forth by the conservation authority (license number: 274/2023/CAPT).

Screening for CoVs

All samples were immediately stored at -20°C until further processing. During preparation for PCR, they were vortexed in 500 µL of PBS and RNA was extracted using the QIAamp viral mini kit (Qiagen, Hilden, Germany). The RNA was stored at -80°C and later tested for CoVs using a pan-CoV nested RT-PCR (Drzewnioková et al. 2021a) assay targeting a conserved region of the RNA-dependent RNA polymerase (*RdRp*) gene, resulting in a final product measuring 440 bp. The sensitivity of the primers used was previously validated for broad-spectrum CoV detection and it has been documented that utilizing a small segment of the *RdRp* from CoVs is an approach for determining taxonomic classifications even at the subgenus level (Drzewnioková et al. 2021a; Wilkinson et al. 2021a). Amplicons of the expected size were purified using the GRS PCR Purification Kit (Grisp, Porto, Portugal). Bidirectional sequencing was then performed using the target gene's specific primers by the Sanger method. Sequence alignment was performed using the

BioEdit Sequence Alignment Editor v7.1.9 software package, version 2.1 (Ibis Biosciences,

Carlsbad, CA, USA). The obtained sequences were trimmed, and consensus sequences were compared with the sequences found online in the nucleotide database NCBI (Gen-Bank, Carlsbad, CA, USA).

Sample preparation and metagenomic sequencing

All seven faecal samples and the one buccal swab sample that tested positive for CoVs by the pan-CoV nested RT-PCR were further analyzed by NGS. For viral metagenomics sample preparation for, the NetoVIR (Novel enrichment technique of VIRomes) protocol was used as previously described (Conceição-Neto et al. 2015b, 2017; Yinda et al. 2017). (Conceição-Neto et al. 2015b)

Subsequently, faecal suspensions and the buccal sample were prepared at a concentration of 10% (w/v) in sterile PBS and homogenized at 3,000 rpm for 1 minute using a tissue homogenizer (Precellys, France). The homogenized samples were centrifuged at $17,000 \times g$ for 3 minutes, and the supernatant containing VLPs was collected. This supernatant was filtered through 0.8 μm PES filters (Sartorius) to remove larger debris. The filtered supernatant was treated with a nuclease cocktail consisting of Benzonase (Novagen) and Micrococcal Nuclease (New England Biolabs) at 37°C for 2 hours to degrade free-floating nucleic acids. After the nuclease treatment, viral RNA and DNA were extracted using the QIAamp Viral RNA Mini Kit (Qiagen), following the manufacturer's protocol with a modification to exclude carrier RNA from the lysis buffer.

For the preparation of sequencing libraries, random amplification was performed using the Whole Transcriptome Amplification (WTA2) Kit (Sigma-Aldrich). Amplification included first and second strand synthesis followed by PCR, which was conducted over 17 cycles with a denaturation temperature set at 95°C to ensure complete denaturation of both dsDNA and dsRNA. The amplified products were then purified using MSB Spin PCRapace spin columns (Stratag).

For library preparation, we followed the Nextera XT Library Preparation Kit protocol (Illumina). The DNA from the WTA2 amplification was tagged by adding 7.5 μL of tagmentation mix to each sample in a 96-well plate, followed by the addition of 2.5 μL of the DNA sample. After a brief spin and incubation in a PCR machine, 2.5 μL of Neutralize Tagment Buffer was added. The samples were then indexed by adding 5 μL of unique indexes from a 96-well plate, followed by the addition of 7.5 μL of Nextera PCR Master Mix. After PCR amplification, the products were purified using AMPure XP beads at a 0.6 $\times$ bead-to-DNA ratio. This included two ethanol washes and resuspension in 26.25 μL of Tris-HCl (Tris(hydroxymethyl)aminomethane hydrochloride) buffer. Finally, the prepared

libraries were sequenced on an Illumina HiSeq 2500 platform, generating 2×150 bp paired-end reads across 300 cycles.

Bioinformatic analysis of metagenomic sequencing

On average, 35M reads per sample were obtained. Raw sequencing reads were processed using the ViPER pipeline (De Coninck et al. 2025). Shortly, low quality reads and adapters were trimmed by Trimmomatic (Bolger et al. 2014) (v.039) and remaining reads were *de novo* assembled with SPAdes (v3.15.3) into contigs (Prjibelski et al. 2020).

The assembled contigs were taxonomically assigned using DIAMOND v.2.0.9, employing the NCBI non-redundant (nr) protein database (accessed on 17 March 2023) for sequence alignment. A lowest common ancestor (LCA) approach was utilized to assign taxonomy, which was facilitated by TaxonKit v.0.8.0. Additionally, KronaTools v.2.8 was employed to visualize the taxonomic composition of the viral communities. Contigs were classified into eukaryotic viruses, phages, bacteria and eukaryotes. We focused exclusively on viruses capable of infecting bats, excluding those infecting archaea, bacteria, fungi, invertebrates, and plants from the analysis.

In parallel, EsVirtu (Tisza et al. 2023) was applied to further refine the taxonomic resolution as a reference-guided assembly approach, particularly focusing on the detection and classification of well represented human and animal viruses in existing databases. EsVirtu's integration with the *de novo* assembly approach provided additional confidence in the classification of the viral contigs.

The genomic organization of the near-complete genomes was assessed using the Genome Detective Virus Tool (<https://www.genomedetective.com/app/typingtool/virus/>). This tool facilitated annotation of open reading frames (ORFs) and detection of conserved genomic features. Detailed genome characterizations, including ORF structure and genome lengths, are available in the Supplementary File.

PCR primers designed for full spike characterization

In one of the Cov sequences (MiSc-F47-CoV-2), a small region of the spike gene was not recovered through NGS. To obtain the missing region of the spike gene, specific primers were designed (Table 1) for targeted amplification and sequencing using the Sanger method.

We employed the one-step RT-PCR kit (GRiSP[®], Porto, Portugal) for the initial round of PCR. The following conditions were used in the Veriti 96-well thermal cycler (Thermo Fisher) for amplification reactions: an initial cycle of 3 min at 95 °C, followed by 40 cycles of 62 °C, then 67 °C, and then temperatures continuing to drop in subsequent cycles to levels to avoid spurious amplification, 50 °C for 15 s, and 72 °C for 2 s, with a final elongation at

72 °C for 10 min.

Table 3: Primers designed for the amplification and sequencing of the missing spike gene region using Sanger sequencing.

Primer	Sequence (5' → 3')
Forward	5- TGG CAC CAT TGG TAA AGG CT -3
Reverse	5- AGG TCA AGC GCT GTT TCA GA -3

Phylogenetic analysis

Phylogenetic trees were constructed using the assembled genome of CoVs, picornaviruses and adenovirus. Reference strains of these viral families with a query coverage exceeding 70% were retrieved from the NCBI database using BLASTn,

After removing duplicate genome sequences with BBMap, alignments were generated using MAFFT, trimmed with Trimal, and maximum likelihood phylogenetic trees were constructed with IQ-TREE 2.

Nucleotide Sequences Accession Numbers

The viruses reported in this article are available in the GenBank database (OQ613362, PV221239, OQ613361, PV359018, PV359019, PV383542-PV383552). The relevant raw high throughput sequencing data obtained in this study were deposited at the NCBI Sequence Read Archives (SRA) under BioProject ID PRJNA1162723.

Homology modelling and protein-protein docking simulations

Protein docking simulations were performed to assess the binding affinity between the F47 CoV spike protein obtained in this study, and bat, porcine and human APNs. The crystal structures of the porcine epidemic diarrhea virus (PEDV) spike protein, as well as human and porcine APNs were obtained from the Protein Data Bank (PDB) (Berman et al. 2000) (<http://www.rcsb.org/>, accessed on October 15, 2024). The PDB IDs for these structures are 6U7K, 6U7G, and 5LDS, respectively.

In the absence of structure models for bat APN, a 3D model was generated via homology modeling using the SWISS-MODEL platform (Waterhouse et al. 2018) (<https://swissmodel.expasy.org/>), with porcine APN (PDB ID: 5LG6) serving as the template. For this purpose, the amino acid sequence of APN from *Miniopterus natalensis* (XP_016072651) was retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>, accessed on October 30, 2024).

The 3D model of the CoV spike protein obtained in this study through full genome sequencing was built through homology modelling using the SWISS-MODEL platform,

with the crystal structure of PEDV spike (PDB ID: 6U7K) as the template.

The spike proteins and bat APN were preprocessed using the Chimera Minimize Structure tool (version 1.18) and saved in PDB format. For the human and porcine APN proteins, preparation was carried out using Chimera's Dock Prep tool (version 1.18), and these structures were also saved in PDB format.

Protein docking studies were conducted to evaluate the risk of cross-species transmission because the spike protein's receptor-binding domain (RBD) determines host specificity by binding to host cell receptors. Protein-protein docking studies between spike RBD and APN proteins were performed in triplicate using Haddock 2.4 (Honorato et al. 2021, 2024), with default parameters. Active residues were specified to provide insights into interacting residues (Deng et al. 2016; Ji et al. 2022; Xu et al. 2023), while passive residues were automatically assigned based on the active residues, including all residues on the interface surface within a 6.5 Å radius of any active residue. The cluster models with the highest HADDOCK scores were saved in PDB format.

RESULTS

Screening for CoVs

In this study, samples were collected from ten bats, including ten buccal swabs, ten anal swabs, and seven faecal samples. Using pan-CoV nested RT-PCR on all 27 samples, three samples (7.41%; 95% confidence interval [CI]: 0% to 17.29) yielded amplicons of the expected size for coronavirus (440 bp), which were subsequently Sanger sequenced. This 440 bp fragment corresponds to a conserved region of the RNA-dependent RNA polymerase (RdRp) gene, chosen because of its high conservation across coronaviruses, enabling reliable comparison among diverse viral strains. Additionally, this region has been widely used in previous studies for coronavirus classification and evolutionary analyses, providing a robust phylogenetic signal for reconstructing evolutionary relationships within the group. Two sequences were retrieved from a faecal sample (MyMy-F52-CoV and, MyMy-F47-CoV), and one from a buccal swab (MyMy-B51-CoV) (Table 2).

BLASTn revealed that sequence F52 was most closely related to several CoV strains from various bat species, including *Miniopterus schreibersii* from Portugal (OQ613364, Bat coronavirus isolate AN25), *Rhinolophus ferrumequinum* from Portugal (OQ613363.1, Bat coronavirus isolate F30), and *Miniopterus schreibersii* from Bulgaria (GU190240.1 /BR98-55/BGR/2008), with percentage sequence identities ranging from 95% to 98%. Sequence B51 showed the closest matches to *Miniopterus schreibersii* from Portugal (OQ613364, Bat coronavirus isolate AN25) and Bulgaria (GU190240.1 /BR98-55/BGR/2008), with sequence identities between 95% and 98%. Sequence F47 was most closely related to a *Miniopterus schreibersii* from Lebanon (MW880977.1, coronavirus isolate Alpha-CoV/LBN/LB20-CO-BAT-51A/2020), bat guano from a mixed colony in Croatia (MZ558551.1 Bat coronavirus isolate BD8 ORF1ab polyprotein), *Miniopterus schreibersii* from France (KY423482.1, Alphacoronavirus sp. isolate FRA_EPI8_Misch35_8D_P11) and *Myotis punicus* from Algeria (MN701038.1, Alphacoronavirus sp. isolate 11F), with sequence identities around 98%.

Table 4: Sample details, including host species, sample type, identified virus, and proposed virus nomenclature for Alphacoronaviruses detected in *M. myotis* and *M. schreibersii* samples through PCR-based screening.

Sample ID	Species	Sample Type	Virus Identified	Proposed Virus Name
F47	<i>M. schreibersii</i>	Faecal	<i>Alphacoronavirus</i>	MiSc-F47-CoV

F52	<i>M. myotis</i>	Faecal	<i>Alphacoronavirus</i>	MyMy-F52-CoV
B51	<i>M. myotis</i>	Buccal swabs	<i>Alphacoronavirus</i>	MyMy-B51-CoV

Metagenomic sequencing of bat faecal samples

Previous studies from our team have shown that faecal samples contain the most viruses, suggesting that replication predominantly occurs in the gastrointestinal tract and emphasizing the potential for faecal-oral transmission (Hemnani et al. 2023a). Based on this, we prioritized the 7 faecal samples for metagenomic analysis, along with the only buccal swab sample that tested positive by RT-PCR for CoV. Contigs were classified into eukaryotic viruses, phages, bacteria and eukaryotes, and we focused only on eukaryotic viruses. We detected viruses belonging to the following families: *Coronaviridae*, *Picornaviridae*, *Adenoviridae* (genus *Mastadenovirus*) and *Parvoviridae* (genus *Dependoparvovirus*) (table 3). Notably, all virus-positive samples resulting from the metagenomic sequencing were faecal, and no viral reads were detected in the buccal swab sample despite its RT-PCR positivity for CoV.

Table 3: Summary of viral sequences detected in *M. myotis* and *M. schreibersii* faecal samples through NGS, including viral classification, proposed nomenclature, and sequence lengths.

Sample ID	Species	Sample Type	Virus Identified	Proposed Virus Name	Sequence length (nt)
F45	<i>M. myotis</i>	Faecal	Picornavirus	MyMy-F45-PicoV-1	7,421
			Parvovirus (<i>Dependoparvovirus</i>)	MyMy-F45-DepPV-1	2,359
				MyMy-F45-DepPV-2	1,120
				MyMy-F45-DepPV-3	3,138
				MyMy-F45-DepPV-4	1,128
			Coronavirus (<i>Alphacoronavirus</i>)	MyMy-F45-CoV-1	27,692
			Adenovirus (<i>Mastadenovirus</i>)	MyMy-F45-Mast-1	36,906
F47	<i>M. schreibersii</i>	Faecal	Coronavirus (<i>Alphacoronavirus</i>)	MiSc-F47-CoV-2	28,712
F49	<i>M. schreibersii</i>	Faecal	Picornavirus	MiSc-F49-PicoV-2	8,557
F50	<i>M. myotis</i>	Faecal	Picornavirus	MyMy-F50.1-PicoV-3	8,351

				MyMy-F50.2-PicoV-3	7,656
F51	<i>M. myotis</i>	Faecal	Picornavirus	MyMy-F51.1-PicoV-4	9,293
				MyMy-F51.2-PicoV-4	8,440

Two near complete genomes of CoVs were identified

Two near complete CoV genomes were assembled (MyMy-F45-CoV-1 and MiSc-F47-CoV-2). BLASTn revealed that sample F45 was most closely related to several CoV strains from various bat species, including *M. petax* from South Korea (ON378804. Alphacoronavirus sp. isolate BatCoV_B20-104-1), *M. adversus* and *M. chinensis* from China (OQ175102 Bat CoV MaJX20 isolate BtMa-AlphaCoV/JX2020-Q82 and MZ328298.1 Jingmen Myotis chinensis alphacoronavirus 1), and *M. dasynceme* from Denmark (MN535734.1 Bat coronavirus isolate BtCoV/18802-1/M.das/DK/2016). The percentage sequence identity between F45 and these related CoV sequences ranged from 81.3% to 87.2%.

Sample 47 (F47 MiSc-F47-CoV-2) collected from *M. schreibersii* was also tested positive through PCR. A small region of the spike protein missing. Specific primers were designed to amplify and characterize this missing region. This sample was most closely related to CoVs previously detected in *M. fuliginosus* and *M. schreibersii* from China (KJ473798.1 BtMf-AlphaCoV/HuB2013 and OQ175329.1 Bat Coronavirus MsYN20 isolate BtMs-AlphaCoV/YN2020-Q81), with percentage sequence identities ranging from 92.8% to 98.0%.

Phylogenetic analysis confirmed that both samples belong to the AlphaCoV based on a 440bp region of the *RdRp* (Figure 1). This gene provides valuable taxonomic information and is highly effective for classification down to the subgenus level (Wilkinson et al. 2021a). To better represent all CoV-positive samples, we generated an additional phylogenetic tree based on a 440 bp fragment of the *RdRp* gene, which includes all four samples testing positive by either RT-PCR or NGS.

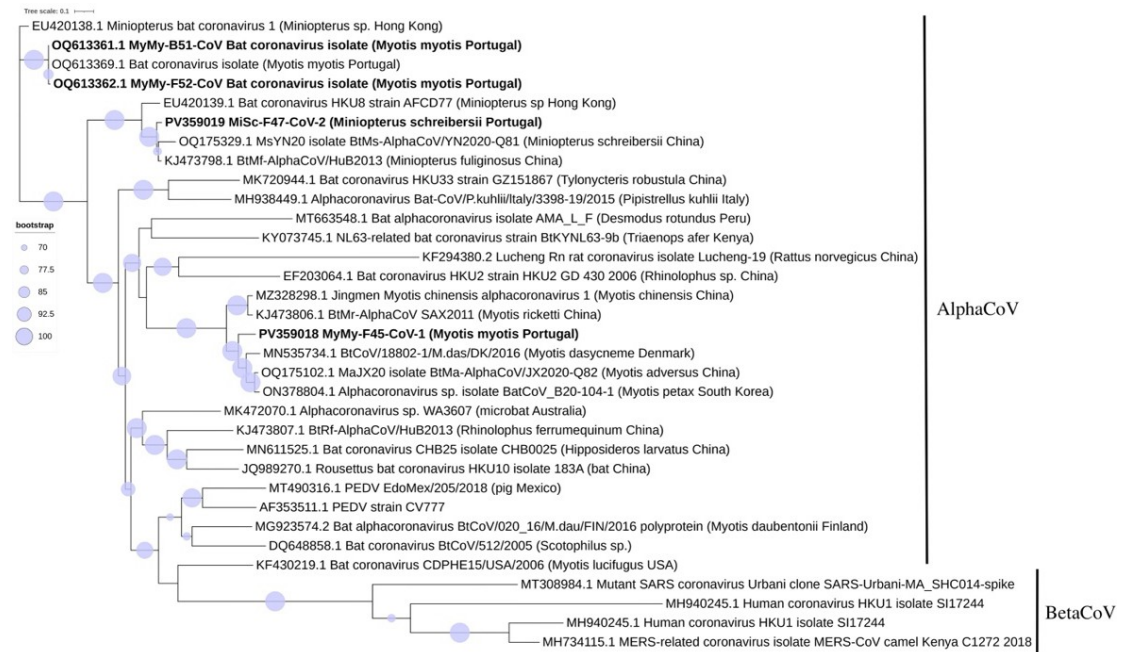


Fig. 9: Phylogeny of the **selected CoV strains** based on the **440 bp partial RdRp gene**. The tree was constructed for **AlphaCoV**, with **BetaCoV** as an outgroup. We used **30 reference strains**, and the four Cov sequences identified in this study via **metagenomics** and PCR are indicated in **bold**. Sequences were aligned with **MAFFT**, and the phylogenetic tree was inferred using **IQ-TREE2** with the **maximum likelihood method based on the GTR+G model**, with **1000 bootstrap replicates**.

The F45 sample initially tested negative for CoV by PCR was detected as positive through NGS analysis. Primer alignment with the F45 genome revealed complete alignment with HU-F primer and mismatches with both Chu-06 and Poon-F primers. A gap in the genome in the region of the HU-R primer hampered alignment analysis (Figure 2).

[illegible]

Fig. 10: Alignment of primers with the F45 sequence. The Hu-F primer aligns perfectly. Chu-06 and Poon-F primers display four and two mismatches, respectively. The mismatches and ambiguous bases may have impaired primer annealing during PCR, contributing to the discrepancy between PCR and NGS results.

We detected six near complete picornavirus genomes in four samples (F45, F49, F50, and F51). BLASTn analysis based on nucleotide sequences revealed varying levels of sequence divergence: while some genomes exhibited high divergence from known genomes (<80% identity), others showed high similarity (up to 97.9% identity). Figure 3 shows the phylogenetic relationships among the picornaviruses identified in this study based on their whole genome nucleotide sequences. Sample F45 (MyMy-F45-PicoV-1), also positive for CoV, was retrieved from *M. myotis* and clustered with picornavirus genome sequences detected from *M. ricketti* and *M. schreibersii* bats from China (OR951291.1 Myotis bat parechovirus 2 isolate GX2017, OR951294.1 Myotis bat parechovirus 2 isolate YN2016,) showing sequence identities ranging from 78.% to 86.8%. Phylogenetic analysis suggests a close relationship within the *Parechovirus* clade, and its level of sequence identity falls within the International Committee on Taxonomy of Viruses (ICTV) threshold for classification within the genus (<30% divergence), supporting its identification as a *Parechovirus* member.

Algeria/2017/Bejaia), with sequence identities between 86.1% and 90.2%. Based on phylogenetic clustering and sequence similarity and following the ICTV classification criteria for *Picornaviridae* (<30% divergence), this virus is classified within the genus *Mischivirus*.

Sample F50 from *M. myotis* contained two genomes (MyMy-F50.1-PicoV-3 and MyMy-F50.2-PicoV-3). BLASTn analysis indicated that F50.1 shared a distant sequence similarity (<78.0%) with picornaviruses previously detected in *M. davidii* and *M. ricketti* from China (OQ716000.1 JM_My.davidii_picorna_1, OQ716001.1 WZ_My.laniger_picorna_2). BLASTn analysis of F50.2 revealed hits with picornaviruses previously detected in *M. myotis* and *M. blythii* from Spain (PP654853 Navarredonda bat picornavirus; PP564854 Almagra bat picornavirus) and *Scotophilus kuhlii* from Vietnam (MZ544240.1 picornavirus isolate 16845x77-12), with sequences identities ranging from 79 to 97%. However, phylogenetic analysis revealed that these sequences are divergent from the reference sequences and may represent novel lineages within the *Picornaviridae*.

Similarly, sample F51, also from *M. myotis*, comprised two picornavirus fragments (MyMy-F51.1-PicoV-4 and MyMy-F51.2-PicoV-4). F51.1 shared similarities with picornaviruses obtained from *Scotophilus kuhlii* from China (PP745854 Picornavirales sp. isolate 184_2515), with a percentage identity of 75.1%. BLASTn analysis of sequence F51.2 showed similarities with picornaviruses previously detected in *M. davidii* and *M. ricketti* from China (OQ716000.1 JM_My.davidii_picorna_1, OQ716001.1 WZ_My.laniger_picorna_2), and with *M. myotis* from Romania (JQ916935.1 BtPV/Rcet88-19/Myo_myo/ROU/2008, a classified *Shanbavirus*) with sequence identities ranging from 78.0% to 95.8%. In the absence of an ICTV-defined divergence threshold for this genus, we propose the tentative assignment of F51.2 to *Shanbavirus* based on its phylogenetic placement.

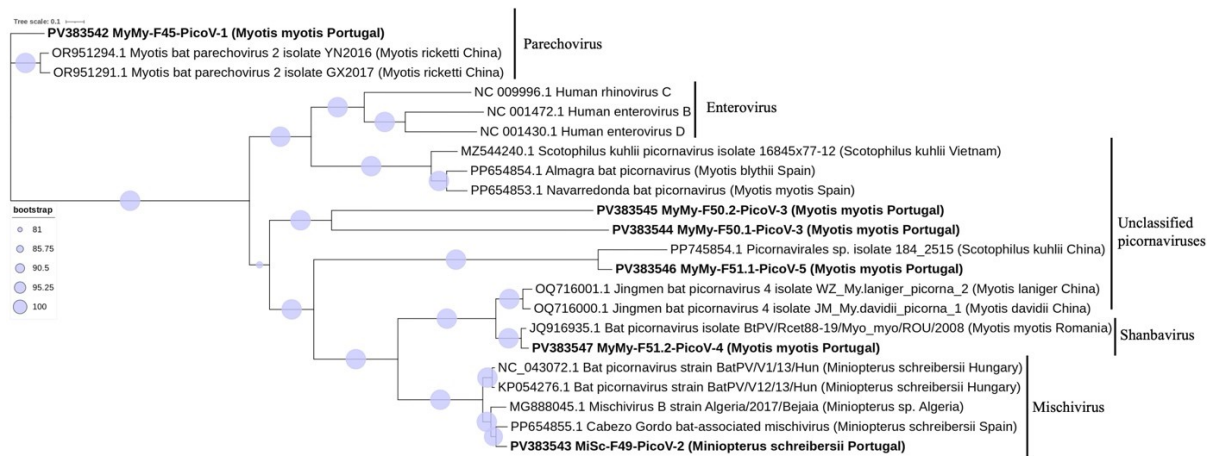


Fig. 3: Phylogeny of the Picornaviruses found in this study. The tree was constructed based on the whole genome nucleotide sequences and using 16 reference strains. The tree was constructed using **IQ-TREE2** with the maximum likelihood method based on the **GTR+F** model, including 1000 bootstrap replicates. Sequences were aligned with **MAFFT**. Sequences from this study are indicated in bold. *Complete genome of a novel bat Mastadenovirus*

A partial *Mastadenovirus* genome sequence of approximately 36,907 nucleotides, representing most of the viral genome, was identified in one bat sample- F45 (MyMy-F45-Mast-1), Phylogenetic analysis placed the adenovirus closely with sequences from *M. chinensis* and *M. ricketti*, all three originating from China (OR998910.1 GD2017_279_2457, OR998906.1 GD2016_278_64183 and OR998845.1 GX2017_216_6860). The percentage sequence identity between our sample and these reference sequences ranged from 84.7% to 88.0%, indicating a moderately high degree of genetic relatedness. The phylogeny of *Mastadenovirus* found in this study can be found in figure 4 and phylogenetic analysis was performed on their whole genome nucleotide sequences.

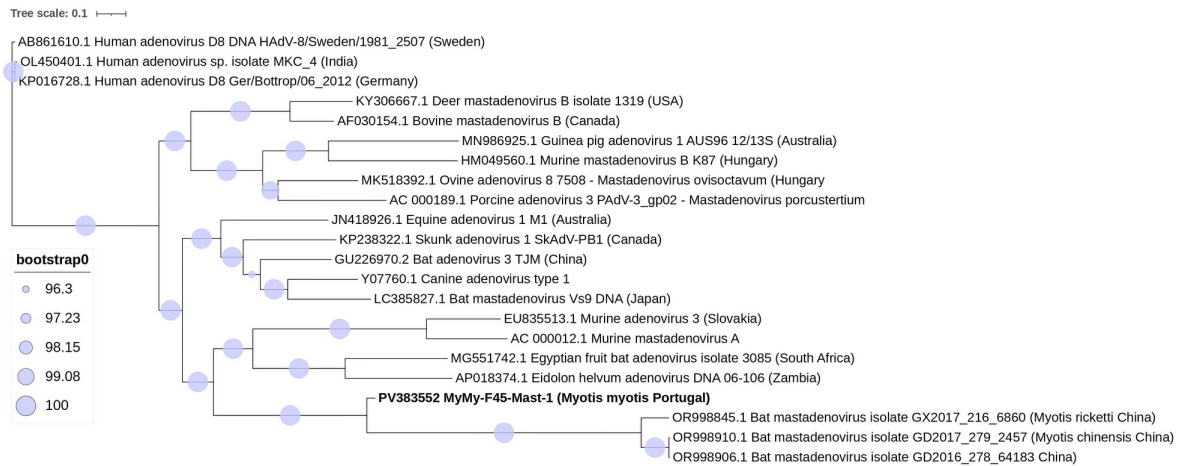


Fig. 11: Phylogeny of the *Mastadenovirus* found in this study. The tree was constructed using **IQ-TREE2** with the maximum likelihood method based on the **GTR+FO** model, including 1000 bootstrap replicates. Sequences were aligned with **MAFFT**. The phylogenetic analysis was performed using the whole genome nucleotide sequences. The tree includes the only *Mastadenovirus*-positive strain identified in this study along with 21 reference strains. Sequence from this study are indicated in bold.

Dependoparvovirus

We also detected four non-overlapping contigs most closely related to members of the genus *Dependoparvovirus* in one bat sample (F45 – *M. myotis*). Due to the lack of overlap and the limited length of two contigs (<2 kb, with poor similarity to known sequences), full genome assembly was not feasible. Nevertheless, a phylogenetic analysis was performed using the two longer contigs containing partial capsid gene sequences. The resulting tree, presented in Supplementary Figure 1, provides preliminary insights into the evolutionary relationships of these sequences. BLASTn analysis of contig MyMy-F45-DepPV-1 showed nucleotide similarities with dependoparvovirus sequences found in *M. ricketti* from China (NC_014468 Bat adeno-associated virus YNM; MH167454.1 Bat adeno-associated virus isolate 1285), with identities around 86%. Contig MyMy-F45-DepPV-2 showed similarities with dependoparvovirus sequences found in *M. ricketti* and *M. chinensis* from China (NC_014468 Bat adeno-associated virus YNM; OR998763.1 Bat adeno-associated virus 2 isolate YN2020), with identities around 80.3%-82.7%. Contig MyMy-F45-DepPV-3 showed highest BLASTn hits with genomes found in *M. ricketti* from China (NC_014468 Bat adeno-associated virus YNM; OR998838.1 Bat adeno-associated virus 2 isolate GD2017_208_21627), with identities around 83%. Contig MyMy-F45-DepPV-4 also

demonstrated similarity with genomes found in *M. ricketti* from China (OR998842.1 Bat adeno-associated virus 2 isolate GD2017_211_127613; OR998836.1 Bat adeno-associated virus 2 isolate GD2017_211_127613), with identities around 81%.

Computational studies of the binding affinity between the spike protein and APN receptors

The interaction of CoV spike proteins with host cell receptors is a determinant of host specificity and viral entry. In this study, we were able to retrieve the full spike of the CoV genome MiSc-F47-CoV-2. To further explore the potential for cross-species receptor usage, we included porcine APN in our simulations, as the *Pedacovirus* subgenus includes exclusively both bat and pig associated CoVs (Han et al. 2023). Moreover, studies have shown that PEDV and bats likely evolved from a shared ancestor, highlighting the potential role of cross-species transmission between bats and pigs in the evolutionary history of Pedacoviruses (Huang et al. 2013). Additionally, we incorporated human APN to investigate whether this bat CoV could theoretically bind to a human receptor, thereby assessing its zoonotic potential. This approach allows us to evaluate the capacity of bat Pedacoviruses to cross species barriers and informs our understanding of potential transmission routes from bats, through intermediate hosts like pigs, to humans.

In this study, protein docking simulations revealed that the binding of the F47 CoV spike protein was strongest with bat APN and similar with human and porcine APNs (Table 4, Figure 5). The HADDOCK scores from the protein docking studies between the spike proteins of bat-CoV (F47) and the APN from different hosts are presented in Table 4. These values represent the mean and standard deviation of three replicates, indicating the stability and consistency of the docking results. The results show that the interaction between F47 and bat APN yielded a score of -126.1 ± 4.8 . For the interaction with porcine APN, the score was -109.0 ± 4.4 , and for human APN, the score was -106.2 ± 7.6 . Figure 5 presents the best structural interaction models obtained from HADDOCK between the RBD of the F47 CoV spike protein and APN from these hosts. The structural differences in these models may reflect variations in binding affinity, as demonstrated by the docking scores. To further explore these findings, docking studies were performed for the PEDV RBD with APN from the same animal species (Table 4, Figure 6). The interaction between PEDV and bat APN resulted in a score of -90.7 ± 7.5 , while with porcine APN, it was -82.8 ± 2.2 , and with human APN, it was -100.2 ± 5.4 .

Table 5: HADDOCK scores obtained in the protein docking studies between the spike proteins from bat-CoV obtained in this study (F47) and the APN from different hosts, using

Haddock 2.4. The values presented correspond to the mean and respective standard deviation of three replicates.

	APN		
	Bat	Porcine	Human
F47	-126.1 +/- 4.8	-109.0 +/- 4.4	-106.2 +/- 7.6
PEDV	-90.7 +/- 7.5	-82.8 +/- 2.2	-100.2 +/- 5.4

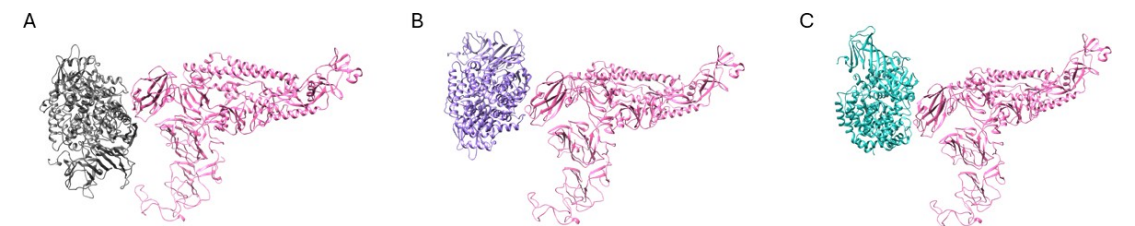


Fig. 5: Models of structural interaction between the F47 CoV spike protein (pink) and aminopeptidase N (APN) from (A) bat (black), (B) porcine (purple), (C) human (green).

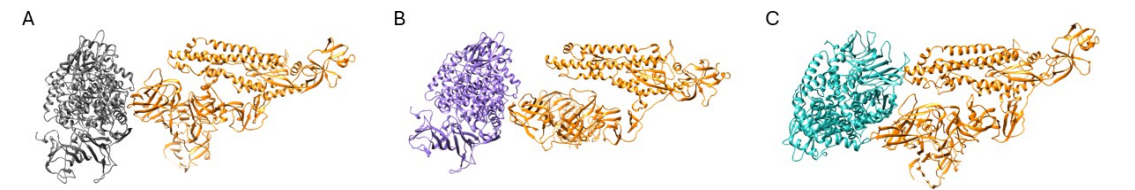


Fig. 6: Models of structural interaction between the PEDV spike protein (orange) and aminopeptidase N (APN) from (A) bat (black), (B) porcine (purple), (C) human (green).

DISCUSSION

The intricate relationship between bats and the viruses they harbor has long been a subject of interest, given the unique ecological role of bats as reservoirs for a diverse range of pathogens (Roffler et al. 2024). In Portugal, interactions between bats and humans occur through various channels, including urban roosting in historical buildings, agricultural landscapes where bats contribute to pest control, and ecotourism activities in caves and natural reserves. These interactions can foster awareness and support bat conservation. Yet, a large gap still persists in the characterization of the viral communities present in bats, particularly in Europe, where most studies remain limited in scope.

In this study, we explored the virome of bat populations in Portugal, uncovering significant viral diversity, including CoVs, picornaviruses, mastadenoviruses (adenoviruses), and dependoparvoviruses. We performed PCR and metagenomic sequencing using the NetoVIR protocol on bat faecal and buccal swab samples collected from Montemor I roost in Portugal. This shelter supports other species of conservation importance, including the Mediterranean horseshoe bat (*Rhinolophus euryale*), greater horseshoe bat (*R. ferrumequinum*), lesser horseshoe bat (*R. hipposideros*), and Mehely's horseshoe bat (*R. mehelyi*). The surrounding area, characterized by oak savanna-like woodland (locally known as *montados*), which is a type of agroforestry system found in the Iberian Peninsula characterized by open pastures and agricultural land, plays an essential role as a feeding ground (Rainho; Batista 2014).

Through PCR analysis of faecal, buccal, and anal swabs, we detected CoV in three samples (F47, F52 and B51), two from distinct *Myotis* individuals, one from a faecal sample and the other from a buccal swab, and one from *M. Schreibersii*, also from a faecal sample. In contrast, our NGS analysis (which focused on all faecal samples and the buccal sample positive via PCR), identified two CoV-positive results from faecal samples (F45 and F47) (two near complete genomes). One of the positive samples (F47) was also positive through PCR, while the other sample (F45) did not show any positive results for both methods (RT-PCR and NGS). As shown in Figure 2, the alignment of primers with the F45 sequence reveals mismatches with Chu-06, and Poon-F primers. These mismatches could explain why the PCR failed to amplify the target, while NGS, with its broader detection capabilities successfully identified the CoVs in the F45 sample.

Previous research highlights that faecal samples often yield the highest detection rates for CoVs, strongly indicating that viral replication predominantly occurs in the gastrointestinal tract (Benkő et al. 2014; Borkenhagen et al. 2019a; Hemnani et al. 2023b;

Méndez-Rodríguez et al. 2024). Given these findings, we focused our NGS analysis primarily on faecal samples, as they are considered the most likely to contain comprehensive viral genomic data. For the metagenomics, we followed the NetoVIR protocol, an optimized and widely used approach for high-throughput, reproducible sample preparation. This method introduces minimal bias and is particularly suited for faecal samples (Conceição- Neto et al. 2015b). Additionally, the buccal swab sample that tested positive for CoV by RT- PCR was included to explore any possible alternative sites of replication or shedding. This approach ensured that our sequencing efforts were strategically directed towards maximizing the recovery of relevant viral sequences. The sequencing revealed a wide range of viruses including mammalian, but also insect viruses (data not shown), which is an expected result given the fact that the diet of the sampled Portuguese bats almost consists of insects (Rodrigues et al. 2003; Herrera et al. 2015; Mata et al. 2021). We concentrated exclusively on viruses that can infect mammals, excluding those that target archaea, bacteria, fungi, invertebrates, and plants.

Our study identified two near complete genomes of AlphaCoVs. The clustering of our sequences with CoVs from bats in South Korea (*M. petax*), China (*M. adversus*, *M. chinensis*, *M. fuliginosus* and *M. schreibersii*), and Denmark (*M. dasyncneme*) highlights the widespread distribution and genetic diversity of bat-associated coronaviruses. However, this pattern may partly reflect sampling bias, as CoV sequences from Asian bats, particularly from China, are more abundantly represented in public databases compared to those from European or Portuguese bats. The limited availability of regional sequences may contribute to the observed phylogenetic clustering with genetically similar but geographically distant strains. In addition, the migratory behaviour of some bat species, may also contribute to the dissemination and genetic mixing of AlphaCoVs across broad geographic regions. Previous studies have documented transboundary bat movements within Europe (Rodrigues and Palmeirim 2008), supporting the possibility of viral exchange along migratory routes. Geographic gaps also exist, with certain regions in Europe lacking comprehensive surveys for bat-associated CoVs, potentially overlooking important reservoirs (Hemnani et al. 2024a). These findings show that Portuguese bats may contribute to the broader circulation of AlphaCoVs, reinforcing their role as crucial reservoirs for this viral group. We also detected picornavirus in several samples, revealing a high degree of sequence divergence both from each other and from known picornavirus sequences in databases. The order *Picornavirales* encompasses nine families, one of which is the family *Picornaviridae* (Carrascosa-Sàez et al. 2024; Zell et al. 2017; Temmam et al. 2020 al. 2024). The identified

picornaviruses displayed clustering patterns similar to picornavirus strains found in bat species from China and Europe, particularly within the *Myotis* and *Miniopterus* genera. The identification of six picornavirus genomes across four samples highlights the presence of diverse picornaviruses in Portuguese bats. The clustering patterns suggest a complex evolutionary history, with some strains showing higher similarity to viruses from geographically close regions (e.g., Spain, Hungary and Algeria), while others are more closely related to strains from distant locations such as China. This likely reflects significant gaps in current knowledge and sampling of bat-associated picornaviruses, which may result in unexpected phylogenetic relationships between viruses from distant geographic locations. Additionally, the observed genetic diversity suggests ongoing viral evolution within these bat species (Kramer and Tavakoli 2021; Jones et al. 2023).

Adenoviruses are medium-sized, non-enveloped viruses belonging to the family *Adenoviridae* (Parija 2023). The family is divided into two genera: *Mastadenovirus*, which includes viruses infecting mammals (Fitzgerald et al. 2020), and *Aviadenovirus*, comprising those that infect birds (Athukorala et al. 2022). The phylogenetic placement of the detected *Mastadenovirus* alongside strains from *Myotis chinensis* and *Myotis ricketti* in China highlights unexpected relationships between viruses from distant regions. Rather than indicating direct viral exchange or contact between bat populations, this pattern likely reflects significant gaps in the global sampling of bat-associated adenoviruses. Such phylogenetic alignments may arise due to underrepresentation of viral sequences from intermediate regions, emphasizing the need for broader surveillance efforts. Moreover, the ability of adenoviruses to infect multiple bat species and cross species barriers is well-documented within the *Adenoviridae* family (Benkő et al. 2014; Borkenhagen et al. 2019a; Méndez-Rodríguez et al. 2024).

The exact mechanisms that allow the transmission of adenoviruses between species remain poorly understood. While some studies suggest that indirect environmental exposure, such as through contaminated water sources, may play a role in facilitating viral exchange among wildlife, domestic animals, and humans (Borkenhagen et al. 2019b; Simonin 2023), these assumptions are largely based on general patterns of pathogen spread rather than adenovirus-specific evidence. Therefore, the intricacies of cross-species transmission and the ecological or behavioral factors that might enable such events require further investigation in the context of adenoviruses.

Dependoparvovirus is a genus within the family *Parvoviridae*. These are parvoviruses that typically require co-infection with a helper virus, such as an adenovirus or a herpesvirus,

to complete their replication cycle (Du et al. 2022). This dependency on a helper virus is what gives them their name, *Dependoparvovirus* (Hildebrandt et al. 2020). In this study, *Dependoparvovirus* was detected in *M. myotis* (F45), the same individual in which adenovirus was also identified. The virus found in this study shared high sequence identity with strains from *Myotis* bats in China, with sequence identities ranging from 81.6% to 86.2%. These similarities likely reflect the limited availability of *Dependoparvovirus* sequences globally, rather than direct viral circulation, underscoring the need for broader sampling to better understand their diversity and evolutionary relationships.

To evaluate the risk of cross-species transmission of the bat CoV sequenced in this study, protein docking studies were conducted to analyze the binding affinity between the bat CoV spike RBD and bat, porcine and human APNs. These interactions are critical for understanding host specificity and the potential for spillover to livestock or zoonotic transmission to humans. By evaluating the binding affinities, we gained insights into the virus's transmission dynamics and its potential to infect other species.

The APN receptors of pigs and humans were included in the docking analysis to assess the potential interaction between the bat CoV spike protein and host receptors. Porcine APN was used due to its known role as the receptor for PEDV. PEDV was included in the docking analysis as a reference alphacoronavirus known to use porcine APN as its entry receptor. Given the sequence similarity between the bat CoV spike protein and PEDV, as well as their shared placement within the *Pedacovirus* subgenus, this analysis aimed to explore whether the bat virus might use a similar entry mechanism. By comparing the binding interactions of the bat CoV spike with those of PEDV, we sought to assess potential similarities in receptor usage and gain insights into possible cross-species transmission pathways (Banerjee et al. 2019; Han et al. 2019). Human APN was included to evaluate the potential risk of zoonotic transmission of this virus. The HADDOCK scores from our protein docking studies reveal important insights into the binding affinities between the spike proteins of bat-CoV (F47) and PEDV with APN receptors from different hosts (bat, porcine, and human). The results suggest that the F47 CoV spike protein exhibits the strongest binding affinity with bat APN, yielding a score of -126.1 ± 4.8 . This high binding affinity could imply that the F47 CoV is well adapted to the bat host, providing insights into the evolutionary history of the virus and its co-evolution with bat species. Interestingly, the binding affinities between the F47 spike protein and the APNs of both humans and pigs are also strong and comparable, with scores of -106.2 ± 7.6 and -109.0 ± 4.4 , respectively.

This finding suggests a potential capacity for cross-species receptor usage, which

could have implications for host range and zoonotic spillover risks. The close binding affinities observed for F47 CoV and human or porcine APN suggest a potential inter-species transmission risk. However, it's important to note that these are computational studies based solely on the spike protein, and the adaptation of other viral proteins has not been considered. Successful viral infection involves more than just spike-APN interactions, as the function of other viral components is also critical for productive infection.

In contrast, the predicted interaction of PEDV is weakest with porcine APN, with a score of -82.8 ± 2.2 , which is quite surprising given that pigs are the natural host for PEDV. Interestingly, the PEDV spike protein demonstrated weaker binding to porcine APN than to human and bat APNs, contrary to the established role of porcine APN as its primary receptor. This unexpected result challenges the established understanding of PEDV spike-receptor interactions and raises intriguing questions about the structural or evolutionary factors influencing binding specificity. In fact, recent studies have suggested that APN may not be the cell receptor of PEDV (Shirato et al. 2016; Whitworth et al. 2019) and that PEDV employs both protein receptors and sugar co-receptors for infection (Chang et al. 2015). Additionally, while the significant binding affinity of PEDV spike for human APN (score of -100.2 ± 5.4) may suggest some potential for cross-species interactions, the absence of documented human infections indicates that other factors likely restrict a PEDV spillover. This could be important in understanding potential zoonotic risks and provide insights into the determinants of host specificity.

These findings highlight the need for further experimental validation to confirm receptor usage and assess the functional relevance of these interactions. Moreover, comparative studies of receptor-binding dynamics across different CoVs could provide valuable insights into the evolutionary pressures shaping spike protein-receptor interactions and their role in determining host range. The data might suggest that the strong binding of F47 CoV's spike to bat APN represents a virus-host relationship optimized through evolutionary pressures, while PEDV's comparatively weaker binding to pig APN could highlight differences in virus-host adaptation strategies. F47 spike's comparable affinity for human and porcine APNs also suggests a possible evolutionary plasticity, where the virus might more easily adapt to new hosts with similar APN structures. This binding pattern could also indicate that, while F47 is primarily adapted to bats, it might possess an intrinsic ability to bind APN from other species, potentially aiding cross-species spillover events. In contrast, PEDV's reliance on additional co-receptors for efficient infection in pigs could suggest a more specialized host relationship, despite the weak APN affinity.

The discovery of CoVs and other viruses in bats in Portugal underscores the importance of monitoring and viral surveillance in bat populations. The detection of viral families commonly associated with mammalian hosts, such as CoVs and picornaviruses, suggests that bats may harbor diverse viral populations with the potential for cross-species transmission.

Given the frequent human-wildlife interactions, such as habitat encroachment, heightened surveillance of bat populations is essential for the early detection of emerging viruses that could pose epidemic risks. This study emphasizes the critical role that bats play in the maintenance of viral diversity, highlighting the need for a "One Health" approach that integrates wildlife monitoring with human health initiatives to prevent potential outbreaks.

This study provided a snapshot of the viral diversity in bat populations in Portugal, contributing to the broader understanding of viral ecology and cross-species transmission. The identification of CoVs and other viral families in these bats underscores the importance of continued research and surveillance to mitigate potential public health threats. Furthermore, it highlights the need for more research on the environmental and ecological factors that influence viral transmission within bat populations. Understanding these dynamics, particularly in regions under high anthropogenic pressure, will be essential for developing effective strategies to mitigate zoonotic risks.

ACKNOWLEDGMENTS

Mahima Hemnani thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of her PhD work under the Maria de Souza Scholarship, contract number 2021.09380.BD. This work was also funded by FCT, under the project numbers UIDB/04750/2020, LA/P/0064/2020, UIDP/00772/2020 (Doi:10.54499/UIDB/00772/2020), and LA/P/0059/2020.

DATA AVAILABILITY DECLARATION

The viruses reported in this article are available in the GenBank database (OQ613362, PV221239, OQ613361, PV359018, PV359019, PV383542-PV383552). The relevant raw high throughput sequencing data obtained in this study were deposited at the NCBI Sequence Read Archives (SRA) under BioProject ID PRJNA1162723.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

- Afelt A, Frutos R, Devaux C (2018) Bats, coronaviruses, and deforestation: Toward the emergence of novel infectious diseases? *Front Microbiol* 9:1–5. <https://doi.org/10.3389/fmicb.2018.00702>
- Ahn M, Anderson DE, Zhang Q, et al (2019) Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nat Microbiol* 4:789–799. <https://doi.org/10.1038/S41564-019-0371-3>;
- Akem ES, Pemunta NV (2020) The bat meat chain and perceptions of the risk of contracting Ebola in the Mount Cameroon region. *BMC Public Health* 20:1–10. <https://doi.org/10.1186/S12889-020-08460-8>/PEER-REVIEW
- Alcalde JT, Jiménez M, Brila I, et al (2021) Transcontinental 2200 km migration of a *Nathusius' pipistrelle* (*Pipistrellus nathusii*) across Europe. *Mammalia* 85:161–163. <https://doi.org/10.1515/mammalia-2020-0069>
- Allen T, Murray KA, Zambrana-Torrel C, et al (2017) Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* 8:. <https://doi.org/10.1038/S41467-017-00923-8>;
- Alves RS, do Canto Olegário J, Weber MN, et al (2022) Detection of coronavirus in vampire bats (*Desmodus rotundus*) in southern Brazil. *Transbound Emerg Dis* 69:2384–2389. <https://doi.org/10.1111/tbed.14150>
- Amengual B, López-Roig M, Serra-Cobo J (2007) First record of seasonal over sea migration of *Miniopterus schreibersii* and *Myotis capaccinii* between Balearic Islands (Spain). *Acta Chiropt* 9:319–322. [https://doi.org/10.3161/1733-5329\(2007\)9\[319:FROSOS\]2.0.CO;2](https://doi.org/10.3161/1733-5329(2007)9[319:FROSOS]2.0.CO;2)
- Ancillotto L, Santini L, Ranc N, et al (2016) Extraordinary range expansion in a common bat: The potential roles of climate change and urbanisation. *Science of Nature* 103:. <https://doi.org/10.1007/S00114-016-1334-7>;
- Anderson SC, Ruxton GD (2020) The evolution of flight in bats: a novel hypothesis. *Mamm Rev* 50:426–439. <https://doi.org/10.1111/MAM.12211>;REQUESTEDJOURNAL:JOURNAL:13652907;PAGE:STRING:ARTICLE/CHAPTER
- Andrusier N, Mashiach E, Nussinov R, Wolfson HJ (2008) Principles of flexible protein-protein docking. *Proteins: Structure, Function and Genetics* 73:271–289. <https://doi.org/10.1002/prot.22170>

- Anthony SJ, Johnson CK, Greig DJ, et al (2017) Global patterns in coronavirus diversity. *Virus Evol* 3:1–15. <https://doi.org/10.1093/ve/vex012>
- Apoorva, Singh SK (2024) A tale of endurance: bats, viruses and immune dynamics. *Future Microbiol* 19:841–856. <https://doi.org/10.2217/fmb-2023-0233>
- Athukorala A, Helbig KJ, Mcsharry BP, et al (2022) Adenoviruses in Avian Hosts: Recent Discoveries Shed New Light on Adenovirus Diversity and Evolution. *Viruses* 14:1767. <https://doi.org/10.3390/v14081767>
- August TA, Mathews F, Nunn MA (2012) Alphacoronavirus detected in bats in the United Kingdom. *Vector-Borne and Zoonotic Diseases* 12:530–533. <https://doi.org/10.1089/vbz.2011.0829>
- Azhar EI, Hashem AM, El-Kafrawy SA, et al (2014) Detection of the Middle East respiratory syndrome coronavirus genome in an air sample originating from a camel barn owned by an infected patient. *mBio* 5:e01450-14. <https://doi.org/10.1128/mBio.01450-14>
- Bakkers MJG, Lang Y, Feitsma LJ, et al (2017) Betacoronavirus Adaptation to Humans Involved Progressive Loss of Hemagglutinin-Esterase Lectin Activity. *Cell Host Microbe* 21:356–366. <https://doi.org/10.1016/j.chom.2017.02.008>
- Banerjee A, Kulcsar K, Misra V, et al (2019) Bats and coronaviruses. *Viruses* 11:7–9. <https://doi.org/10.3390/v11010041>
- Batista T (2014) Paisagem e ecologia da região de Montemor-o-Novo Conversas à volta do espírito dos lugares
- Beere HM, Wolf BB, Cain K, et al (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2:469–475. <https://doi.org/10.1038/35019501;KWRD=LIFE+SCIENCES>
- Beniac DR, Andonov A, Grudeski E, Booth TF (2006) Architecture of the SARS coronavirus prefusion spike. *Nat Struct Mol Biol* 13:751–752. <https://doi.org/10.1038/NSMB1123;KWRD=LIFE+SCIENCES>
- Benkő M, Harrach B, Kremer EJ (2014) Do Nonhuman Primate or Bat Adenoviruses Pose a Risk for Human Health? *Future Microbiol* 9:269–272. <https://doi.org/10.2217/fmb.13.170>
- Bergner LM, Orton RJ, Benavides JA, et al (2020) Demographic and environmental drivers of metagenomic viral diversity in vampire bats. *Mol Ecol* 29:26–39. <https://doi.org/10.1111/MEC.15250>

- Berman HM, Westbrook J, Feng Z, et al (2000) The Protein Data Bank. *Nucleic Acids Res* 28:235–242. <https://doi.org/10.1093/nar/28.1.235>
- Biol Sci BJ, de Jesus Santos N, Santos da Paz E, et al (2019) Evaluation of bat-related knowledge, perceptions, and practices in an urban community: A strategy for Conservation Biology and health promotion. *Brazilian Journal of Biological Sciences* 6:e392–e392. <https://doi.org/10.21472/BJBS.061303>
- Bittar C, Machado RRG, Comelis MT, et al (2020) Alphacoronavirus Detection in Lungs, Liver, and Intestines of Bats from Brazil. *Microb Ecol* 79:203–212. <https://doi.org/10.1007/s00248-019-01391-x>
- Blatteis CM (2003) Fever: pathological or physiological, injurious or beneficial? *J Therm Biol* 28:1–13. [https://doi.org/10.1016/S0306-4565\(02\)00034-7](https://doi.org/10.1016/S0306-4565(02)00034-7)
- Boardman WSJ, Baker ML, Boyd V, et al (2020) Seroprevalence of three paramyxoviruses; Hendra virus, Tioman virus, Cedar virus and a rhabdovirus, Australian bat lyssavirus, in a range expanding fruit bat, the Grey-headed flying fox (*Pteropus poliocephalus*). *PLoS One* 15:. <https://doi.org/10.1371/JOURNAL.PONE.0232339>;
- Bodewes R, Ruiz-Gonzalez A, Schapendonk CME, et al (2014) Viral metagenomic analysis of feces of wild small carnivores. *Virol J* 11:1–13. <https://doi.org/10.1186/1743-422X-11-89>
- Bokelmann M, Balkema-buschmann A (2021a) Coronaviruses in Bats. *Berl Munch Tierarztl Wochenschr* 134:1–16. <https://doi.org/10.2376/1439-0299-2020-44>
- Bokelmann M, Balkema-buschmann A (2021b) Coronaviruses in Bats. *Berl Munch Tierarztl Wochenschr* 134:1–16. <https://doi.org/10.2376/1439-0299-2020-44>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Bonny T, Driver J, Paisie T, et al (2017) Detection of Alphacoronavirus vRNA in the Feces of Brazilian Free-Tailed Bats (*Tadarida brasiliensis*) from a Colony in Florida, USA. *Diseases* 5:7. <https://doi.org/10.3390/diseases5010007>
- Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019a) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect* 8:1679–1687. <https://doi.org/10.1080/22221751.2019.1690953>
- Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019b) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect* 8:1679–1687. <https://doi.org/10.1080/22221751.2019.1690953>

- Botto Nuñez G, Becker DJ, Lawrence RL, Plowright RK (2020) Synergistic Effects of Grassland Fragmentation and Temperature on Bovine Rabies Emergence. *Ecohealth* 17:203–216. <https://doi.org/10.1007/S10393-020-01486-9>;
- Bredenbeek PJ, Pachuk CJ, Noten AFH, et al (1990) The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59; a highly conserved polymerase is expressed by an efficient ribosomal frameshifting mechanism. *Nucleic Acids Res* 18:1825–1832. <https://doi.org/10.1093/NAR/18.7.1825>
- Breed AC, Field HE, Smith CS, et al (2010) Bats without borders: Long-distance movements and implications for disease risk management. *Ecohealth* 7:204–212. <https://doi.org/10.1007/S10393-010-0332-Z/FIGURES/3>
- Breshears LE, Nguyen BT, Mata Robles S, et al (2022) Biosensor detection of airborne respiratory viruses such as SARS-CoV-2. *SLAS Technol* 27:4–17. <https://doi.org/10.1016/j.slant.2021.12.004>
- Brook CE, Dobson AP (2015) Bats as “special” reservoirs for emerging zoonotic pathogens. *Trends Microbiol* 23:. <https://doi.org/10.1016/j.tim.2014.12.004>
- Bryja J, Kaňuch P, Fornůsková A, et al (2009) Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe. *Biological Journal of the Linnean Society* 96:103–114. <https://doi.org/10.1111/j.1095-8312.2008.01093.x>
- Bueno LM, Rizotto LS, Viana A de O, et al (2022) High genetic diversity of alphacoronaviruses in bat species (Mammalia: Chiroptera) from the Atlantic Forest in Brazil. *Transbound Emerg Dis* 69:e2863–e2875. <https://doi.org/10.1111/tbed.14636>
- Burgin CJ, Colella JP, Kahn PL, Upham NS (2018) How many species of mammals are there? *J Mammal* 99:1–14. <https://doi.org/10.1093/JMAMMAL/GYX147>
- Cadet J, Richard Wagner J (2013) DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harb Perspect Biol* 5:. <https://doi.org/10.1101/CSHPERSPECT.A012559>,
- Calisher CH, Childs JE, Field HE, et al (2006a) Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19:531–545. <https://doi.org/10.1128/CMR.00017-06>
- Calisher CH, Childs JE, Field HE, et al (2006b) Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19:531–545. <https://doi.org/10.1128/CMR.00017-06>

- Cantlay JC, Ingram DJ, Meredith AL (2017) A Review of Zoonotic Infection Risks Associated with the Wild Meat Trade in Malaysia. *Ecohealth* 14:361–388. <https://doi.org/10.1007/S10393-017-1229-X>;
- Cao T, Jin JP (2020) Evolution of Flight Muscle Contractility and Energetic Efficiency. *Front Physiol* 11:565310. <https://doi.org/10.3389/FPHYS.2020.01038/BIBTEX>
- Cappelli F, Costantini V, Consoli D (2021) The trap of climate change-induced “natural” disasters and inequality. *Global Environmental Change* 70:. <https://doi.org/10.1016/J.GLOENVCHA.2021.102329>;
- Carlson CJ, Albery GF, Merow C, et al (2022) Climate change increases cross-species viral transmission risk. *Nature* 607:555–562. <https://doi.org/10.1038/S41586-022-04788-W>;
- Chang CK, Sue SC, Yu TH, et al (2006) Modular organization of SARS coronavirus nucleocapsid protein. *J Biomed Sci* 13:59–72. <https://doi.org/10.1007/S11373-005-9035-9/METRICS>
- Chaverri G, Ancillotto L, Russo D (2018) Social communication in bats. *Biological Reviews* 93:1938–1954. <https://doi.org/10.1111/brv.12427>
- CHEEVER FS, DANIELS JB (1949) A murine virus (JHM) causing disseminated encephalomyelitis with. *J Exp Med* 90:181–210. <https://doi.org/10.1084/JEM.90.3.181>,
- Chen Y, Liu Q, Guo D (2020) Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol* 92:418–423. <https://doi.org/10.1002/JMV.25681>
- Chua KB, Lek Koh C, Hooi PS, et al (2002) Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4:145–151. [https://doi.org/10.1016/S1286-4579\(01\)01522-2](https://doi.org/10.1016/S1286-4579(01)01522-2);
- Ciotti M, Ciccozzi M, Terrinoni A, et al (2020) The COVID-19 pandemic. *Crit Rev Clin Lab Sci* 0:365–388. <https://doi.org/10.1080/10408363.2020.1783198>
- Clark JS, Bell DM, Hersh MH, et al (2011) Individual-scale variation, species-scale differences: inference needed to understand diversity. *Ecol Lett* 14:1273–1287. <https://doi.org/10.1111/J.1461-0248.2011.01685.X>
- Cohen LE, Fagre AC, Chen B, et al (2023) Coronavirus sampling and surveillance in bats from 1996–2019: a systematic review and meta-analysis. *Nat Microbiol* 8:1176–1186. <https://doi.org/10.1038/s41564-023-01375-1>
- Collen A (2012) The evolution of echolocation in bats: a comparative approach. Doctoral thesis, UCL (University College London)

- Conceição-Neto N, Godinho R, Álvares F, et al (2017) Viral gut metagenomics of sympatric wild and domestic canids, and monitoring of viruses: Insights from an endangered wolf population. *Ecol Evol* 7:4135–4146. <https://doi.org/10.1002/ece3.2991>
- Conceição-Neto N, Zeller M, Lefrère H, et al (2015a) Modular approach to customise sample preparation procedures for viral metagenomics: A reproducible protocol for virome analysis. *Sci Rep* 5:1–14. <https://doi.org/10.1038/srep16532>
- Conceição-Neto N, Zeller M, Lefrère H, et al (2015b) Modular approach to customise sample preparation procedures for viral metagenomics: A reproducible protocol for virome analysis. *Sci Rep* 5:1–14. <https://doi.org/10.1038/srep16532>
- Conenna I, Muñoz-Garcia A, Korine C (2025) Physiological and Behavioural Strategies of Bats From Arid Environments. *Mamm Rev* e70005. <https://doi.org/10.1111/MAM.70005>
- Contini C, Di Nuzzo M, Barp N, et al (2020) The novel zoonotic COVID-19 pandemic: An expected global health concern. *J Infect Dev Ctries* 14:254–264. <https://doi.org/10.3855/jidc.12671>
- Cooper LN, Cretokos CJ, Sears KE (2012) The evolution and development of mammalian flight. *Wiley Interdiscip Rev Dev Biol* 1:773–779. <https://doi.org/10.1002/WDEV.50;CTYPE:STRING:JOURNAL>
- Corman VM, Kallies R, Philipps H, et al (2014a) Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *J Virol* 88:717–724. <https://doi.org/10.1128/JVI.01600-13>,
- Corman VM, Kallies R, Philipps H, et al (2014b) Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *J Virol* 88:717–724. <https://doi.org/10.1128/JVI.01600-13>,
- Cui X, Fan K, Liang X, et al (2023) Virus diversity, wildlife-domestic animal circulation and potential zoonotic viruses of small mammals, pangolins and zoo animals. *Nat Commun* 14:2488. <https://doi.org/10.1038/s41467-023-38202-4>
- da Silva PG, Gonçalves J, Nascimento MS, et al (2022) Detection of SARS-CoV-2 in the Indoor and Outdoor Areas of Urban Public Transport Systems of Three Major Cities of Portugal in 2021. *Int J Environ Res Public Health* 19
- da Silva PG, Mesquita JR, de São José Nascimento M, Ferreira VAM (2021a) Viral, host and environmental factors that favor anthroozoonotic spillover of coronaviruses: An opinionated review, focusing on SARS-CoV, MERS-CoV and SARS-CoV-2. *Science of the Total Environment* 750:141483. <https://doi.org/10.1016/j.scitotenv.2020.141483>

- da Silva PG, Nascimento MSJ, Soares RRG, et al (2021b) Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV. *Science of the Total Environment* 764:.. <https://doi.org/10.1016/j.scitotenv.2020.142802>
- da Silva PG, Nascimento MSJ, Soares RRG, et al (2021c) Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV. *Science of the Total Environment* 764:.. <https://doi.org/10.1016/j.scitotenv.2020.142802>
- De Benedictis P, Marciano S, Scaravelli D, et al (2014) Alpha and lineage C betaCoV infections in Italian bats. *Virus Genes* 48:366–371. <https://doi.org/10.1007/s11262-013-1008-x>
- De Coninck L, Faye L, Basler N, et al (2025) ViPER
- de Groot RJ, Baker SC, Baric RS, et al (2013) Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *J Virol* 87:7790–7792. <https://doi.org/10.1128/JVI.01244-13>
- De Oliveira HFM, Camargo NF, Hemprich-Bennett DR, et al (2020) Wing morphology predicts individual niche specialization in *Pteronotus mesoamericanus* (Mammalia: Chiroptera). *PLoS One* 15:e0232601. <https://doi.org/10.1371/JOURNAL.PONE.0232601>
- DeDiego ML, Álvarez E, Almazán F, et al (2007) A Severe Acute Respiratory Syndrome Coronavirus That Lacks the E Gene Is Attenuated In Vitro and In Vivo. *J Virol* 81:1701–1713. <https://doi.org/10.1128/JVI.01467-06/ASSET/983812D0-AAA7-4C88-8679-A844299EF1C1/ASSETS/GRAPHIC/ZJV0040787220008.JPEG>
- Delaplace M, Huet H, Gambino A, Le Poder S (2021) Feline Coronavirus Antivirals: A Review. *Pathogens* 2021, Vol 10, Page 1150 10:1150. <https://doi.org/10.3390/PATHOGENS10091150>
- Delmas B, Gelfi J, L'Haridon R, et al (1992) Amino peptidase N is a major receptor for the enteropathogenic coronavirus TGEV. *Nature* 357:417–420. <https://doi.org/10.1038/357417A0>,
- Deng F, Ye G, Liu Q, et al (2016) Identification and Comparison of Receptor Binding Characteristics of the Spike Protein of Two Porcine Epidemic Diarrhea Virus Strains. *Viruses* 8:55

- Denzinger A, Schnitzler HU (2013) Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front Physiol* 4 JUL:48812. <https://doi.org/10.3389/FPHYS.2013.00164/BIBTEX>
- Diengdoh VL, Onde S, Hunt M, Brook BW (2022) Predicted impacts of climate change and extreme temperature events on the future distribution of fruit bat species in Australia. *Glob Ecol Conserv* 37:. <https://doi.org/10.1016/J.GECCO.2022.E02181>;
- Dietz M, Brombacher M, Erasmy M, et al (2018) Bat Community and Roost Site Selection of Tree-Dwelling Bats in a Well-Preserved European Lowland Forest. *Acta Chiropt* 20:117–127. <https://doi.org/10.3161/15081109ACC2018.20.1.008>
- Domańska-Blicharz K, Miłek-Krupa J, Pikuła A (2021) Diversity of coronaviruses in wild representatives of the aves class in poland. *Viruses* 13:1–19. <https://doi.org/10.3390/v13081497>
- Dong N, Fang L, Zeng S, et al (2015) Porcine Deltacoronavirus in mainland China. *Emerg Infect Dis* 21:2254–2255. <https://doi.org/10.3201/EID2112.150283>,
- Drexler JF, Corman VM, Drosten C (2014a) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Res* 101:45–56. <https://doi.org/10.1016/j.antiviral.2013.10.013>
- Drexler JF, Corman VM, Drosten C (2014b) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Res* 101:45–56. <https://doi.org/10.1016/j.antiviral.2013.10.013>
- Drexler JF, Corman VM, Müller MA, et al (2012) Bats host major mammalian paramyxoviruses. *Nat Commun* 3:. <https://doi.org/10.1038/ncomms1796>
- Drosten C, Günther S, Preiser W, et al (2003) Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1967–1976. <https://doi.org/10.1056/NEJMOA030747>,
- Drosten C, Meyer B, Müller MA, et al (2014) Transmission of MERS-Coronavirus in Household Contacts. *New England Journal of Medicine* 371:828–835. <https://doi.org/10.1056/NEJMOA1405858>,
- Drzewnioková P, Festa F, Panzarin V, et al (2021a) Best molecular tools to investigate coronavirus diversity in mammals: A comparison. *Viruses* 13:. <https://doi.org/10.3390/v13101975>
- Drzewnioková P, Festa F, Panzarin V, et al (2021b) Best molecular tools to investigate coronavirus diversity in mammals: A comparison. *Viruses* 13:. <https://doi.org/10.3390/v13101975>

- Du Y, Wang C, Zhang Y (2022) Viral Coinfections. *Viruses* 2022, Vol 14, Page 2645 14:2645. <https://doi.org/10.3390/V14122645>
- Edson D, Field H, McMichael L, et al (2015a) Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One* 10:e0140670. <https://doi.org/10.1371/JOURNAL.PONE.0140670>
- Edson D, Field H, McMichael L, et al (2015b) Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One* 10:e0140670. <https://doi.org/10.1371/JOURNAL.PONE.0140670>
- El-Sayed A, Kamel M (2021) Coronaviruses in humans and animals: the role of bats in viral evolution. *Environmental Science and Pollution Research* 2021 28:16 28:19589–19600. <https://doi.org/10.1007/S11356-021-12553-1>
- Farsani SMJ, Dijkman R, Jebbink MF, et al (2012) The first complete genome sequences of clinical isolates of human coronavirus 229E. *Virus Genes* 45:433–439. <https://doi.org/10.1007/S11262-012-0807-9>,
- Faust CL, McCallum HI, Bloomfield LSP, et al (2018) Pathogen spillover during land conversion. *Ecol Lett* 21:471–483. <https://doi.org/10.1111/ELE.12904>;
- Fehr AR, Perlman S (2015) Coronaviruses: An overview of their replication and pathogenesis. *Coronaviruses: Methods and Protocols* 1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1/TABLES/2
- Field H, Young P, Yob JM, et al (2001) The natural history of Hendra and Nipah viruses. *Microbes Infect* 3:307–314. [https://doi.org/10.1016/S1286-4579\(01\)01384-3](https://doi.org/10.1016/S1286-4579(01)01384-3)
- Figueiroa T, Galvão Bueno M, Bento Moura PE, et al (2025) Alpha and Betacoronavirus Detection in Neotropical Bats from Northeast Brazil Suggests Wide Geographical Distribution and Persistence in Natural Populations. *Animals* 2025, Vol 15, Page 332 15:332. <https://doi.org/10.3390/ANI15030332>
- Findley JS, Wilson DE (1982) Ecological Significance of Chiropteran Morphology. *Ecology of bats* 243–260. https://doi.org/10.1007/978-1-4613-3421-7_6
- Fitzgerald SD, Rautenschlein S, Mahsoub HM, et al (2020) Adenovirus Infections. In: *Diseases of Poultry*. Wiley, pp 321–363
- Frick WF, Kingston T, Flanders J (2020a) A review of the major threats and challenges to global bat conservation. *Ann N Y Acad Sci* 1469:5–25. <https://doi.org/10.1111/nyas.14045>

- Frick WF, Kingston T, Flanders J (2020b) A review of the major threats and challenges to global bat conservation. *Ann N Y Acad Sci* 1469:5–25. <https://doi.org/10.1111/nyas.14045>
- Fritz SA, Bininda-Emonds ORP, Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: Big is bad, but only in the tropics. *Ecol Lett* 12:538–549. <https://doi.org/10.1111/J.1461-0248.2009.01307.X>;
- Galindo-González J (2024) Avoiding novel, unwanted interactions among species to decrease risk of zoonoses. *Conservation Biology* 38:. <https://doi.org/10.1111/COBI.14232>;
- Galindo-González J (2022) Live animal markets: Identifying the origins of emerging infectious diseases. *Curr Opin Environ Sci Health* 25:. <https://doi.org/10.1016/J.COESH.2021.100310>;
- Gangopadhyaya A, Bhukya PL (2023) Factors Contributing to the Emergence of Viral Diseases. In: *Emerging Human Viral Diseases, Volume I*. Springer Nature Singapore, Singapore, pp 3–69
- Gannon MR, Bovard BN (2016) The Value of Bats : Keystone
- Ge XY, Li JL, Yang X Lou, et al (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503:535–538. <https://doi.org/10.1038/nature12711>
- Geng R, Zhou P (2021) Severe acute respiratory syndrome (SARS) related coronavirus in bats. *Animal Diseases* 1:1–8. <https://doi.org/10.1186/s44149-021-00004-w>
- Gerow CM, Rapin N, Voordouw MJ, et al (2019) Arousal from hibernation and reactivation of *Eptesicus fuscus* gammaherpesvirus (EfHV) in big brown bats. *Transbound Emerg Dis* 66:1054–1062. <https://doi.org/10.1111/TBED.13102>
- Ghosh P, Jayaram S, Patwardhan D, et al (2021) Diversity of coronavirus receptors. 6–8. <https://doi.org/10.20944/preprints202108.0071.v1>
- Gloza-Rausch F, Ipsen A, Seebens A, et al (2008a) Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14:626–631. <https://doi.org/10.3201/eid1404.071439>
- Gloza-Rausch F, Ipsen A, Seebens A, et al (2008b) Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14:626–631. <https://doi.org/10.3201/eid1404.071439>

- Goffard A, Demanche C, Arthur L, et al (2015) Alphacoronaviruses detected in french bats are phylogeographically linked to coronaviruses of european bats. *Viruses* 7:6279–6290. <https://doi.org/10.3390/v7122937>
- Gonzalez V, Banerjee A (2022) Molecular, ecological, and behavioral drivers of the bat-virus relationship. *iScience* 25:104779. <https://doi.org/10.1016/j.isci.2022.104779>
- Grellet E, L'Hôte I, Goulet A, Imbert I (2022) Replication of the coronavirus genome: A paradox among positive-strand RNA viruses. *Journal of Biological Chemistry* 298:101923. <https://doi.org/10.1016/j.jbc.2022.101923>
- Gupta P, Singh MP, Goyal K, et al (2021a) Bats and viruses: a death-defying friendship. *Virusdisease* 32:467–479. <https://doi.org/10.1007/s13337-021-00716-0>
- Gupta P, Singh MP, Goyal K, et al (2021b) Bats and viruses: a death-defying friendship. *Virusdisease* 32:467–479. <https://doi.org/10.1007/s13337-021-00716-0>
- Gupta R (2005) Recent outbreak of rabies infections in Brazil transmitted by vampire bats. *Euro Surveill* 10:. <https://doi.org/10.2807/ESW.10.45.02831-EN>
- Guruprasad L (2021) Human coronavirus spike protein-host receptor recognition. *Prog Biophys Mol Biol* 161:39–53. <https://doi.org/10.1016/j.pbiomolbio.2020.10.006>
- Guthid S, Hanley KA, Althouse BM, Boots M (2020) Ecological processes underlying the emergence of novel enzootic cycles: Arboviruses in the neotropics as a case study. *PLoS Negl Trop Dis* 14:1–22. <https://doi.org/10.1371/JOURNAL.PNTD.0008338>;
- Haagmans BL, Al Dhahiry SHS, Reusken CBEM, et al (2014) Middle East respiratory syndrome coronavirus in dromedary camels: An outbreak investigation. *Lancet Infect Dis* 14:140–145. [https://doi.org/10.1016/S1473-3099\(13\)70690-X](https://doi.org/10.1016/S1473-3099(13)70690-X)
- Han Y, Du J, Su H, et al (2019) Identification of diverse bat alphacoronaviruses and betacoronaviruses in china provides new insights into the evolution and origin of coronavirus-related diseases. *Front Microbiol* 10:454784. <https://doi.org/10.3389/FMICB.2019.01900/BIBTEX>
- Han Y, Xu P, Wang Y, et al (2023) Panoramic analysis of coronaviruses carried by representative bat species in Southern China to better understand the coronavirus sphere. *Nature Communications* 2023 14:1 14:1–20. <https://doi.org/10.1038/s41467-023-41264-z>
- Hanley DE, Bourassa MA, O'Brien JJ, et al (2003) A Quantitative Evaluation of ENSO Indices. *J Clim* 16:1249–1258. [https://doi.org/10.1175/1520-0442\(2003\)16<1249:AQEOEI>2.0.CO;2](https://doi.org/10.1175/1520-0442(2003)16<1249:AQEOEI>2.0.CO;2);

- Hao X, Qin LU, Zhao H (2024) A molecular phylogeny for all 21 families within Chiroptera (bats). *Integr Zool* 19:989–998. <https://doi.org/10.1111/1749-4877.12772>
- Hasday JD, Fairchild KD, Shanholtz C (2000) The role of fever in the infected host. *Microbes Infect* 2:1891–1904. [https://doi.org/10.1016/S1286-4579\(00\)01337-X](https://doi.org/10.1016/S1286-4579(00)01337-X)
- Hayman DTS (2016) Bats as Viral Reservoirs. *Annu Rev Virol* 3:77–99. <https://doi.org/10.1146/annurev-virology-110615-042203>
- Hemnani M, da Silva PG, Thompson G, et al (2024a) Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *Ecohealth*. <https://doi.org/10.1007/s10393-024-01688-5>
- Hemnani M, da Silva PG, Thompson G, et al (2024b) Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *Ecohealth*. <https://doi.org/10.1007/s10393-024-01688-5>
- Hemnani M, Silva PG da, Thompson G, et al (2023a) First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* 15:1–12. <https://doi.org/10.3390/v15071521>
- Hemnani M, Silva PG da, Thompson G, et al (2023b) First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* 15:1–12. <https://doi.org/10.3390/v15071521>
- Herrera JM, Costa P, Medinas D, et al (2015) Community composition and activity of insectivorous bats in Mediterranean olive farms. *Anim Conserv* 18:557–566. <https://doi.org/10.1111/ACV.12209/SUPPINFO>
- Hildebrandt E, Penzes JJ, Gifford RJ, et al (2020) Evolution of dependoparvoviruses across geological timescales—implications for design of AAV-based gene therapy vectors. *Virus Evol* 6:. <https://doi.org/10.1093/ve/veaa043>
- Hofmann H, Pyrc K, Van Der Hoek L, et al (2005) Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci U S A* 102:7988–7993. <https://doi.org/10.1073/PNAS.0409465102>,
- Honorato R V, Koukos PI, Jiménez-García B, et al (2021) Structural Biology in the Clouds: The WeNMR-EOSC Ecosystem. *Front Mol Biosci* 8
- Honorato R V, Trellet ME, Jiménez-García B, et al (2024) The HADDOCK2.4 web server for integrative modeling of biomolecular complexes. *Nat Protoc* 19:3219–3241. <https://doi.org/10.1038/s41596-024-01011-0>

- Huang YW, Dickerman AW, Piñeyro P, et al (2013) Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the united states. *mBio* 4:737–750. https://doi.org/10.1128/MBIO.00737-13/SUPPL_FILE/MBO005131645ST2.DOCX
- Hulva P, Horáček I, Strelkov PP, Benda P (2004) Molecular architecture of *Pipistrellus pipistrellus*/*Pipistrellus pygmaeus* complex (Chiroptera: Vespertilionidae): Further cryptic species and Mediterranean origin of the divergence. *Mol Phylogenet Evol* 32:1023–1035. <https://doi.org/10.1016/j.ympev.2004.04.007>
- Ingala M, Fernandes H, De Oliveira M, et al (2025) Bats in Habitats, Bats as Habitats: An integrative ecological framework for understanding synergistic interactions across levels of community organization. <https://doi.org/10.32942/X2RW59>
- Irving AT, Ahn M, Goh G, et al (2021a) Lessons from the host defences of bats, a unique viral reservoir. *Nature* 589:363–370. <https://doi.org/10.1038/s41586-020-03128-0>
- Irving AT, Ahn M, Goh G, et al (2021b) Lessons from the host defences of bats, a unique viral reservoir. *Nature* 589:363–370. <https://doi.org/10.1038/s41586-020-03128-0>
- Islam MS, Sazzad HMS, Satter SM, et al (2016) Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011–2014. *Emerg Infect Dis* 22:664. <https://doi.org/10.3201/EID2204.151747>
- Janetanakit T, Lumyai M, Bunpapong N, et al (2016) Porcine deltacoronavirus, Thailand, 2015. *Emerg Infect Dis* 22:757–759. <https://doi.org/10.3201/EID2204.151852>,
- Jebb D, Foley NM, Whelan C V., et al (2018) Population level mitogenomics of long-lived bats reveals dynamic heteroplasmy and challenges the Free Radical Theory of Ageing. *Scientific Reports* 2018 8:1 8:1–12. <https://doi.org/10.1038/s41598-018-31093-2>
- Ji W, Peng Q, Fang X, et al (2022) Structures of a deltacoronavirus spike protein bound to porcine and human receptors. *Nat Commun* 13:. <https://doi.org/10.1038/s41467-022-29062-5>
- Jones BD, Kaufman EJ, Peel AJ (2023) Viral Co-Infection in Bats: A Systematic Review. *Viruses* 15:. <https://doi.org/10.3390/v15091860>
- Jones DN, Ravelomanantsoa NAF, Yeoman CJ, et al (2022) Do gastrointestinal microbiomes play a role in bats' unique viral hosting capacity? *Trends Microbiol* 30:632–642. <https://doi.org/10.1016/J.TIM.2021.12.009/ASSET/BF65FB14-03F2-4E41-9A61-77CCA8033999/MAIN.ASSETS/GR2.JPG>
- Jones G, Jacobs D, Kunz T, et al (2009) Carpe noctem: the importance of bats as bioindicators. *Endanger Species Res* 8:93–115. <https://doi.org/10.3354/esr00182>

- Jones G, Teeling EC (2006) The evolution of echolocation in bats. *Trends Ecol Evol* 21:149–156. <https://doi.org/10.1016/J.TREE.2006.01.001/ATTACHMENT/C0C308DE-5941-4D66-AA93-3F77C85F48FF/MMC1.PDF>
- Kamins AO, Restif O, Ntiamoa-Baidu Y, et al (2011) Uncovering the fruit bat bushmeat commodity chain and the true extent of fruit bat hunting in Ghana, West Africa. *Biol Conserv* 144:3000–3008. <https://doi.org/10.1016/J.BIOCON.2011.09.003>
- Kasso M, Balakrishnan M (2013) Ecological and Economic Importance of Bats (Order Chiroptera). *ISRN Biodiversity* 2013:1–9. <https://doi.org/10.1155/2013/187415>
- Kim N, Ma J, Kim W, et al (2024) Genome-resolved metagenomics: a game changer for microbiome medicine. *Exp Mol Med* 56:1501–1512. <https://doi.org/10.1038/s12276-024-01262-7>
- Kim SH, Chang SY, Sung M, et al (2016) Extensive Viable Middle East Respiratory Syndrome (MERS) Coronavirus Contamination in Air and Surrounding Environment in MERS Isolation Wards. *Clinical Infectious Diseases* 63:363–369. <https://doi.org/10.1093/cid/ciw239>
- Korine C, Oelbaum PJ, Muñoz-Garcia A (2022) Stress Physiology, Foraging, and Ecophysiology of Bats in Urban Environments. 3–18. https://doi.org/10.1007/978-3-031-13173-8_1
- Kramer LD, Tavakoli NP (2021) VIRUSES OF TERRESTRIAL MAMMALS. In: *Studies in Viral Ecology*. Wiley, pp 541–583
- Ksiazek TG, Erdman D, Goldsmith CS, et al (2003a) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1953–1966. <https://doi.org/10.1056/NEJMOA030781>,
- Ksiazek TG, Erdman D, Goldsmith CS, et al (2003b) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1953–1966. <https://doi.org/10.1056/NEJMOA030781>,
- Kunz TH (1982a) Roosting ecology of bats. *Ecology of bats* 1–55. https://doi.org/10.1007/978-1-4613-3421-7_1
- Kunz TH (ed) (1982b) *Ecology of Bats*. <https://doi.org/10.1007/978-1-4613-3421-7>
- Kunz TH, de Torrez EB, Bauer D, et al (2011) Ecosystem services provided by bats. *Ann N Y Acad Sci* 1223:1–38. <https://doi.org/10.1111/j.1749-6632.2011.06004.x>
- Kunz TH., Fenton MBrock (2003a) *Bat ecology*. 779
- Kunz TH., Fenton MBrock (2003b) *Bat ecology*. 779

- Latinne A, Morand S (2022) Climate Anomalies and Spillover of Bat-Borne Viral Diseases in the Asia-Pacific Region and the Arabian Peninsula. *Viruses* 14:. <https://doi.org/10.3390/V14051100>;
- Lau SKP, Luk HKH, Wong ACP, et al (2019) Identification of a novel betacoronavirus (merbecovirus) in amur hedgehogs from China. *Viruses* 11:. <https://doi.org/10.3390/V11110980>,
- Lau SKP, Wong EYM, Tsang C-C, et al (2018a) Discovery and Sequence Analysis of Four Deltacoronaviruses from Birds in the Middle East Reveal Interspecies Jumping with Recombination as a Potential Mechanism for Avian-to-Avian and Avian-to-Mammalian Transmission. *J Virol* 92:. <https://doi.org/10.1128/JVI.00265-18>,
- Lau SKP, Woo PCY, Li KSM, et al (2005) Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 102:14040–14045. <https://doi.org/10.1073/PNAS.0506735102>,
- Lau SKP, Zhang L, Luk HKH, et al (2018b) Receptor usage of a novel bat lineage C betacoronavirus reveals evolution of middle east respiratory syndrome-related coronavirus spike proteins for human dipeptidyl peptidase 4 binding. *Journal of Infectious Diseases* 218:197–207. <https://doi.org/10.1093/INFDIS/JIY018>,
- Lazov CM, Chriél M, Baagøe HJ, et al (2018) Detection and characterization of distinct alphacoronaviruses in five different bat species in Denmark. *Viruses* 10:. <https://doi.org/10.3390/v10090486>
- Le VP, Song S, An BH, et al (2018) A novel strain of porcine deltacoronavirus in Vietnam. *Arch Virol* 163:203–207. <https://doi.org/10.1007/S00705-017-3594-8>,
- Lednicky J, Lauzard M, Fan ZH, et al (2020) Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *International Journal of Infectious Diseases* 100:476–482. <https://doi.org/10.1016/j.ijid.2020.09.025>
- Lednicky JA, Tagliamonte MS, White SK, et al (2021) Independent infections of porcine deltacoronavirus among Haitian children. *Nature* 600:133–137. <https://doi.org/10.1038/S41586-021-04111-Z>,
- Lee JH, Chung HC, Nguyen VG, et al (2016) Detection and Phylogenetic Analysis of Porcine Deltacoronavirus in Korean Swine Farms, 2015. *Transbound Emerg Dis* 63:248–252. <https://doi.org/10.1111/TBED.12490>,
- Lee YF, McCracken GF (2002) Foraging activity and food resource use of Brazilian free-tailed bats, *Tadarida brasiliensis* (Molossidae). *Ecoscience* 9:306–313.

<https://doi.org/10.1080/11956860.2002.11682717>;WGROUP:STRING:PUBLICATION

- Lelli D, Papetti A, Sabelli C, et al (2013) Detection of coronaviruses in bats of various species in Italy. *Viruses* 5:2679–2689. <https://doi.org/10.3390/v5112679>
- Li F, Li W, Farzan M, Harrison SC (2005a) Structural biology: Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* (1979) 309:1864–1868. <https://doi.org/10.1126/SCIENCE.1116480>,
- Li W, Shi Z, Yu M, et al (2005b) Bats are natural reservoirs of SARS-like coronaviruses. *Science* (1979) 310:676–679. <https://doi.org/10.1126/SCIENCE.1118391>,
- Lindenmayer DB, Laurance WF (2017) The ecology, distribution, conservation and management of large old trees. *Biological Reviews* 92:1434–1458. <https://doi.org/10.1111/BRV.12290>;WGROUP:STRING:PUBLICATION
- Lu M, Wang X, Ye H, et al (2021) Does public fear that bats spread COVID-19 jeopardize bat conservation? *Biol Conserv* 254:108952. <https://doi.org/10.1016/j.biocon.2021.108952>
- Lu R, Zhao X, Li J, et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* 395:565–574. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8)
- Luby SP, Rahman M, Hossain MJ, et al (2006) Foodborne Transmission of Nipah Virus, Bangladesh. *Emerg Infect Dis* 12:1888. <https://doi.org/10.3201/EID1212.060732>
- Lucero Arteaga F, Miragaya M, Molina N, et al (2023) Identification of coronaviruses in bats and rodents in northern and central Argentina. *Arch Virol* 168:1–7. <https://doi.org/10.1007/s00705-023-05703-y>
- Luis AD, Hayman DTS, O’Shea TJ, et al (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? *Proceedings of the Royal Society B: Biological Sciences* 280:. <https://doi.org/10.1098/rspb.2012.2753>
- Lynteris C (2016) The Prophetic Faculty of Epidemic Photography: Chinese Wet Markets and the Imagination of the Next Pandemic. *Vis Anthropol* 29:118–132. <https://doi.org/10.1080/08949468.2016.1131484>;
- Madapong A, Saeng-chuto K, Lorsirigool A, et al (2016) Complete genome sequence of porcine deltacoronavirus isolated in Thailand in 2015. *Genome Announc* 4:. <https://doi.org/10.1128/GENOMEA.00408-16>,

- Maina JN (2000) What it Takes to Fly: The Structural and Functional Respiratory Refinements in Birds and Bats. *Journal of Experimental Biology* 203:3045–3064. <https://doi.org/10.1242/JEB.203.20.3045>
- Marinello MM, Bernard E (2014) Wing morphology of neotropical bats: A quantitative and qualitative analysis with implications for habitat use. *Can J Zool* 92:141–147. <https://doi.org/10.1139/CJZ-2013-0127>;PAGE:STRING:ARTICLE/CHAPTER
- Martínez-Abraín A, Jiménez J (2016) Anthropogenic areas as incidental substitutes for original habitat. *Conservation Biology* 30:593–598. <https://doi.org/10.1111/COBI.12644>;REQUESTEDJOURNAL:JOURNAL:15231739;WGROUPE:STRING:PUBLICATION
- Mata VA, Da Silva LP, Issimo J Ver, et al (2021) Combining DNA metabarcoding and ecological networks to inform conservation biocontrol by small vertebrate predators. *Ecological Applications* 31:e02457. <https://doi.org/10.1002/EAP.2457>
- Mathias ML, Fonseca C, Rodrigues L, Grilo C, Lopes-Fernandes M, Palmeirim JM, Santos-Reis M, Alves PC, Cabral JA FM, Mira A, Eira C, Negrões N, Paupério J, Pita R, Rainho A, Rosalino LM TJ& VJ Livro vermelho dos mamíferos de Portugal continental, 2023rd edn. FCIências.ID, ICNF, Lisboa
- Mekibib B, Ariën KK (2016) Aerosol Transmission of Filoviruses. *Viruses* 2016, Vol 8, Page 148 8:148. <https://doi.org/10.3390/V8050148>
- Méndez-Rodríguez A, Horta P, Zarza H, et al (2024) Surveying Bat-Hosted Adenoviruses and Herpesviruses: A Comprehensive Analysis. *Diversity (Basel)* 16:46. <https://doi.org/10.3390/d16010046>
- Metzner W, Müller R (2016) Ultrasound Production, Emission, and Reception. 55–91. https://doi.org/10.1007/978-1-4939-3527-7_3
- Mihindukulasuriya KA, Wu G, St. Leger J, et al (2008a) Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J Virol* 82:5084–5088. <https://doi.org/10.1128/jvi.02722-07>
- Mihindukulasuriya KA, Wu G, St. Leger J, et al (2008b) Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J Virol* 82:5084–5088. <https://doi.org/10.1128/jvi.02722-07>
- Miller AK, Mifsud JCO, Costa VA, et al (2021) Slippery when wet: Cross-species transmission of divergent coronaviruses in bony and jawless fish and the evolutionary history of the Coronaviridae. *Virus Evol* 7:1–10. <https://doi.org/10.1093/ve/veab050>

- Mo M, Roache M, Davies J, et al (2021) Estimating flying-fox mortality associated with abandonments of pups and extreme heat events during the austral summer of 2019–20. *Pacific Conservation Biology* 28:124–139. <https://doi.org/10.1071/PC21003>
- Monto AS, Cowling BJ, Peiris JSM (2014) Coronaviruses. *Viral Infections of Humans: Epidemiology and Control* 199–223. https://doi.org/10.1007/978-1-4899-7448-8_10/TABLES/3
- Moratelli R, Calisher CH (2015) Bats and zoonotic viruses: Can we confidently link bats with emerging deadly viruses? *Mem Inst Oswaldo Cruz* 110:1–22. <https://doi.org/10.1590/0074-02760150048>
- Morawska L, Milton DK (2020) It is Time to Address Airborne Transmission of COVID-19. *Clinical Infectious Diseases*. <https://doi.org/10.1093/cid/ciaa939>
- Moreno A, Lelli D, De Sabato L, et al (2017) Detection and full genome characterization of two beta CoV viruses related to Middle East respiratory syndrome from bats in Italy. *Virol J* 14:1–11. <https://doi.org/10.1186/s12985-017-0907-1>
- Moussy C, Hosken DJ, Mathews F, et al (2013) Migration and dispersal patterns of bats and their influence on genetic structure. *Mamm Rev* 43:183–195. <https://doi.org/10.1111/J.1365-2907.2012.00218.X>
- Mühldorfer K, Speck S, Kurth A, et al (2011) Diseases and Causes of Death in European Bats: Dynamics in Disease Susceptibility and Infection Rates. *PLoS One* 6:e29773. <https://doi.org/10.1371/JOURNAL.PONE.0029773>
- Naguib MM, Li R, Ling J, et al (2021) Live and Wet Markets: Food Access versus the Risk of Disease Emergence. *Trends Microbiol* 29:573–581. <https://doi.org/10.1016/J.TIM.2021.02.007/ASSET/25E2E3F1-49B1-4501-87B4-09B52F7908FC/MAIN.ASSETS/GR1.JPG>
- Nahar N, Asaduzzaman M, Mandal UK, et al (2020) Hunting Bats for Human Consumption in Bangladesh. *Ecohealth* 17:139–151. <https://doi.org/10.1007/S10393-020-01468-X/TABLES/8>
- Nakagawa S, Miyazawa T (2020) Genome evolution of SARS-CoV-2 and its virological characteristics. *Inflamm Regen* 40:0–6. <https://doi.org/10.1186/s41232-020-00126-7>
- Nal B, Chan C, Kien F, et al (2005) Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. *Journal of General Virology* 86:1423–1434. <https://doi.org/10.1099/VIR.0.80671-0/CITE/REFWORKS>

- Neuman BW, Kiss G, Kunding AH, et al (2011) A structural analysis of M protein in coronavirus assembly and morphology. *J Struct Biol* 174:11–22. <https://doi.org/10.1016/J.JSB.2010.11.021>
- Niederwerder MC, Hesse RA (2018) Swine enteric coronavirus disease: A review of 4 years with porcine epidemic diarrhoea virus and porcine deltacoronavirus in the United States and Canada. *Transbound Emerg Dis* 65:660–675. <https://doi.org/10.1111/TBED.12823>
- Nieto-Torres JL, DeDiego ML, Verdía-Báguena C, et al (2014) Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. *PLoS Pathog* 10:e1004077. <https://doi.org/10.1371/JOURNAL.PPAT.1004077>
- Nojiri T, Wilson LAB, López-Aguirre C, et al (2021) Embryonic evidence uncovers convergent origins of laryngeal echolocation in bats. *Current Biology* 31:1353–1365.e3. <https://doi.org/10.1016/j.cub.2020.12.043>
- Nusová G, Uhrin M, Voigt CC, Kaňuch P (2020) Tracing the geographic origin of common pipistrelles (*Pipistrellus pipistrellus*) swarming at a mass hibernaculum. *Mammalian Biology* 100:601–610. <https://doi.org/10.1007/s42991-020-00057-3>
- Nyberg T, Ferguson NM, Nash SG, et al (2022) Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *The Lancet* 399:1303–1312. [https://doi.org/10.1016/S0140-6736\(22\)00462-7](https://doi.org/10.1016/S0140-6736(22)00462-7)
- Oedin M, Brescia F, Vidal E, Millon A (2022) Make flying-fox hunting sustainable again: Comparing expected demographic effectiveness and hunters' acceptance of more restrictive regulations. *Ambio* 51:1078–1089. <https://doi.org/10.1007/S13280-021-01630-X/FIGURES/6>
- Olival KJ, Cryan PM, Amman BR, et al (2020) Possibility for reverse zoonotic transmission of sars-cov-2 to free-ranging wildlife: A case study of bats. *PLoS Pathog* 16:1–19. <https://doi.org/10.1371/journal.ppat.1008758>
- Olsen SJ, Chang HL, Cheung TYY, et al (2003) Transmission of the Severe Acute Respiratory Syndrome on Aircraft. *New England Journal of Medicine* 349:2416–2422. <https://doi.org/10.1056/NEJMoa031349>
- Openshaw JJ, Hegde S, Sazzad HMS, et al (2017a) Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. *Transbound Emerg Dis* 64:1287–1293. <https://doi.org/10.1111/tbed.12505>

- Openshaw JJ, Hegde S, Sazzad HMS, et al (2017b) Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. *Transbound Emerg Dis* 64:1287–1293. <https://doi.org/10.1111/TBED.12505>
- O’Shea TJ, Cryan PM, Cunningham AA, et al (2014) Bat flight and zoonotic viruses. *Emerg Infect Dis* 20:741–745. <https://doi.org/10.3201/eid2005.130539>
- Page RA, Bernal XE (2020) The challenge of detecting prey: Private and social information use in predatory bats. *Funct Ecol* 34:344–363. <https://doi.org/10.1111/1365-2435.13439/SUPPINFO>
- Papineau A, Berhane Y, Wylie TN, et al (2019) Genome Organization of Canada Goose Coronavirus, A Novel Species Identified in a Mass Die-off of Canada Geese. *Sci Rep* 9:. <https://doi.org/10.1038/S41598-019-42355-Y>,
- Peiris JSM, Guan Y, Yuen KY (2004) Severe acute respiratory syndrome. *Nat Med* 10:S88–S97. <https://doi.org/10.1038/nm1143>
- Pensaert MB, de Bouck P (1978) A new coronavirus-like particle associated with diarrhea in swine. *Arch Virol* 58:243–247. <https://doi.org/10.1007/BF01317606>,
- Perry RW (2013) A review of factors affecting cave climates for hibernating bats in temperate North America. *Environmental Reviews* 21:28–39. <https://doi.org/10.1139/ER-2012-0042;PAGEGROUP:STRING:PUBLICATION>
- Phelps KL, Hamel L, Alhmoud N, et al (2019) Bat research networks and viral surveillance: Gaps and opportunities in western Asia. *Viruses* 11:. <https://doi.org/10.3390/v11030240>
- Phillips AM, Gonzalez LO, Nekongo EE, et al (2017) Host proteostasis modulates influenza evolution. *Elife* 6:. <https://doi.org/10.7554/ELIFE.28652>
- Platto S, Zhou J, Wang Y, et al (2021a) Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease. *Biochem Biophys Res Commun* 538:2–13. <https://doi.org/10.1016/j.bbrc.2020.10.028>
- Platto S, Zhou J, Wang Y, et al (2021b) Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease. *Biochem Biophys Res Commun* 538:2–13. <https://doi.org/10.1016/j.bbrc.2020.10.028>
- Pratelli A, Tempesta M, Elia G, et al (2022) The knotty biology of canine coronavirus: A worrying model of coronaviruses’ danger. *Res Vet Sci* 144:190–195. <https://doi.org/10.1016/J.RVSC.2021.11.014>

- Prentice MB, Gilbertson MLJ, Storm DJ, et al (2024) Metagenomic sequencing sheds light on microbes putatively associated with pneumonia-related fatalities of white-tailed deer (*Odocoileus virginianus*). *Microb Genom* 10:. <https://doi.org/10.1099/mgen.0.001214>
- Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes De Novo Assembler. *Curr Protoc Bioinformatics* 70:1–29. <https://doi.org/10.1002/cpbi.102>
- Pujol FH, Branda F, Ceccarelli G, et al (2025) Nipah Virus: A Zoonotic Threat Re-Emerging in the Wake of Global Public Health Challenges. *Microorganisms* 2025, Vol 13, Page 124 13:124. <https://doi.org/10.3390/MICROORGANISMS13010124>
- Pyankov O V, Bodnev SA, Pyankova OG, Agranovski IE (2018a) Survival of aerosolized coronavirus in the ambient air. *J Aerosol Sci* 115:158–163. <https://doi.org/10.1016/j.jaerosci.2017.09.009>
- Pyankov O V, Bodnev SA, Pyankova OG, Agranovski IE (2018b) Survival of aerosolized coronavirus in the ambient air. *J Aerosol Sci* 115:158–163. <https://doi.org/10.1016/j.jaerosci.2017.09.009>
- Racey PA (2015) The Uniqueness of Bats. *Bats and Viruses: A New Frontier of Emerging Infectious Diseases* 1–22. <https://doi.org/10.1002/9781118818824.CH1>
- Rainho A Summer foraging habitats of bats in a Mediterranean region of the Iberian Peninsula. <https://doi.org/10.3161/150811007781694408>
- Ramanathan K, Antognini D, Combes A, et al (2020) Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. *Journal of Hospital Infection* 92:235–250
- Ramírez-Fráncel LA, García-Herrera LV, Losada-Prado S, et al (2022) Bats and their vital ecosystem services: a global review. *Integr Zool* 17:2–23. <https://doi.org/10.1111/1749-4877.12552>
- Ravi V, Saxena S, Panda PS (2022) Basic virology of SARS-CoV 2. *Indian J Med Microbiol* 40:182–186. <https://doi.org/10.1016/J.IJMMB.2022.02.005>
- Reguera J, Santiago C, Mudgal G, et al (2012) Structural Bases of Coronavirus Attachment to Host Aminopeptidase N and Its Inhibition by Neutralizing Antibodies. *PLoS Pathog* 8:e1002859. <https://doi.org/10.1371/journal.ppat.1002859>
- Reusken CBEM, Lina PHC, Pielaat A, et al (2010) Circulation of group 2 coronaviruses in a bat species common to urban areas in Western Europe. *Vector Borne Zoonotic Dis* 10:785–791. <https://doi.org/10.1089/vbz.2009.0173>

- Reyes A, Semenkovich NP, Whiteson K, et al (2012) Going viral: Next-generation sequencing applied to phage populations in the human gut. *Nat Rev Microbiol* 10:607–617. <https://doi.org/10.1038/nrmicro2853>
- Reyes-del Valle J, Chávez-Salinas S, Medina F, del Angel RM (2005) Heat Shock Protein 90 and Heat Shock Protein 70 Are Components of Dengue Virus Receptor Complex in Human Cells. *J Virol* 79:4557–4567. <https://doi.org/10.1128/JVI.79.8.4557-4567.2005/ASSET/D00B8FCF-B4BA-4E36-84D5-BAC18F19286A/ASSETS/GRAPHIC/ZJV0080560520008.JPEG>
- Richard M, Kok A, de Meulder D, et al (2020) SARS-CoV-2 is transmitted via contact and via the air between ferrets. *Nat Commun* 11:. <https://doi.org/10.1038/S41467-020-17367-2>,
- Riskin DK, Racey PA (2010) How do sucker-footed bats hold on, and why do they roost head-up? *Biological Journal of the Linnean Society* 99:233–240. <https://doi.org/10.1111/J.1095-8312.2009.01362.X>
- Rizzo F, Edenborough KM, Toffoli R, et al (2017) Coronavirus and paramyxovirus in bats from Northwest Italy. *BMC Vet Res* 13:1–11. <https://doi.org/10.1186/s12917-017-1307-x>
- Rodrigues L, Zahn A, Rainho A, Palmeirim JM (2003) Contrasting the roosting behaviour and phenology of an insectivorous bat (*Myotis myotis*) in its southern and northern distribution ranges. *Mammalia* 67:321–335. <https://doi.org/10.1515/MAMM.2003.67.3.321/MACHINEREADABLECITATION/RIS>
- Roffler AA, Maurer DP, Lunn TJ, et al (2024) Bat humoral immunity and its role in viral pathogenesis, transmission, and zoonosis. *Front Immunol* 15:1–12. <https://doi.org/10.3389/fimmu.2024.1269760>
- Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun* 109:102433. <https://doi.org/https://doi.org/10.1016/j.jaut.2020.102433>
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022a) Ecology, evolution and spillover of coronaviruses from bats. Springer US
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022b) Ecology, evolution and spillover of coronaviruses from bats. Springer US
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022c) Ecology, evolution and spillover of coronaviruses from bats. Springer US

- Russo D (2023a) Chiroptera., 2023rd edn. Springer
- Russo D, Cistrone L, Budinski I, et al (2017) Sociality influences thermoregulation and roost switching in a forest bat using ephemeral roosts. *Ecol Evol* 7:5310–5321. <https://doi.org/10.1002/ECE3.3111>
- Saeng-chuto K, Lorsirigool A, Temeeyasen G, et al (2017) Different Lineage of Porcine Deltacoronavirus in Thailand, Vietnam and Lao PDR in 2015. *Transbound Emerg Dis* 64:3–10. <https://doi.org/10.1111/TBED.12585>,
- Santana SE, Dial TO, Eiting TP, Alfaro ME (2011) Roosting Ecology and the Evolution of Pelage Markings in Bats. *PLoS One* 6:e25845. <https://doi.org/10.1371/JOURNAL.PONE.0025845>
- Schnitzler HU, Denzinger A (2024) Foraging strategies of echolocating bats. A Natural History of Bat Foraging: Evolution, Physiology, Ecology, Behavior, and Conservation 83–107. <https://doi.org/10.1016/B978-0-323-91820-6.16003-6>
- Schnitzler HU, Moss CF, Denzinger A (2003) From spatial orientation to food acquisition in echolocating bats. *Trends Ecol Evol* 18:386–394. [https://doi.org/10.1016/S0169-5347\(03\)00185-X/ASSET/ECEFFF0C-8B89-45C9-A7C1-D3C758339FC8/MAIN.ASSETS/GR5.GIF](https://doi.org/10.1016/S0169-5347(03)00185-X/ASSET/ECEFFF0C-8B89-45C9-A7C1-D3C758339FC8/MAIN.ASSETS/GR5.GIF)
- Serra-Cobo J, López-Roig M (2017) Bats and emerging infections: An ecological and virological puzzle. *Adv Exp Med Biol* 972:35–48. https://doi.org/10.1007/5584_2016_131
- Shen YY, Liang L, Zhu ZH, et al (2010) Adaptive evolution of energy metabolism genes and the origin of flight in bats. *Proc Natl Acad Sci U S A* 107:8666–8671. https://doi.org/10.1073/PNAS.0912613107/SUPPL_FILE/STXT01.PDF
- Shipley R, Wright E, Selden D, et al (2019) Bats and viruses: Emergence of novel lyssaviruses and association of bats with viral zoonoses in the EU. *Trop Med Infect Dis* 4:1–22. <https://doi.org/10.3390/tropicalmed4010031>
- Silva PG, Branco PTBS, Soares RRG, et al (2022a) SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* 32:e13083. <https://doi.org/https://doi.org/10.1111/ina.13083>
- Silva PG, Branco PTBS, Soares RRG, et al (2022b) SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* 32:e13083. <https://doi.org/https://doi.org/10.1111/ina.13083>
- Simonin Y (2023) Emerging Diseases/Viruses Prevention, Control, Surveillance, and One Health. *Trop Med Infect Dis* 8:257. <https://doi.org/10.3390/tropicalmed8050257>

- Singh R, Kølvrå S, Bross P, et al (2006) Heat-shock protein 70 genes and human longevity: A view from Denmark. *Ann N Y Acad Sci* 1067:301–308. <https://doi.org/10.1196/ANNALS.1354.040;PAGE:STRING:ARTICLE/CHAPTER>
- Smith I, Wang LF (2013) Bats and their virome: An important source of emerging viruses capable of infecting humans. *Curr Opin Virol* 3:84–91. <https://doi.org/10.1016/j.coviro.2012.11.006>
- Smith SH, Somsen GA, Van Rijn C, et al (2020) Aerosol persistence in relation to possible transmission of SARS-CoV-2. *Physics of Fluids* 32:.. <https://doi.org/10.1063/5.0027844>,
- Soler-Tovar D, Escobar LE (2025) Rabies transmitted from vampires to cattle: An overview. *PLoS One* 20:e0317214. <https://doi.org/10.1371/JOURNAL.PONE.0317214>
- Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. *Nat Rev Immunol* 2:185–194. <https://doi.org/10.1038/NRI749;KWRD=BIOMEDICINE>
- Stalin Raj V, Farag EABA, Reusken CBEM, et al (2014) Isolation of MERS coronavirus from dromedary camel, Qatar, 2014. *Emerg Infect Dis* 20:1339–1342. <https://doi.org/10.3201/EID2008.140663>,
- Stidsholt L (2025) Unlocking the mysteries of biosonar use and foraging behaviour in wild bats with on-board acoustic recording tags. *Journal of Experimental Biology* 228:.. <https://doi.org/10.1242/JEB.250777/368992>
- Subudhi S, Rapin N, Misra V (2019) Immune System Modulation and Viral Persistence in Bats: Understanding Viral Spillover. *Viruses* 2019, Vol 11, Page 192 11:192. <https://doi.org/10.3390/V11020192>
- Teeling EC, Jones G, Rossiter SJ (2016) Phylogeny, Genes, and Hearing: Implications for the Evolution of Echolocation in Bats. 25–54. https://doi.org/10.1007/978-1-4939-3527-7_2
- Tellier R, Li Y, Cowling BJ, Tang JW (2019) Recognition of aerosol transmission of infectious agents: A commentary. *BMC Infect Dis* 19:1–9. <https://doi.org/10.1186/s12879-019-3707-y>
- Thomas SP, Suthers RA (1972) The Physiology and Energetics of Bat Flight. *Journal of Experimental Biology* 57:317–335. <https://doi.org/10.1242/JEB.57.2.317>
- Tisza M, Javornik Cregeen S, Avadhanula V, et al (2023) Wastewater sequencing reveals community and variant dynamics of the collective human virome. *Nat Commun* 14:1–10. <https://doi.org/10.1038/s41467-023-42064-1>

- Tiwari R, Dhama K, Sharun K, et al (2020) COVID-19: animals, veterinary and zoonotic links. *Veterinary Quarterly* 40:169–182. <https://doi.org/10.1080/01652176.2020.1766725>
- Torquetti CG, Guimarães ATB, Soto-Blanco B (2021) Exposure to pesticides in bats. *Science of The Total Environment* 755:142509. <https://doi.org/10.1016/j.scitotenv.2020.142509>
- T.S. YI, Yuguo L, Wai WT, et al (2024) Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus. *New England Journal of Medicine* 350:1731–1739. <https://doi.org/10.1056/NEJMoa032867>
- Vakulenko Y, Deviatkin A, Drexler JF, Lukashev A (2021) Modular evolution of coronavirus genomes. *Viruses* 13:1270. <https://doi.org/10.3390/V13071270/S1>
- Verreault D, Moineau S, Duchaine C (2008) Methods for Sampling of Airborne Viruses. *Microbiology and Molecular Biology Reviews* 72:413–444. <https://doi.org/10.1128/membr.00002-08>
- Vlasova AN, Halpin R, Wang S, et al (2011) Molecular characterization of a new species in the genus Alphacoronavirus associated with mink epizootic catarrhal gastroenteritis. *Journal of General Virology* 92:1369–1379. <https://doi.org/10.1099/VIR.0.025353-0>,
- Voigt CC, Kingston T (2015) *Bats in the anthropocene: Conservation of bats in a changing world*. Springer International Publishing
- Wang B, Zhang A, Sun JL, et al (2005) Study of SARS transmission via liquid droplets in air. *J Biomech Eng* 127:32–38. <https://doi.org/10.1115/1.1835350>
- Wang L, Zhang Y, Byrum B (2014) Complete genome sequence of porcine coronavirus HKU15 strain IN2847 from the United States. *Genome Announc* 2:. <https://doi.org/10.1128/GENOMEA.00291-14>,
- Wang LF, Anderson DE (2019) Viruses in bats and potential spillover to animals and humans. *Curr Opin Virol* 34:79–89. <https://doi.org/10.1016/j.coviro.2018.12.007>
- Wang W, Lin XD, Guo WP, et al (2015) Discovery, diversity and evolution of novel coronaviruses sampled from rodents in China. *Virology* 474:19–27. <https://doi.org/10.1016/j.virol.2014.10.017>
- Wang Z, Huang G, Huang M, et al (2023) Global patterns of phylogenetic diversity and transmission of bat coronavirus. *Sci China Life Sci* 66:861–874. <https://doi.org/10.1007/s11427-022-2221-5>

- Waterhouse A, Bertoni M, Bienert S, et al (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46:W296–W303. <https://doi.org/10.1093/nar/gky427>
- Whittaker GR, André NM, Millet JK (2018) Improving Virus Taxonomy by Recontextualizing Sequence-Based Classification with Biologically Relevant Data: the Case of the Alphacoronavirus 1 Species . *mSphere* 3:. <https://doi.org/10.1128/MSPHEREDIRECT.00463-17>,
- WHO (2021) Coronavirus disease (COVID-19): How is it transmitted?
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2021a) Analysis of partial sequences of the RNA-dependent RNA polymerase gene as a tool for genus and subgenus classification of coronaviruses. *Journal of General Virology* 101:1261–1269. <https://doi.org/10.1099/jgv.0.001494>
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2020) Partial RdRp sequences offer a robust method for Coronavirus subgenus classification. *bioRxiv* 2020.03.02.974311. <https://doi.org/10.1101/2020.03.02.974311>
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2021b) Analysis of partial sequences of the RNA-dependent RNA polymerase gene as a tool for genus and subgenus classification of coronaviruses. *Journal of General Virology* 101:1261–1269. <https://doi.org/10.1099/JGV.0.001494/CITE/REFWORKS>
- Wong ACP, Lau SKP, Woo PCY (2021) Interspecies jumping of bat coronaviruses. *Viruses* 13:. <https://doi.org/10.3390/V13112188>,
- Wong S, Lau S, Woo P, Yuen KY (2007a) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91. <https://doi.org/10.1002/rmv.520>
- Wong S, Lau S, Woo P, Yuen KY (2007b) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91. <https://doi.org/10.1002/rmv.520>
- Woo PCY, De Groot RJ, Haagmans B, et al (2023a) ICTV Virus Taxonomy Profile: Coronaviridae 2023. *Journal of General Virology* 104:. <https://doi.org/10.1099/JGV.0.001843>
- Woo PCY, Groot RJ De, Haagmans B, et al (2023b) Family : Coronaviridae. 1–27
- Woo PCY, Lau SKP (2019) Viruses and bats. *Viruses* 11:2–5. <https://doi.org/10.3390/v11100884>
- Woo PCY, Lau SKP, Chu C, et al (2005) Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. *J Virol* 79:884–895. <https://doi.org/10.1128/JVI.79.2.884-895.2005>,

- Woo PCY, Lau SKP, Huang Y, Yuen KY (2009a) Coronavirus diversity, phylogeny and interspecies jumping. *Exp Biol Med* 234:1117–1127. <https://doi.org/10.3181/0903-MR-94>
- Woo PCY, Lau SKP, Lam CSF, et al (2012a) Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavi. *J Virol* 86:3995–4008. <https://doi.org/10.1128/jvi.06540-11>
- Woo PCY, Lau SKP, Lam CSF, et al (2014a) Discovery of a Novel Bottlenose Dolphin Coronavirus Reveals a Distinct Species of Marine Mammal Coronavirus in Gammacoronavirus. *J Virol* 88:1318–1331. <https://doi.org/10.1128/jvi.02351-13>
- Woo PCY, Lau SKP, Lam CSF, et al (2009b) Comparative Analysis of Complete Genome Sequences of Three Avian Coronaviruses Reveals a Novel Group 3c Coronavirus. *J Virol* 83:908–917. <https://doi.org/10.1128/JVI.01977-08>,
- Woo PCY, Lau SKP, Lam CSF, et al (2012b) Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavirus. *J Virol* 86:3995–4008. <https://doi.org/10.1128/JVI.06540-11>,
- Woo PCY, Lau SKP, Teng JLL, et al (2014b) New hepatitis E virus genotype in camels, the Middle East. *Emerg Infect Dis* 20:1044–1048. <https://doi.org/10.3201/eid2006.140140>
- Woo PCY, Lau SKP, Wernery U, et al (2014c) Novel betacoronavirus in dromedaries of the Middle East, 2013. *Emerg Infect Dis* 20:560–572. <https://doi.org/10.3201/EID2004.131769>,
- Woo PCY, Wang M, Lau SKP, et al (2007) Comparative Analysis of Twelve Genomes of Three Novel Group 2c and Group 2d Coronaviruses Reveals Unique Group and Subgroup Features. *J Virol* 81:1574–1585. <https://doi.org/10.1128/JVI.02182-06>,
- Wood JLN, Leach M, Waldman L, et al (2012) A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:2881–2892. <https://doi.org/10.1098/RSTB.2012.0228>
- Wu Z, Lu L, Du J, et al (2018) Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. *Microbiome* 6:. <https://doi.org/10.1186/S40168-018-0554-9>,

- Xiao W, Wang M, Wei W, et al (2004) [Detection of SARS-CoV and RNA on aerosol samples from SARS-patients admitted to hospital]. *Zhonghua Liu Xing Bing Xue Za Zhi* 25:882–885
- Xu Q, Wang F, Jiao W, et al (2023) Virtual Screening-Based Peptides Targeting Spike Protein to Inhibit Porcine Epidemic Diarrhea Virus (PEDV) Infection. *Viruses* 15:381. <https://doi.org/10.3390/v15020381>
- Yang D, Leibowitz JL (2015) The structure and functions of coronavirus genomic 3' and 5' ends. *Virus Res* 206:120–133. <https://doi.org/10.1016/J.VIRUSRES.2015.02.025>
- Yen HL, Sit THC, Brackman CJ, et al (2022) Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. *The Lancet* 399:1070–1078. [https://doi.org/10.1016/S0140-6736\(22\)00326-9](https://doi.org/10.1016/S0140-6736(22)00326-9)
- Yinda CK, Zell R, Deboutte W, et al (2017) Highly diverse population of Picornaviridae and other members of the Picornavirales, in Cameroonian fruit bats. *BMC Genomics* 18:249. <https://doi.org/10.1186/s12864-017-3632-7>
- Yuan Y, Cao D, Zhang Y, et al (2017) Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nature Communications* 2017 8:1 8:1–9. <https://doi.org/10.1038/ncomms15092>
- Zagmajster M (2019) Bats. *Encyclopedia of Caves*, Third Edition 94–101. <https://doi.org/10.1016/B978-0-12-814124-3.00013-3>
- Zaki AM, van Boheemen S, Bestebroer TM, et al (2012) Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New England Journal of Medicine* 367:1814–1820. <https://doi.org/10.1056/NEJMOA1211721>,
- Zhao B, Chen C, Yang X, Li Y (2011) Role of two-way airflow owing to temperature difference in severe acute respiratory syndrome transmission: Revisiting the largest nosocomial severe acute respiratory syndrome outbreak in Hong Kong. *J R Soc Interface* 8:699–710. <https://doi.org/10.1098/rsif.2010.0486>
- Zhou P, Fan H, Lan T, et al (2018) Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* 556:255–259. <https://doi.org/10.1038/s41586-018-0010-9>
- Zhou P, Yang X Lou, Wang XG, et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579:270–273. <https://doi.org/10.1038/s41586-020-2012-7>

- Zhu N, Zhang D, Wang W, et al (2020a) A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 382:727–733. <https://doi.org/10.1056/NEJMOA2001017>,
- Zhu N, Zhang D, Wang W, et al (2020b) A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 382:727–733. <https://doi.org/10.1056/NEJMOA2001017>,
- Zukal J, Pikula J, Bandouchova H (2015) Bats as bioindicators of heavy metal pollution: history and prospect. *Mammalian Biology* 80:220–227. <https://doi.org/10.1016/j.mambio.2015.01.001>
- Zumla A, Hui DS, Perlman S (2015) Middle East respiratory syndrome. *The Lancet* 386:995–1007. [https://doi.org/10.1016/S0140-6736\(15\)60454-8](https://doi.org/10.1016/S0140-6736(15)60454-8)

Chapter 5- General Discussion and Conclusions

General discussion

The intricate relationship between bats and the viruses they harbour has long been a subject of intense interest, particularly given the unique ecological role bats play as reservoirs for a diverse range of pathogens. In Europe, and specifically in Portugal, interactions between bats and humans occur through various channels, including urban roosting in historical buildings, agricultural landscapes where bats contribute to pest control, and ecotourism activities in caves and natural reserves. While these interactions can foster awareness and support for bat conservation, a significant gap persists in the comprehensive characterization of the viral communities present in bats, with most existing studies remaining limited in scope.

This research aimed to bridge some of these gaps by investigating the circulation of CoVs in distinct epidemiological aspects: airborne CoVs at bat roosts, CoVs found specifically in cave-dwelling bats, and CoVs within crevice and tree-dwelling bat populations in Portugal. Furthermore, the study explored the broader virome of bat populations in Portugal, uncovering significant viral diversity beyond CoVs, including picornaviruses, mastadenoviruses (adenoviruses), and dependoparvoviruses. These investigations provide crucial insights into the viral ecology and diversity of these animals, contributing significantly to the collective understanding of zoonotic disease dynamics.

In the first study of this thesis, where the first-ever description of CoVs in bats in Portugal is presented, I aimed to detect and characterize CoVs in cave and building roosts across central and southern Portugal, collecting biological samples and performing environmental air sampling to assess the potential for airborne virus presence.

A range of sample types was collected, including stool samples, anal and buccal swabs, as well as airborne particles captured using a Coriolis air sampler, given that viruses may be present in biological fluids, on surfaces, and in the surrounding environment. Out of the 98 samples obtained from bats, seven samples (8.87%) exhibited amplicons of the expected size for CoVs. These seven samples were further analysed through bidirectional sequencing and nucleotide BLAST analysis.

The results revealed that all seven samples were characterized as AlphaCovs. Notably, although faecal samples were fewer in number compared to anal and buccal swabs, they accounted for most positive detections ($n = 6$), supporting the hypothesis that viral replication occurs primarily in the gastrointestinal tract and indicating the potential for faecal–oral transmission. In contrast, only a single anal swab tested positive, and no buccal

swabs were positive. The anal swab that yielded a positive result originated from the same *Miniopterus schreibersii* individual that also provided a positive faecal sample. None of the six air samples tested positive for CoVs.

The CoV strains found in the bat populations in this study are closely related to AlphaCovs strains retrieved from the bat species *M. schreibersii* from Bulgaria and Italy and *Hypsugo savii* from Spain. The bat species sampled in this study do not migrate over long distances (Amengual et al. 2007), hence no long-distance transmission has likely occurred, and viruses are probably circulating solely in the studied region.

The approach for detection and characterization of CoVs has resourced to a partial RdRp region with primers described by (Drzewnioková et al. 2021b), this region is sufficiently informative to allow classification within known CoV genera (Wilkinson et al. 2020). The RdRp exhibits a certain degree of sequence conservation across different CoVs subgenus (Wilkinson et al. 2021b). Focusing on this specific region, we can obtain valuable taxonomic information without the need for analyzing the entire viral genome and is highly effective in determining taxonomic classifications, reaching the subgenus-level (Wilkinson et al. 2021a).

To date, both Alpha and Beta CoVs have been found in bats (Platto et al. 2021b). Bat CoVs are known to be excreted at higher viral loads in stools, making the enteric route a major environmental source for the CoV spillover events (Wong et al. 2007b). The CoVs detected in this present study were detected mostly in faeces, confirming the enteric route of transmission as the most significant, being consistent with other studies with bats (Bonny et al. 2017; Alves et al. 2022). The faecal-oral route has also been described in CoVs from other animals such as with feline CoVs (FCoV) (Delaplace et al. 2021), canine CoVs (Pratelli et al. 2022) and swine CoVs (SADS-CoV) (Niederwerder and Hesse 2018), all of them classified as AlphaCoV. The replication of CoVs in the enteric location suggests an adaptation of the virus to the bat host's gastrointestinal environment (Jones et al. 2022). Further investigations into the mechanisms underlying this viral replication in the gastrointestinal tract may help unravel the unique interplay between the CoVs and the bats' immune system (Ruiz-Aravena et al. 2022c). However, the detection of CoVs in faecal samples in this study does not preclude the possibility of alternative transmission routes, including respiratory or direct contact pathways.

Previously, it has been reported the presence of SARS-CoV (Xiao et al. 2004; Wang et al. 2005) and MERS-CoV (Azhar et al. 2014; Kim et al. 2016; Pyankov et al. 2018b) in air samples, with the discussion of the airborne route of transmission for these human CoVs

gaining notoriety during the SARS-CoV-2 pandemic, with many reports of SARS-CoV-2 presence in indoor and outdoor samples throughout the world (Lednický et al. 2020; Morawska and Milton 2020; da Silva et al. 2021b, 2022) and the World Health Organization acknowledging the airborne route as a transmission route for SARS-CoV-2 (WHO 2021). In response to the aforementioned findings, we conducted air sampling within bat caves and buildings to investigate the potential presence of CoVs in the air within these environments.

Despite rigorous sampling efforts, CoVs were not detected in any of the collected air samples. This absence of detection may be attributable to several factors, with sampling conditions hypothesized to be a significant determinant. Specifically, the duration of air sampling is critical for the adequate capture of airborne particulates, including viral aerosols. Insufficient sampling duration could consequently reduce the probability of detecting CoVs present in the atmospheric environment. Current knowledge regarding the airborne transmission of bat CoVs remains limited. It is plausible that bats excrete low viral copy numbers, leading to aerosolized viral loads below the limits of detection (Mekibib and Ariën 2016). Therefore, the optimization of air sampling duration for CoV detection may not have been achieved in the present study, potentially contributing to the observed negative findings.

Additionally, the type of sampler used in this study was cyclone-based. While cyclone-based samplers are commonly employed for air sampling, they might not be the most effective option for capturing CoVs. Notwithstanding, both the choice of air sampler and duration of air sampling has been successfully applied in detecting SARS-CoV-2, an airborne CoV (da Silva et al. 2021c, a; Silva et al. 2022a). All in all, it is important to acknowledge these limitations and consider alternative sampling strategies for future investigations such as alternative sampling durations and utilizing samplers specifically designed for capturing viral particles that could enhance the sensitivity of CoV detection in the air (Breshears et al. 2022).

When interpreting these results, it is important to consider that viral concentrations in air may be too low at the specific place and time of sampling. Consequently, negative results do not necessarily indicate the absence of virus in the environment at the time of collection (Silva et al. 2022b). Future efforts to sample airborne viruses in bat environments should include protocol adjustments, such as extending sampling durations and employing alternative devices that may be better suited for pathogen recovery, including impinger-based, filter-based, or water-based condensation samplers. Moreover, the air sampling conducted in this study took place during the dry summer season; it is possible that other

times of the year may provide more favourable climatic conditions for viral persistence in the air.

Prior to this study, no information was available regarding the diversity of CoVs in bats or in the air of bat-inhabited environments in mainland Portugal, where 27 species are recognized. Our findings provide the first evidence of AlphaCoVs circulation in cave-dwelling bats from Portugal, with sequences showing close phylogenetic relationships to those previously reported in bat species from Bulgaria, Italy, and Spain.

In the subsequent study, the aim was to investigate CoV prevalence in crevice and tree-dwelling bats in Portugal. It was collected 87 stool samples from bats from 3 sites. Only one sample (1.15%) was positive for AlphaCoV, belonging to *P. pipistrellus*, and was collected during the first day at Serra da Estrela (central Portugal).

These results are low compared to other studies that reported prevalence rates usually ranging from 3% to 17% (Gloza-Rausch et al. 2008a; Lelli et al. 2013; Bittar et al. 2020; Bueno et al. 2022). Unexpectedly, the first investigation into CoV excretion in Portugal revealed an occurrence of 8.87%, indicating a higher prevalence of CoVs in cave-dwelling bats than crevice and tree-dwelling bats (Hemnani et al. 2023a). This could have happened because of differences habitat characteristics, variations in bat species behaviour, proximity to potential virus reservoirs, and fluctuations in bat population density. These factors can potentially influence the likelihood of detecting CoV in bat populations.

Even if the study had opted for a country-wide and multi-year surveillance, the prevalence rate might not necessarily increase. This is because the effectiveness of surveillance efforts depends on various factors beyond the scope of the study design, such as the choice of sampling locations. Conducting surveillance in different habitats, such as caves or other bat habitats, may yield different results due to variations in environmental conditions and bat species composition, and more importantly animal densities (Frick et al. 2020a). The CoV strain identified in the bat in our study exhibited close genetic relationships with AlphaCoVs strains previously isolated from the same bat species, *P. pipistrellus*, from the United Kingdom (West Sussex) (August et al. 2012), more specifically from the *Pedacovirus* subgenus, and it also clustered with other strains previously isolated also from *P. pipistrellus*, from France and Italy (De Benedictis et al. 2014; Goffard et al. 2015). The retrieved *Pedacovirus* sequence also clustered with sequences found in other *Pipistrellus* spp. throughout Europe, namely in *P. nathusii* and *P. pygmaeus*, suggesting a certain degree in host specificity and raising important questions on viral circulation. This clustering strongly implies that closely related viruses are prevalent within the same host genus, even when

geographically separated. This phenomenon has been corroborated by previous research findings, indicating a compelling pattern of viral association with specific bat hosts irrespective of geographical location (Rizzo et al. 2017; Bittar et al. 2020).

In contrast, the initial research involving cave-dwelling bats revealed that the clustering of bat CoVs was not primarily determined by the species of bats. Instead, it appeared that geographical location played a more significant role in influencing the evolution and dissemination of these viruses (Hemnani et al. 2023a). This suggests that environmental factors, such as habitat characteristics and local ecological dynamics, may exert a more significant influence on the genetic diversity and distribution of CoVs in cave-dwelling bat populations.

Pipistrellus pipistrellus distribution encompasses a substantial portion of Europe, with confirmed sightings extending as far north as Norway and southern Finland. To the south, its presence has been established in Greece, Cyprus, and Turkey (Hulva et al. 2004). Beyond the borders of Europe, it has also been documented in northwest Africa, Asia Minor, and across the Middle East to Iran and Afghanistan (Hulva et al. 2004). In Portugal, this species is widely distributed across the entire mainland territory being considered one of the most abundant bat species in the country (Salgueiro et al. 2002). These bats, characterized by their preference for dwelling in crevices, commonly seek refuge in various human-made structures, leading them to roost in buildings, warehouses, and architectural relics such as churches and castles, among others. Their adaptation to utilizing human infrastructures for roosting highlights their status as synanthropic species, demonstrating their ability to thrive in close proximity to human populations (Frick et al. 2020b). This close association with human habitats not only shows the remarkable adaptability of these bats but also the importance of understanding their ecological dynamics in urban environments. However, due to their high plasticity, it can also utilize crevices in rocks, cliffs, tree cavities, occasionally underground shelters during the hibernation season, additionally, also seek shelter beneath tree bark (Dietz et al. 2018).

Pipistrellus pipistrellus are bats that are not very known for flying long distances, but recent findings regarding their genetic information indicates a significant degree of genetic exchange within summer colonies in central Europe (Bryja et al. 2009). This observation implies two distinct possibilities: the occurrence migrations motivated by the search for suitable mates in distant habitats and propelled by factors such as competition for resources or territory, or the need to establish new breeding grounds (Nusová et al. 2020). Although, this species does not have a strong migratory character, since they are usually known to

remain confined to their own colonies even if another colony is located 10km away (Goffard et al. 2015). This also indicates that these viruses may not primarily evolve within a specific bat species, instead, their evolution and spread appear to be significantly influenced by geographical location. This suggests that factors such as environmental conditions, host availability, and interactions with other wildlife species could play pivotal roles in shaping the evolutionary dynamics and spread of these viruses across different regions. Another possible interpretation is that the presence of similar strains in geographically separated areas could be attributed to transmission occurring during the migration of bats (Moussy et al. 2013). Considering the genetic divergence observed between the sampled strain and others, it is likely that direct transmission has not occurred, but rather, it would likely entail the involvement of intermediate strains that have not been included in the sampled data (Bittar et al. 2020).

All these findings combined to our results suggest the existence of *Pedacovirus*-like AlphaCoV strains specific to bats genus (*Pipistrellus*), although with low occurrence, underscores the significance of understanding viral diversity within bat populations. While the occurrence of these strains may be relatively low, their presence highlights the potential for novel viral variants to emerge and circulate among bat species (Brook and Dobson 2015). Moreover, the observation that this pattern may extend to synanthropic bat species, which inhabit a wide range of roosting habitats including natural environments and human-built structures like roofs and windows, is of considerable importance.

The last study of the thesis aimed to evaluate the occurrence of CoVs in Portuguese bats and predict the affinity of their spike proteins with the aminopeptidase N (APN) receptor of several host species. The study also aimed to explore the viral diversity in bat samples using metagenomic sequencing. Samples were collected from ten bats from two species (*M. myotis* and *M. schreibersii*), including ten buccal swabs, ten anal swabs, and seven faecal samples.

Using pan-CoV nested RT-PCR on all 27 samples, three samples (7.41%; 95% confidence interval [CI]: 0% to 17.29) yielded amplicons of the expected size for CoVs. Two sequences were retrieved from a faecal sample (MyMy-F52-CoV and, MyMy-F47-CoV), and one from a buccal swab (MyMy-B51-CoV).

Previous studies have shown that faecal samples contain the most viruses, suggesting that replication predominantly occurs in the gastrointestinal tract and emphasizing the potential for faecal-oral transmission (Benkő et al. 2014; Borkenhagen et al. 2019a; Hemnani et al. 2023b; Méndez-Rodríguez et al. 2024). Based on this, the seven faecal samples were

prioritized for metagenomic analysis, as they are considered the most likely to contain comprehensive viral genomic data. The only buccal swab sample that tested positive by RT-PCR for CoV was also included to explore any possible alternative sites of replication or shedding. This approach ensured that our sequencing efforts were strategically directed towards maximizing the recovery of relevant viral sequences. The metagenomics was done following the NetoVIR protocol, an optimized and widely used approach for high-throughput, reproducible sample preparation. This method introduces minimal bias and is particularly suited for faecal samples (Conceição-Neto et al. 2015b).

All virus-positive samples resulting from the metagenomic sequencing were faecal, and no viral reads were detected in the buccal swab sample despite its RT-PCR positivity for CoV. The sequencing revealed a wide range of viruses including mammalian, but also insect viruses (data not shown), which is an expected result given the fact that the diet of the sampled Portuguese bats almost consists of insects (Rodrigues et al. 2003; Herrera et al. 2015; Mata et al. 2021). We concentrated exclusively on viruses that can infect mammals, excluding those that target archaea, bacteria, fungi, invertebrates, and plants.

Two near complete CoV genomes were assembled (MyMy-F45-CoV-1 and MiSc-F47-CoV-2). It was also detected six near complete picornavirus genomes in four samples, a partial *Mastadenovirus* genome sequence and four non-overlapping contigs most closely related to members of the genus *Dependoparvovirus* in one bat sample.

The clustering of our sequences with CoVs from bats in South Korea (*M. petax*), China (*M. adversus*, *M. chinensis*, *M. fuliginosus* and *M. schreibersii*), and Denmark (*M. dasycneme*) highlights the widespread distribution and genetic diversity of bat-associated coronaviruses. However, this pattern may partly reflect sampling bias, as CoV sequences from Asian bats, particularly from China, are more abundantly represented in public databases compared to those from European or Portuguese bats. The limited availability of regional sequences may contribute to the observed phylogenetic clustering with genetically similar but geographically distant strains. In addition, the migratory behaviour of some bat species, may also contribute to the dissemination and genetic mixing of AlphaCoVs across broad geographic regions. Previous studies have documented transboundary bat movements within Europe (Rodrigues and Palmeirim 2008), supporting the possibility of viral exchange along migratory routes. Geographic gaps also exist, with certain regions in Europe lacking comprehensive surveys for bat-associated CoVs, potentially overlooking important reservoirs (Hemnani et al. 2024a). These findings show that Portuguese bats may contribute

to the broader circulation of AlphaCoVs, reinforcing their role as crucial reservoirs for this viral group.

The detection of picornaviruses in several samples reveals a high degree of sequence divergence both from each other and from known picornavirus sequences in databases. The order *Picornavirales* encompasses nine families, one of which is the family *Picornaviridae* (Carrascosa-Sàez et al. 2024; Zell et al. 2017; Temmam et al. 2020). The identified picornaviruses displayed clustering patterns similar to picornavirus strains found in bat species from China and Europe, particularly within the *Myotis* and *Miniopterus* genera. The identification of six picornavirus genomes across four samples highlights the presence of diverse picornaviruses in Portuguese bats. The clustering patterns suggest a complex evolutionary history, with some strains showing higher similarity to viruses from geographically close regions (e.g., Spain, Hungary and Algeria), while others are more closely related to strains from distant locations such as China. This likely reflects significant gaps in current knowledge and sampling of bat-associated picornaviruses, which may result in unexpected phylogenetic relationships between viruses from distant geographic locations. Additionally, the observed genetic diversity suggests ongoing viral evolution within these bat species (Kramer and Tavakoli 2021; Jones et al. 2023).

Adenoviruses are medium-sized, non-enveloped viruses belonging to the family *Adenoviridae* (Parija 2023). The family is divided into two genera: *Mastadenovirus*, which includes viruses infecting mammals (Fitzgerald et al. 2020), and *Aviadenovirus*, comprising those that infect birds (Athukorala et al. 2022). The phylogenetic placement of the detected *Mastadenovirus* alongside strains from *Myotis chinensis* and *Myotis ricketti* in China highlights unexpected relationships between viruses from distant regions. Rather than indicating direct viral exchange or contact between bat populations, this pattern likely reflects significant gaps in the global sampling of bat-associated adenoviruses. Such phylogenetic alignments may arise due to underrepresentation of viral sequences from intermediate regions, emphasizing the need for broader surveillance efforts. Moreover, the ability of adenoviruses to infect multiple bat species and cross species barriers is well-documented within the *Adenoviridae* family (Benkő et al. 2014; Borkenhagen et al. 2019a; Méndez-Rodríguez et al. 2024).

The exact mechanisms that allow the transmission of adenoviruses between species remain poorly understood. While some studies suggest that indirect environmental exposure, such as through contaminated water sources, may play a role in facilitating viral exchange among wildlife, domestic animals, and humans (Borkenhagen et al. 2019b; Simonin 2023),

these assumptions are largely based on general patterns of pathogen spread rather than adenovirus-specific evidence. Therefore, the intricacies of cross-species transmission and the ecological or behavioural factors that might enable such events require further investigation in the context of adenoviruses.

Dependoparvovirus is a genus within the family *Parvoviridae*. These are parvoviruses that typically require co-infection with a helper virus, such as an adenovirus or a herpesvirus, to complete their replication cycle (Du et al. 2022). This dependency on a helper virus is what gives them their name, *Dependoparvovirus* (Hildebrandt et al. 2020). In this study, *Dependoparvovirus* was detected in *M. myotis* (F45), the same individual in which adenovirus was also identified. The virus found in this study shared high sequence identity with strains from *Myotis* bats in China, with sequence identities ranging from 81.6% to 86.2%. These similarities likely reflect the limited availability of *Dependoparvovirus* sequences globally, rather than direct viral circulation, underscoring the need for broader sampling to better understand their diversity and evolutionary relationships.

To evaluate the risk of cross-species transmission of the bat CoV sequenced in this study, protein docking studies were conducted to analyse the binding affinity between the bat CoV spike RBD and bat, porcine and human APNs. These interactions are critical for understanding host specificity and the potential for spillover to livestock or zoonotic transmission to humans. By evaluating the binding affinities, we gained insights into the virus's transmission dynamics and its potential to infect other species.

Protein docking simulations revealed that the binding of the F47 CoV spike protein was strongest with bat APN and similar with human and porcine APNs. The interaction between F47 and bat APN yielded a score of -126.1 ± 4.8 . For the interaction with porcine APN, the score was -109.0 ± 4.4 , and for human APN, the score was -106.2 ± 7.6 . The docking studies performed for the PEDV RBD with APN from the same animal species resulted in a score of -90.7 ± 7.5 , while with porcine APN, it was -82.8 ± 2.2 , and with human APN, it was -100.2 ± 5.4 .

The APN receptors of pigs and humans were included in the docking analysis to assess the potential interaction between the bat CoV spike protein and host receptors. Porcine APN was used due to its known role as the receptor for PEDV. PEDV was included in the docking analysis as a reference for AlphaCoVs known to use porcine APN as its entry receptor. Given the sequence similarity between the bat CoV spike protein and PEDV, as well as their shared placement within the *Pedacovirus* subgenus, this analysis aimed to explore whether the bat virus might use a similar entry mechanism. By comparing the binding interactions of the bat

CoV spike with those of PEDV, we sought to assess potential similarities in receptor usage and gain insights into possible cross-species transmission pathways (Banerjee et al. 2019; Han et al. 2019). Human APN was included to evaluate the potential risk of zoonotic transmission of this virus. The HADDOCK scores from our protein docking studies reveal important insights into the binding affinities between the spike proteins of bat CoV (F47) and PEDV with APN receptors from different hosts (bat, porcine, and human). The results suggest that the F47 CoV spike protein exhibits the strongest binding affinity with bat APN, yielding a score of -126.1 ± 4.8 . This high binding affinity could imply that the F47 CoV is well adapted to the bat host, providing insights into the evolutionary history of the virus and its co-evolution with bat species. Interestingly, the binding affinities between the F47 spike protein and the APNs of both humans and pigs are also strong and comparable, with scores of -106.2 ± 7.6 and -109.0 ± 4.4 , respectively.

This finding suggests a potential capacity for cross-species receptor usage, which could have implications for host range and zoonotic spillover risks. The close binding affinities observed for F47 CoV and human or porcine APN suggest a potential inter-species transmission risk. However, it is important to note that these are computational studies based solely on the spike protein, and the adaptation of other viral proteins has not been considered. Successful viral infection involves more than just spike-APN interactions, as the function of other viral components is also critical for productive infection.

In contrast, the predicted interaction of PEDV is weakest with porcine APN, with a score of -82.8 ± 2.2 , which is quite surprising given that pigs are the natural host for PEDV. Interestingly, the PEDV spike protein demonstrated weaker binding to porcine APN than to human and bat APNs, contrary to the established role of porcine APN as its primary receptor. This unexpected result challenges the established understanding of PEDV spike-receptor interactions and raises intriguing questions about the structural or evolutionary factors influencing binding specificity. In fact, recent studies have suggested that APN may not be the cell receptor of PEDV (Shirato et al. 2016; Whitworth et al. 2019) and that PEDV employs both protein receptors and sugar co-receptors for infection (Chang et al. 2015). Additionally, while the significant binding affinity of PEDV spike for human APN (score of -100.2 ± 5.4) may suggest some potential for cross-species interactions, the absence of documented human infections indicates that other factors likely restrict a PEDV spillover. This could be important in understanding potential zoonotic risks and provide insights into the determinants of host specificity.

These findings highlight the need for further experimental validation to confirm receptor usage and assess the functional relevance of these interactions. Moreover, comparative studies of receptor-binding dynamics across different CoVs could provide valuable insights into the evolutionary pressures shaping spike protein-receptor interactions and their role in determining host range. The data might suggest that the strong binding of F47 CoV's spike to bat APN represents a virus-host relationship optimized through evolutionary pressures, while PEDV's comparatively weaker binding to pig APN could highlight differences in virus-host adaptation strategies. F47 spike's comparable affinity for human and porcine APNs also suggests a possible evolutionary plasticity, where the virus might more easily adapt to new hosts with similar APN structures. This binding pattern could also indicate that, while F47 is primarily adapted to bats, it might possess an intrinsic ability to bind APN from other species, potentially aiding cross-species spillover events. In contrast, PEDV's reliance on additional co-receptors for efficient infection in pigs could suggest a more specialized host relationship, despite the weak APN affinity.

REFERENCES

- Afelt A, Frutos R, Devaux C (2018) Bats, coronaviruses, and deforestation: Toward the emergence of novel infectious diseases? *Front Microbiol* 9:1–5. <https://doi.org/10.3389/fmicb.2018.00702>
- Ahn M, Anderson DE, Zhang Q, et al (2019) Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nat Microbiol* 4:789–799. <https://doi.org/10.1038/S41564-019-0371-3>;
- Akem ES, Pemunta NV (2020) The bat meat chain and perceptions of the risk of contracting Ebola in the Mount Cameroon region. *BMC Public Health* 20:1–10. <https://doi.org/10.1186/S12889-020-08460-8>/PEER-REVIEW
- Alcalde JT, Jiménez M, Brila I, et al (2021) Transcontinental 2200 km migration of a *Nathusius' pipistrelle* (*Pipistrellus nathusii*) across Europe. *Mammalia* 85:161–163. <https://doi.org/10.1515/mammalia-2020-0069>
- Allen T, Murray KA, Zambrana-Torrel C, et al (2017) Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* 8:. <https://doi.org/10.1038/S41467-017-00923-8>;
- Alves RS, do Canto Olegário J, Weber MN, et al (2022) Detection of coronavirus in vampire bats (*Desmodus rotundus*) in southern Brazil. *Transbound Emerg Dis* 69:2384–2389. <https://doi.org/10.1111/tbed.14150>
- Amengual B, López-Roig M, Serra-Cobo J (2007) First record of seasonal over sea migration of *Miniopterus schreibersii* and *Myotis capaccinii* between Balearic Islands (Spain). *Acta Chiropt* 9:319–322. [https://doi.org/10.3161/1733-5329\(2007\)9\[319:FROSOS\]2.0.CO;2](https://doi.org/10.3161/1733-5329(2007)9[319:FROSOS]2.0.CO;2)
- Ancillotto L, Santini L, Ranc N, et al (2016) Extraordinary range expansion in a common bat: The potential roles of climate change and urbanisation. *Science of Nature* 103:. <https://doi.org/10.1007/S00114-016-1334-7>;
- Anderson SC, Ruxton GD (2020) The evolution of flight in bats: a novel hypothesis. *Mamm Rev* 50:426–439. <https://doi.org/10.1111/MAM.12211>;REQUESTEDJOURNAL:JOURNAL:13652907;PAGE:STRING:ARTICLE/CHAPTER
- Andrusier N, Mashiach E, Nussinov R, Wolfson HJ (2008) Principles of flexible protein-protein docking. *Proteins: Structure, Function and Genetics* 73:271–289. <https://doi.org/10.1002/prot.22170>

- Anthony SJ, Johnson CK, Greig DJ, et al (2017) Global patterns in coronavirus diversity. *Virus Evol* 3:1–15. <https://doi.org/10.1093/ve/vex012>
- Apoorva, Singh SK (2024) A tale of endurance: bats, viruses and immune dynamics. *Future Microbiol* 19:841–856. <https://doi.org/10.2217/fmb-2023-0233>
- Athukorala A, Helbig KJ, Mcsharry BP, et al (2022) Adenoviruses in Avian Hosts: Recent Discoveries Shed New Light on Adenovirus Diversity and Evolution. *Viruses* 14:1767. <https://doi.org/10.3390/v14081767>
- August TA, Mathews F, Nunn MA (2012) Alphacoronavirus detected in bats in the United Kingdom. *Vector-Borne and Zoonotic Diseases* 12:530–533. <https://doi.org/10.1089/vbz.2011.0829>
- Azhar EI, Hashem AM, El-Kafrawy SA, et al (2014) Detection of the Middle East respiratory syndrome coronavirus genome in an air sample originating from a camel barn owned by an infected patient. *mBio* 5:e01450-14. <https://doi.org/10.1128/mBio.01450-14>
- Bakkers MJG, Lang Y, Feitsma LJ, et al (2017) Betacoronavirus Adaptation to Humans Involved Progressive Loss of Hemagglutinin-Esterase Lectin Activity. *Cell Host Microbe* 21:356–366. <https://doi.org/10.1016/j.chom.2017.02.008>
- Banerjee A, Kulcsar K, Misra V, et al (2019) Bats and coronaviruses. *Viruses* 11:7–9. <https://doi.org/10.3390/v11010041>
- Batista T (2014) Paisagem e ecologia da região de Montemor-o-Novo Conversas à volta do espírito dos lugares
- Beere HM, Wolf BB, Cain K, et al (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2:469–475. <https://doi.org/10.1038/35019501;KWRD=LIFE+SCIENCES>
- Beniac DR, Andonov A, Grudeski E, Booth TF (2006) Architecture of the SARS coronavirus prefusion spike. *Nat Struct Mol Biol* 13:751–752. <https://doi.org/10.1038/NSMB1123;KWRD=LIFE+SCIENCES>
- Benkő M, Harrach B, Kremer EJ (2014) Do Nonhuman Primate or Bat Adenoviruses Pose a Risk for Human Health? *Future Microbiol* 9:269–272. <https://doi.org/10.2217/fmb.13.170>
- Bergner LM, Orton RJ, Benavides JA, et al (2020) Demographic and environmental drivers of metagenomic viral diversity in vampire bats. *Mol Ecol* 29:26–39. <https://doi.org/10.1111/MEC.15250>

- Berman HM, Westbrook J, Feng Z, et al (2000) The Protein Data Bank. *Nucleic Acids Res* 28:235–242. <https://doi.org/10.1093/nar/28.1.235>
- Biol Sci BJ, de Jesus Santos N, Santos da Paz E, et al (2019) Evaluation of bat-related knowledge, perceptions, and practices in an urban community: A strategy for Conservation Biology and health promotion. *Brazilian Journal of Biological Sciences* 6:e392–e392. <https://doi.org/10.21472/BJBS.061303>
- Bittar C, Machado RRG, Comelis MT, et al (2020) Alphacoronavirus Detection in Lungs, Liver, and Intestines of Bats from Brazil. *Microb Ecol* 79:203–212. <https://doi.org/10.1007/s00248-019-01391-x>
- Blatteis CM (2003) Fever: pathological or physiological, injurious or beneficial? *J Therm Biol* 28:1–13. [https://doi.org/10.1016/S0306-4565\(02\)00034-7](https://doi.org/10.1016/S0306-4565(02)00034-7)
- Boardman WSJ, Baker ML, Boyd V, et al (2020) Seroprevalence of three paramyxoviruses; Hendra virus, Tioman virus, Cedar virus and a rhabdovirus, Australian bat lyssavirus, in a range expanding fruit bat, the Grey-headed flying fox (*Pteropus poliocephalus*). *PLoS One* 15:. <https://doi.org/10.1371/JOURNAL.PONE.0232339>;
- Bodewes R, Ruiz-Gonzalez A, Schapendonk CME, et al (2014) Viral metagenomic analysis of feces of wild small carnivores. *Virol J* 11:1–13. <https://doi.org/10.1186/1743-422X-11-89>
- Bokelmann M, Balkema-buschmann A (2021a) Coronaviruses in Bats. *Berl Munch Tierarztl Wochenschr* 134:1–16. <https://doi.org/10.2376/1439-0299-2020-44>
- Bokelmann M, Balkema-buschmann A (2021b) Coronaviruses in Bats. *Berl Munch Tierarztl Wochenschr* 134:1–16. <https://doi.org/10.2376/1439-0299-2020-44>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Bonny T, Driver J, Paisie T, et al (2017) Detection of Alphacoronavirus vRNA in the Feces of Brazilian Free-Tailed Bats (*Tadarida brasiliensis*) from a Colony in Florida, USA. *Diseases* 5:7. <https://doi.org/10.3390/diseases5010007>
- Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019a) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect* 8:1679–1687. <https://doi.org/10.1080/22221751.2019.1690953>
- Borkenhagen LK, Fieldhouse JK, Seto D, Gray GC (2019b) Are adenoviruses zoonotic? A systematic review of the evidence. *Emerg Microbes Infect* 8:1679–1687. <https://doi.org/10.1080/22221751.2019.1690953>

- Botto Nuñez G, Becker DJ, Lawrence RL, Plowright RK (2020) Synergistic Effects of Grassland Fragmentation and Temperature on Bovine Rabies Emergence. *Ecohealth* 17:203–216. <https://doi.org/10.1007/S10393-020-01486-9>;
- Bredenbeek PJ, Pachuk CJ, Noten AFH, et al (1990) The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59; a highly conserved polymerase is expressed by an efficient ribosomal frameshifting mechanism. *Nucleic Acids Res* 18:1825–1832. <https://doi.org/10.1093/NAR/18.7.1825>
- Breed AC, Field HE, Smith CS, et al (2010) Bats without borders: Long-distance movements and implications for disease risk management. *Ecohealth* 7:204–212. <https://doi.org/10.1007/S10393-010-0332-Z/FIGURES/3>
- Breshears LE, Nguyen BT, Mata Robles S, et al (2022) Biosensor detection of airborne respiratory viruses such as SARS-CoV-2. *SLAS Technol* 27:4–17. <https://doi.org/10.1016/j.slant.2021.12.004>
- Brook CE, Dobson AP (2015) Bats as “special” reservoirs for emerging zoonotic pathogens. *Trends Microbiol* 23:. <https://doi.org/10.1016/j.tim.2014.12.004>
- Bryja J, Kaňuch P, Fornůsková A, et al (2009) Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe. *Biological Journal of the Linnean Society* 96:103–114. <https://doi.org/10.1111/j.1095-8312.2008.01093.x>
- Bueno LM, Rizotto LS, Viana A de O, et al (2022) High genetic diversity of alphacoronaviruses in bat species (Mammalia: Chiroptera) from the Atlantic Forest in Brazil. *Transbound Emerg Dis* 69:e2863–e2875. <https://doi.org/10.1111/tbed.14636>
- Burgin CJ, Colella JP, Kahn PL, Upham NS (2018) How many species of mammals are there? *J Mammal* 99:1–14. <https://doi.org/10.1093/JMAMMAL/GYX147>
- Cadet J, Richard Wagner J (2013) DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harb Perspect Biol* 5:. <https://doi.org/10.1101/CSHPERSPECT.A012559>,
- Calisher CH, Childs JE, Field HE, et al (2006a) Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19:531–545. <https://doi.org/10.1128/CMR.00017-06>
- Calisher CH, Childs JE, Field HE, et al (2006b) Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19:531–545. <https://doi.org/10.1128/CMR.00017-06>

- Cantlay JC, Ingram DJ, Meredith AL (2017) A Review of Zoonotic Infection Risks Associated with the Wild Meat Trade in Malaysia. *Ecohealth* 14:361–388. <https://doi.org/10.1007/S10393-017-1229-X>;
- Cao T, Jin JP (2020) Evolution of Flight Muscle Contractility and Energetic Efficiency. *Front Physiol* 11:565310. <https://doi.org/10.3389/FPHYS.2020.01038/BIBTEX>
- Cappelli F, Costantini V, Consoli D (2021) The trap of climate change-induced “natural” disasters and inequality. *Global Environmental Change* 70:. <https://doi.org/10.1016/J.GLOENVCHA.2021.102329>;
- Carlson CJ, Albery GF, Merow C, et al (2022) Climate change increases cross-species viral transmission risk. *Nature* 607:555–562. <https://doi.org/10.1038/S41586-022-04788-W>;
- Chang CK, Sue SC, Yu TH, et al (2006) Modular organization of SARS coronavirus nucleocapsid protein. *J Biomed Sci* 13:59–72. <https://doi.org/10.1007/S11373-005-9035-9/METRICS>
- Chaverri G, Ancillotto L, Russo D (2018) Social communication in bats. *Biological Reviews* 93:1938–1954. <https://doi.org/10.1111/brv.12427>
- CHEEVER FS, DANIELS JB (1949) A murine virus (JHM) causing disseminated encephalomyelitis with. *J Exp Med* 90:181–210. <https://doi.org/10.1084/JEM.90.3.181>,
- Chen Y, Liu Q, Guo D (2020) Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol* 92:418–423. <https://doi.org/10.1002/JMV.25681>
- Chua KB, Lek Koh C, Hooi PS, et al (2002) Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4:145–151. [https://doi.org/10.1016/S1286-4579\(01\)01522-2](https://doi.org/10.1016/S1286-4579(01)01522-2);
- Ciotti M, Ciccozzi M, Terrinoni A, et al (2020) The COVID-19 pandemic. *Crit Rev Clin Lab Sci* 0:365–388. <https://doi.org/10.1080/10408363.2020.1783198>
- Clark JS, Bell DM, Hersh MH, et al (2011) Individual-scale variation, species-scale differences: inference needed to understand diversity. *Ecol Lett* 14:1273–1287. <https://doi.org/10.1111/J.1461-0248.2011.01685.X>
- Cohen LE, Fagre AC, Chen B, et al (2023) Coronavirus sampling and surveillance in bats from 1996–2019: a systematic review and meta-analysis. *Nat Microbiol* 8:1176–1186. <https://doi.org/10.1038/s41564-023-01375-1>
- Collen A (2012) The evolution of echolocation in bats: a comparative approach. Doctoral thesis, UCL (University College London)

- Conceição-Neto N, Godinho R, Álvares F, et al (2017) Viral gut metagenomics of sympatric wild and domestic canids, and monitoring of viruses: Insights from an endangered wolf population. *Ecol Evol* 7:4135–4146. <https://doi.org/10.1002/ece3.2991>
- Conceição-Neto N, Zeller M, Lefrère H, et al (2015a) Modular approach to customise sample preparation procedures for viral metagenomics: A reproducible protocol for virome analysis. *Sci Rep* 5:1–14. <https://doi.org/10.1038/srep16532>
- Conceição-Neto N, Zeller M, Lefrère H, et al (2015b) Modular approach to customise sample preparation procedures for viral metagenomics: A reproducible protocol for virome analysis. *Sci Rep* 5:1–14. <https://doi.org/10.1038/srep16532>
- Conenna I, Muñoz-Garcia A, Korine C (2025) Physiological and Behavioural Strategies of Bats From Arid Environments. *Mamm Rev* e70005. <https://doi.org/10.1111/MAM.70005>
- Contini C, Di Nuzzo M, Barp N, et al (2020) The novel zoonotic COVID-19 pandemic: An expected global health concern. *J Infect Dev Ctries* 14:254–264. <https://doi.org/10.3855/jidc.12671>
- Cooper LN, Cretokos CJ, Sears KE (2012) The evolution and development of mammalian flight. *Wiley Interdiscip Rev Dev Biol* 1:773–779. <https://doi.org/10.1002/WDEV.50;CTYPE:STRING:JOURNAL>
- Corman VM, Kallies R, Philipps H, et al (2014a) Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *J Virol* 88:717–724. <https://doi.org/10.1128/JVI.01600-13>,
- Corman VM, Kallies R, Philipps H, et al (2014b) Characterization of a Novel Betacoronavirus Related to Middle East Respiratory Syndrome Coronavirus in European Hedgehogs. *J Virol* 88:717–724. <https://doi.org/10.1128/JVI.01600-13>,
- Cui X, Fan K, Liang X, et al (2023) Virus diversity, wildlife-domestic animal circulation and potential zoonotic viruses of small mammals, pangolins and zoo animals. *Nat Commun* 14:2488. <https://doi.org/10.1038/s41467-023-38202-4>
- da Silva PG, Gonçalves J, Nascimento MS, et al (2022) Detection of SARS-CoV-2 in the Indoor and Outdoor Areas of Urban Public Transport Systems of Three Major Cities of Portugal in 2021. *Int J Environ Res Public Health* 19
- da Silva PG, Mesquita JR, de São José Nascimento M, Ferreira VAM (2021a) Viral, host and environmental factors that favor anthroponotic spillover of coronaviruses: An opinionated review, focusing on SARS-CoV, MERS-CoV and SARS-CoV-2. *Science of the Total Environment* 750:141483. <https://doi.org/10.1016/j.scitotenv.2020.141483>

- da Silva PG, Nascimento MSJ, Soares RRG, et al (2021b) Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV. *Science of the Total Environment* 764:.. <https://doi.org/10.1016/j.scitotenv.2020.142802>
- da Silva PG, Nascimento MSJ, Soares RRG, et al (2021c) Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV. *Science of the Total Environment* 764:.. <https://doi.org/10.1016/j.scitotenv.2020.142802>
- De Benedictis P, Marciano S, Scaravelli D, et al (2014) Alpha and lineage C betaCoV infections in Italian bats. *Virus Genes* 48:366–371. <https://doi.org/10.1007/s11262-013-1008-x>
- De Coninck L, Faye L, Basler N, et al (2025) ViPER
- de Groot RJ, Baker SC, Baric RS, et al (2013) Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *J Virol* 87:7790–7792. <https://doi.org/10.1128/JVI.01244-13>
- De Oliveira HFM, Camargo NF, Hemprich-Bennett DR, et al (2020) Wing morphology predicts individual niche specialization in *Pteronotus mesoamericanus* (Mammalia: Chiroptera). *PLoS One* 15:e0232601. <https://doi.org/10.1371/JOURNAL.PONE.0232601>
- DeDiego ML, Álvarez E, Almazán F, et al (2007) A Severe Acute Respiratory Syndrome Coronavirus That Lacks the E Gene Is Attenuated In Vitro and In Vivo. *J Virol* 81:1701–1713. <https://doi.org/10.1128/JVI.01467-06/ASSET/983812D0-AAA7-4C88-8679-A844299EF1C1/ASSETS/GRAPHIC/ZJV0040787220008.JPEG>
- Delaplace M, Huet H, Gambino A, Le Poder S (2021) Feline Coronavirus Antivirals: A Review. *Pathogens* 2021, Vol 10, Page 1150 10:1150. <https://doi.org/10.3390/PATHOGENS10091150>
- Delmas B, Gelfi J, L'Haridon R, et al (1992) Amino peptidase N is a major receptor for the enteropathogenic coronavirus TGEV. *Nature* 357:417–420. <https://doi.org/10.1038/357417A0>,
- Deng F, Ye G, Liu Q, et al (2016) Identification and Comparison of Receptor Binding Characteristics of the Spike Protein of Two Porcine Epidemic Diarrhea Virus Strains. *Viruses* 8:55

- Denzinger A, Schnitzler HU (2013) Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Front Physiol* 4 JUL:48812. <https://doi.org/10.3389/FPHYS.2013.00164/BIBTEX>
- Diengdoh VL, Onde S, Hunt M, Brook BW (2022) Predicted impacts of climate change and extreme temperature events on the future distribution of fruit bat species in Australia. *Glob Ecol Conserv* 37:. <https://doi.org/10.1016/J.GECCO.2022.E02181>;
- Dietz M, Brombacher M, Erasmy M, et al (2018) Bat Community and Roost Site Selection of Tree-Dwelling Bats in a Well-Preserved European Lowland Forest. *Acta Chiropt* 20:117–127. <https://doi.org/10.3161/15081109ACC2018.20.1.008>
- Domańska-Blicharz K, Miłek-Krupa J, Pikuła A (2021) Diversity of coronaviruses in wild representatives of the aves class in poland. *Viruses* 13:1–19. <https://doi.org/10.3390/v13081497>
- Dong N, Fang L, Zeng S, et al (2015) Porcine Deltacoronavirus in mainland China. *Emerg Infect Dis* 21:2254–2255. <https://doi.org/10.3201/EID2112.150283>,
- Drexler JF, Corman VM, Drosten C (2014a) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Res* 101:45–56. <https://doi.org/10.1016/j.antiviral.2013.10.013>
- Drexler JF, Corman VM, Drosten C (2014b) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Res* 101:45–56. <https://doi.org/10.1016/j.antiviral.2013.10.013>
- Drexler JF, Corman VM, Müller MA, et al (2012) Bats host major mammalian paramyxoviruses. *Nat Commun* 3:. <https://doi.org/10.1038/ncomms1796>
- Drosten C, Günther S, Preiser W, et al (2003) Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1967–1976. <https://doi.org/10.1056/NEJMOA030747>,
- Drosten C, Meyer B, Müller MA, et al (2014) Transmission of MERS-Coronavirus in Household Contacts. *New England Journal of Medicine* 371:828–835. <https://doi.org/10.1056/NEJMOA1405858>,
- Drzewnioková P, Festa F, Panzarin V, et al (2021a) Best molecular tools to investigate coronavirus diversity in mammals: A comparison. *Viruses* 13:. <https://doi.org/10.3390/v13101975>
- Drzewnioková P, Festa F, Panzarin V, et al (2021b) Best molecular tools to investigate coronavirus diversity in mammals: A comparison. *Viruses* 13:. <https://doi.org/10.3390/v13101975>

- Du Y, Wang C, Zhang Y (2022) Viral Coinfections. *Viruses* 2022, Vol 14, Page 2645 14:2645. <https://doi.org/10.3390/V14122645>
- Edson D, Field H, McMichael L, et al (2015a) Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One* 10:e0140670. <https://doi.org/10.1371/JOURNAL.PONE.0140670>
- Edson D, Field H, McMichael L, et al (2015b) Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One* 10:e0140670. <https://doi.org/10.1371/JOURNAL.PONE.0140670>
- El-Sayed A, Kamel M (2021) Coronaviruses in humans and animals: the role of bats in viral evolution. *Environmental Science and Pollution Research* 2021 28:16 28:19589–19600. <https://doi.org/10.1007/S11356-021-12553-1>
- Farsani SMJ, Dijkman R, Jebbink MF, et al (2012) The first complete genome sequences of clinical isolates of human coronavirus 229E. *Virus Genes* 45:433–439. <https://doi.org/10.1007/S11262-012-0807-9>,
- Faust CL, McCallum HI, Bloomfield LSP, et al (2018) Pathogen spillover during land conversion. *Ecol Lett* 21:471–483. <https://doi.org/10.1111/ELE.12904>;
- Fehr AR, Perlman S (2015) Coronaviruses: An overview of their replication and pathogenesis. *Coronaviruses: Methods and Protocols* 1282:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1/TABLES/2
- Field H, Young P, Yob JM, et al (2001) The natural history of Hendra and Nipah viruses. *Microbes Infect* 3:307–314. [https://doi.org/10.1016/S1286-4579\(01\)01384-3](https://doi.org/10.1016/S1286-4579(01)01384-3)
- Figueiroa T, Galvão Bueno M, Bento Moura PE, et al (2025) Alpha and Betacoronavirus Detection in Neotropical Bats from Northeast Brazil Suggests Wide Geographical Distribution and Persistence in Natural Populations. *Animals* 2025, Vol 15, Page 332 15:332. <https://doi.org/10.3390/ANI15030332>
- Findley JS, Wilson DE (1982) Ecological Significance of Chiropteran Morphology. *Ecology of bats* 243–260. https://doi.org/10.1007/978-1-4613-3421-7_6
- Fitzgerald SD, Rautenschlein S, Mahsoub HM, et al (2020) Adenovirus Infections. In: *Diseases of Poultry*. Wiley, pp 321–363
- Frick WF, Kingston T, Flanders J (2020a) A review of the major threats and challenges to global bat conservation. *Ann N Y Acad Sci* 1469:5–25. <https://doi.org/10.1111/nyas.14045>

- Frick WF, Kingston T, Flanders J (2020b) A review of the major threats and challenges to global bat conservation. *Ann N Y Acad Sci* 1469:5–25. <https://doi.org/10.1111/nyas.14045>
- Fritz SA, Bininda-Emonds ORP, Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: Big is bad, but only in the tropics. *Ecol Lett* 12:538–549. <https://doi.org/10.1111/J.1461-0248.2009.01307.X>;
- Galindo-González J (2024) Avoiding novel, unwanted interactions among species to decrease risk of zoonoses. *Conservation Biology* 38:. <https://doi.org/10.1111/COBI.14232>;
- Galindo-González J (2022) Live animal markets: Identifying the origins of emerging infectious diseases. *Curr Opin Environ Sci Health* 25:. <https://doi.org/10.1016/J.COESH.2021.100310>;
- Gangopadhyaya A, Bhukya PL (2023) Factors Contributing to the Emergence of Viral Diseases. In: *Emerging Human Viral Diseases, Volume I*. Springer Nature Singapore, Singapore, pp 3–69
- Gannon MR, Bovard BN (2016) *The Value of Bats : Keystone*
- Ge XY, Li JL, Yang X Lou, et al (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503:535–538. <https://doi.org/10.1038/nature12711>
- Geng R, Zhou P (2021) Severe acute respiratory syndrome (SARS) related coronavirus in bats. *Animal Diseases* 1:1–8. <https://doi.org/10.1186/s44149-021-00004-w>
- Gerow CM, Rapin N, Voordouw MJ, et al (2019) Arousal from hibernation and reactivation of *Eptesicus fuscus* gammaherpesvirus (EfHV) in big brown bats. *Transbound Emerg Dis* 66:1054–1062. <https://doi.org/10.1111/TBED.13102>
- Ghosh P, Jayaram S, Patwardhan D, et al (2021) Diversity of coronavirus receptors. 6–8. <https://doi.org/10.20944/preprints202108.0071.v1>
- Gloza-Rausch F, Ipsen A, Seebens A, et al (2008a) Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14:626–631. <https://doi.org/10.3201/eid1404.071439>
- Gloza-Rausch F, Ipsen A, Seebens A, et al (2008b) Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14:626–631. <https://doi.org/10.3201/eid1404.071439>

- Goffard A, Demanche C, Arthur L, et al (2015) Alphacoronaviruses detected in french bats are phylogeographically linked to coronaviruses of european bats. *Viruses* 7:6279–6290. <https://doi.org/10.3390/v7122937>
- Gonzalez V, Banerjee A (2022) Molecular, ecological, and behavioral drivers of the bat-virus relationship. *iScience* 25:104779. <https://doi.org/10.1016/j.isci.2022.104779>
- Grellet E, L'Hôte I, Goulet A, Imbert I (2022) Replication of the coronavirus genome: A paradox among positive-strand RNA viruses. *Journal of Biological Chemistry* 298:101923. <https://doi.org/10.1016/j.jbc.2022.101923>
- Gupta P, Singh MP, Goyal K, et al (2021a) Bats and viruses: a death-defying friendship. *Virusdisease* 32:467–479. <https://doi.org/10.1007/s13337-021-00716-0>
- Gupta P, Singh MP, Goyal K, et al (2021b) Bats and viruses: a death-defying friendship. *Virusdisease* 32:467–479. <https://doi.org/10.1007/s13337-021-00716-0>
- Gupta R (2005) Recent outbreak of rabies infections in Brazil transmitted by vampire bats. *Euro Surveill* 10:. <https://doi.org/10.2807/ESW.10.45.02831-EN>
- Guruprasad L (2021) Human coronavirus spike protein-host receptor recognition. *Prog Biophys Mol Biol* 161:39–53. <https://doi.org/10.1016/j.pbiomolbio.2020.10.006>
- Guthid S, Hanley KA, Althouse BM, Boots M (2020) Ecological processes underlying the emergence of novel enzootic cycles: Arboviruses in the neotropics as a case study. *PLoS Negl Trop Dis* 14:1–22. <https://doi.org/10.1371/JOURNAL.PNTD.0008338>;
- Haagmans BL, Al Dhahiry SHS, Reusken CBEM, et al (2014) Middle East respiratory syndrome coronavirus in dromedary camels: An outbreak investigation. *Lancet Infect Dis* 14:140–145. [https://doi.org/10.1016/S1473-3099\(13\)70690-X](https://doi.org/10.1016/S1473-3099(13)70690-X)
- Han Y, Du J, Su H, et al (2019) Identification of diverse bat alphacoronaviruses and betacoronaviruses in china provides new insights into the evolution and origin of coronavirus-related diseases. *Front Microbiol* 10:454784. <https://doi.org/10.3389/FMICB.2019.01900/BIBTEX>
- Han Y, Xu P, Wang Y, et al (2023) Panoramic analysis of coronaviruses carried by representative bat species in Southern China to better understand the coronavirus sphere. *Nature Communications* 2023 14:1 14:1–20. <https://doi.org/10.1038/s41467-023-41264-z>
- Hanley DE, Bourassa MA, O'Brien JJ, et al (2003) A Quantitative Evaluation of ENSO Indices. *J Clim* 16:1249–1258. [https://doi.org/10.1175/1520-0442\(2003\)16<1249:AQEOEI>2.0.CO;2](https://doi.org/10.1175/1520-0442(2003)16<1249:AQEOEI>2.0.CO;2);

- Hao X, Qin LU, Zhao H (2024) A molecular phylogeny for all 21 families within Chiroptera (bats). *Integr Zool* 19:989–998. <https://doi.org/10.1111/1749-4877.12772>
- Hasday JD, Fairchild KD, Shanholtz C (2000) The role of fever in the infected host. *Microbes Infect* 2:1891–1904. [https://doi.org/10.1016/S1286-4579\(00\)01337-X](https://doi.org/10.1016/S1286-4579(00)01337-X)
- Hayman DTS (2016) Bats as Viral Reservoirs. *Annu Rev Virol* 3:77–99. <https://doi.org/10.1146/annurev-virology-110615-042203>
- Hemnani M, da Silva PG, Thompson G, et al (2024a) Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *Ecohealth*. <https://doi.org/10.1007/s10393-024-01688-5>
- Hemnani M, da Silva PG, Thompson G, et al (2024b) Detection and Prevalence of Coronaviruses in European Bats: A Systematic Review. *Ecohealth*. <https://doi.org/10.1007/s10393-024-01688-5>
- Hemnani M, Silva PG da, Thompson G, et al (2023a) First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* 15:1–12. <https://doi.org/10.3390/v15071521>
- Hemnani M, Silva PG da, Thompson G, et al (2023b) First Report of Alphacoronavirus Circulating in Cavernicolous Bats from Portugal. *Viruses* 15:1–12. <https://doi.org/10.3390/v15071521>
- Herrera JM, Costa P, Medinas D, et al (2015) Community composition and activity of insectivorous bats in Mediterranean olive farms. *Anim Conserv* 18:557–566. <https://doi.org/10.1111/ACV.12209/SUPPINFO>
- Hildebrandt E, Penzes JJ, Gifford RJ, et al (2020) Evolution of dependoparvoviruses across geological timescales—implications for design of AAV-based gene therapy vectors. *Virus Evol* 6:. <https://doi.org/10.1093/ve/veaa043>
- Hofmann H, Pyrc K, Van Der Hoek L, et al (2005) Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci U S A* 102:7988–7993. <https://doi.org/10.1073/PNAS.0409465102>,
- Honorato R V, Koukos PI, Jiménez-García B, et al (2021) Structural Biology in the Clouds: The WeNMR-EOSC Ecosystem. *Front Mol Biosci* 8
- Honorato R V, Trellet ME, Jiménez-García B, et al (2024) The HADDOCK2.4 web server for integrative modeling of biomolecular complexes. *Nat Protoc* 19:3219–3241. <https://doi.org/10.1038/s41596-024-01011-0>

- Huang YW, Dickerman AW, Piñeyro P, et al (2013) Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the united states. *mBio* 4:737–750. https://doi.org/10.1128/MBIO.00737-13/SUPPL_FILE/MBO005131645ST2.DOCX
- Hulva P, Horáček I, Strelkov PP, Benda P (2004) Molecular architecture of *Pipistrellus pipistrellus*/*Pipistrellus pygmaeus* complex (Chiroptera: Vespertilionidae): Further cryptic species and Mediterranean origin of the divergence. *Mol Phylogenet Evol* 32:1023–1035. <https://doi.org/10.1016/j.ympev.2004.04.007>
- Ingala M, Fernandes H, De Oliveira M, et al (2025) Bats in Habitats, Bats as Habitats: An integrative ecological framework for understanding synergistic interactions across levels of community organization. <https://doi.org/10.32942/X2RW59>
- Irving AT, Ahn M, Goh G, et al (2021a) Lessons from the host defences of bats, a unique viral reservoir. *Nature* 589:363–370. <https://doi.org/10.1038/s41586-020-03128-0>
- Irving AT, Ahn M, Goh G, et al (2021b) Lessons from the host defences of bats, a unique viral reservoir. *Nature* 589:363–370. <https://doi.org/10.1038/s41586-020-03128-0>
- Islam MS, Sazzad HMS, Satter SM, et al (2016) Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011–2014. *Emerg Infect Dis* 22:664. <https://doi.org/10.3201/EID2204.151747>
- Janetanakit T, Lumyai M, Bunpapong N, et al (2016) Porcine deltacoronavirus, Thailand, 2015. *Emerg Infect Dis* 22:757–759. <https://doi.org/10.3201/EID2204.151852>,
- Jebb D, Foley NM, Whelan C V., et al (2018) Population level mitogenomics of long-lived bats reveals dynamic heteroplasmy and challenges the Free Radical Theory of Ageing. *Scientific Reports* 2018 8:1 8:1–12. <https://doi.org/10.1038/s41598-018-31093-2>
- Ji W, Peng Q, Fang X, et al (2022) Structures of a deltacoronavirus spike protein bound to porcine and human receptors. *Nat Commun* 13:. <https://doi.org/10.1038/s41467-022-29062-5>
- Jones BD, Kaufman EJ, Peel AJ (2023) Viral Co-Infection in Bats: A Systematic Review. *Viruses* 15:. <https://doi.org/10.3390/v15091860>
- Jones DN, Ravelomanantsoa NAF, Yeoman CJ, et al (2022) Do gastrointestinal microbiomes play a role in bats' unique viral hosting capacity? *Trends Microbiol* 30:632–642. <https://doi.org/10.1016/J.TIM.2021.12.009/ASSET/BF65FB14-03F2-4E41-9A61-77CCA8033999/MAIN.ASSETS/GR2.JPG>
- Jones G, Jacobs D, Kunz T, et al (2009) Carpe noctem: the importance of bats as bioindicators. *Endanger Species Res* 8:93–115. <https://doi.org/10.3354/esr00182>

- Jones G, Teeling EC (2006) The evolution of echolocation in bats. *Trends Ecol Evol* 21:149–156. <https://doi.org/10.1016/J.TREE.2006.01.001/ATTACHMENT/C0C308DE-5941-4D66-AA93-3F77C85F48FF/MMC1.PDF>
- Kamins AO, Restif O, Ntiamoa-Baidu Y, et al (2011) Uncovering the fruit bat bushmeat commodity chain and the true extent of fruit bat hunting in Ghana, West Africa. *Biol Conserv* 144:3000–3008. <https://doi.org/10.1016/J.BIOCON.2011.09.003>
- Kasso M, Balakrishnan M (2013) Ecological and Economic Importance of Bats (Order Chiroptera). *ISRN Biodiversity* 2013:1–9. <https://doi.org/10.1155/2013/187415>
- Kim N, Ma J, Kim W, et al (2024) Genome-resolved metagenomics: a game changer for microbiome medicine. *Exp Mol Med* 56:1501–1512. <https://doi.org/10.1038/s12276-024-01262-7>
- Kim SH, Chang SY, Sung M, et al (2016) Extensive Viable Middle East Respiratory Syndrome (MERS) Coronavirus Contamination in Air and Surrounding Environment in MERS Isolation Wards. *Clinical Infectious Diseases* 63:363–369. <https://doi.org/10.1093/cid/ciw239>
- Korine C, Oelbaum PJ, Muñoz-Garcia A (2022) Stress Physiology, Foraging, and Ecophysiology of Bats in Urban Environments. 3–18. https://doi.org/10.1007/978-3-031-13173-8_1
- Kramer LD, Tavakoli NP (2021) VIRUSES OF TERRESTRIAL MAMMALS. In: *Studies in Viral Ecology*. Wiley, pp 541–583
- Ksiazek TG, Erdman D, Goldsmith CS, et al (2003a) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1953–1966. <https://doi.org/10.1056/NEJMOA030781>,
- Ksiazek TG, Erdman D, Goldsmith CS, et al (2003b) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine* 348:1953–1966. <https://doi.org/10.1056/NEJMOA030781>,
- Kunz TH (1982a) Roosting ecology of bats. *Ecology of bats* 1–55. https://doi.org/10.1007/978-1-4613-3421-7_1
- Kunz TH (ed) (1982b) *Ecology of Bats*. <https://doi.org/10.1007/978-1-4613-3421-7>
- Kunz TH, de Torrez EB, Bauer D, et al (2011) Ecosystem services provided by bats. *Ann N Y Acad Sci* 1223:1–38. <https://doi.org/10.1111/j.1749-6632.2011.06004.x>
- Kunz TH., Fenton MBrock (2003a) *Bat ecology*. 779
- Kunz TH., Fenton MBrock (2003b) *Bat ecology*. 779

- Latinne A, Morand S (2022) Climate Anomalies and Spillover of Bat-Borne Viral Diseases in the Asia-Pacific Region and the Arabian Peninsula. *Viruses* 14:. <https://doi.org/10.3390/V14051100>;
- Lau SKP, Luk HKH, Wong ACP, et al (2019) Identification of a novel betacoronavirus (merbecovirus) in amur hedgehogs from China. *Viruses* 11:. <https://doi.org/10.3390/V11110980>,
- Lau SKP, Wong EYM, Tsang C-C, et al (2018a) Discovery and Sequence Analysis of Four Deltacoronaviruses from Birds in the Middle East Reveal Interspecies Jumping with Recombination as a Potential Mechanism for Avian-to-Avian and Avian-to-Mammalian Transmission. *J Virol* 92:. <https://doi.org/10.1128/JVI.00265-18>,
- Lau SKP, Woo PCY, Li KSM, et al (2005) Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 102:14040–14045. <https://doi.org/10.1073/PNAS.0506735102>,
- Lau SKP, Zhang L, Luk HKH, et al (2018b) Receptor usage of a novel bat lineage C betacoronavirus reveals evolution of middle east respiratory syndrome-related coronavirus spike proteins for human dipeptidyl peptidase 4 binding. *Journal of Infectious Diseases* 218:197–207. <https://doi.org/10.1093/INFDIS/JIY018>,
- Lazov CM, Chriél M, Baagøe HJ, et al (2018) Detection and characterization of distinct alphacoronaviruses in five different bat species in Denmark. *Viruses* 10:. <https://doi.org/10.3390/v10090486>
- Le VP, Song S, An BH, et al (2018) A novel strain of porcine deltacoronavirus in Vietnam. *Arch Virol* 163:203–207. <https://doi.org/10.1007/S00705-017-3594-8>,
- Lednický J, Lauzard M, Fan ZH, et al (2020) Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *International Journal of Infectious Diseases* 100:476–482. <https://doi.org/10.1016/j.ijid.2020.09.025>
- Lednický JA, Tagliamonte MS, White SK, et al (2021) Independent infections of porcine deltacoronavirus among Haitian children. *Nature* 600:133–137. <https://doi.org/10.1038/S41586-021-04111-Z>,
- Lee JH, Chung HC, Nguyen VG, et al (2016) Detection and Phylogenetic Analysis of Porcine Deltacoronavirus in Korean Swine Farms, 2015. *Transbound Emerg Dis* 63:248–252. <https://doi.org/10.1111/TBED.12490>,
- Lee YF, McCracken GF (2002) Foraging activity and food resource use of Brazilian free-tailed bats, *Tadarida brasiliensis* (Molossidae). *Ecoscience* 9:306–313.

<https://doi.org/10.1080/11956860.2002.11682717>;WGROUP:STRING:PUBLICATION

- Lelli D, Papetti A, Sabelli C, et al (2013) Detection of coronaviruses in bats of various species in Italy. *Viruses* 5:2679–2689. <https://doi.org/10.3390/v5112679>
- Li F, Li W, Farzan M, Harrison SC (2005a) Structural biology: Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* (1979) 309:1864–1868. <https://doi.org/10.1126/SCIENCE.1116480>,
- Li W, Shi Z, Yu M, et al (2005b) Bats are natural reservoirs of SARS-like coronaviruses. *Science* (1979) 310:676–679. <https://doi.org/10.1126/SCIENCE.1118391>,
- Lindenmayer DB, Laurance WF (2017) The ecology, distribution, conservation and management of large old trees. *Biological Reviews* 92:1434–1458. <https://doi.org/10.1111/BRV.12290>;WGROUP:STRING:PUBLICATION
- Lu M, Wang X, Ye H, et al (2021) Does public fear that bats spread COVID-19 jeopardize bat conservation? *Biol Conserv* 254:108952. <https://doi.org/10.1016/j.biocon.2021.108952>
- Lu R, Zhao X, Li J, et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* 395:565–574. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8)
- Luby SP, Rahman M, Hossain MJ, et al (2006) Foodborne Transmission of Nipah Virus, Bangladesh. *Emerg Infect Dis* 12:1888. <https://doi.org/10.3201/EID1212.060732>
- Lucero Arteaga F, Miragaya M, Molina N, et al (2023) Identification of coronaviruses in bats and rodents in northern and central Argentina. *Arch Virol* 168:1–7. <https://doi.org/10.1007/s00705-023-05703-y>
- Luis AD, Hayman DTS, O’Shea TJ, et al (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? *Proceedings of the Royal Society B: Biological Sciences* 280:. <https://doi.org/10.1098/rspb.2012.2753>
- Lynteris C (2016) The Prophetic Faculty of Epidemic Photography: Chinese Wet Markets and the Imagination of the Next Pandemic. *Vis Anthropol* 29:118–132. <https://doi.org/10.1080/08949468.2016.1131484>;
- Madapong A, Saeng-chuto K, Lorsirigool A, et al (2016) Complete genome sequence of porcine deltacoronavirus isolated in Thailand in 2015. *Genome Announc* 4:. <https://doi.org/10.1128/GENOMEA.00408-16>,

- Maina JN (2000) What it Takes to Fly: The Structural and Functional Respiratory Refinements in Birds and Bats. *Journal of Experimental Biology* 203:3045–3064. <https://doi.org/10.1242/JEB.203.20.3045>
- Marinello MM, Bernard E (2014) Wing morphology of neotropical bats: A quantitative and qualitative analysis with implications for habitat use. *Can J Zool* 92:141–147. <https://doi.org/10.1139/CJZ-2013-0127>;PAGE:STRING:ARTICLE/CHAPTER
- Martínez-Abraín A, Jiménez J (2016) Anthropogenic areas as incidental substitutes for original habitat. *Conservation Biology* 30:593–598. <https://doi.org/10.1111/COBI.12644>;REQUESTEDJOURNAL:JOURNAL:15231739;WGROUPE:STRING:PUBLICATION
- Mata VA, Da Silva LP, Issimo J Ver, et al (2021) Combining DNA metabarcoding and ecological networks to inform conservation biocontrol by small vertebrate predators. *Ecological Applications* 31:e02457. <https://doi.org/10.1002/EAP.2457>
- Mathias ML, Fonseca C, Rodrigues L, Grilo C, Lopes-Fernandes M, Palmeirim JM, Santos-Reis M, Alves PC, Cabral JA FM, Mira A, Eira C, Negrões N, Paupério J, Pita R, Rainho A, Rosalino LM TJ& VJ Livro vermelho dos mamíferos de Portugal continental, 2023rd edn. FCIências.ID, ICNF, Lisboa
- Mekibib B, Ariën KK (2016) Aerosol Transmission of Filoviruses. *Viruses* 2016, Vol 8, Page 148 8:148. <https://doi.org/10.3390/V8050148>
- Méndez-Rodríguez A, Horta P, Zarza H, et al (2024) Surveying Bat-Hosted Adenoviruses and Herpesviruses: A Comprehensive Analysis. *Diversity (Basel)* 16:46. <https://doi.org/10.3390/d16010046>
- Metzner W, Müller R (2016) Ultrasound Production, Emission, and Reception. 55–91. https://doi.org/10.1007/978-1-4939-3527-7_3
- Mihindukulasuriya KA, Wu G, St. Leger J, et al (2008a) Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J Virol* 82:5084–5088. <https://doi.org/10.1128/jvi.02722-07>
- Mihindukulasuriya KA, Wu G, St. Leger J, et al (2008b) Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J Virol* 82:5084–5088. <https://doi.org/10.1128/jvi.02722-07>
- Miller AK, Mifsud JCO, Costa VA, et al (2021) Slippery when wet: Cross-species transmission of divergent coronaviruses in bony and jawless fish and the evolutionary history of the Coronaviridae. *Virus Evol* 7:1–10. <https://doi.org/10.1093/ve/veab050>

- Mo M, Roache M, Davies J, et al (2021) Estimating flying-fox mortality associated with abandonments of pups and extreme heat events during the austral summer of 2019–20. *Pacific Conservation Biology* 28:124–139. <https://doi.org/10.1071/PC21003>
- Monto AS, Cowling BJ, Peiris JSM (2014) Coronaviruses. *Viral Infections of Humans: Epidemiology and Control* 199–223. https://doi.org/10.1007/978-1-4899-7448-8_10/TABLES/3
- Moratelli R, Calisher CH (2015) Bats and zoonotic viruses: Can we confidently link bats with emerging deadly viruses? *Mem Inst Oswaldo Cruz* 110:1–22. <https://doi.org/10.1590/0074-02760150048>
- Morawska L, Milton DK (2020) It is Time to Address Airborne Transmission of COVID-19. *Clinical Infectious Diseases*. <https://doi.org/10.1093/cid/ciaa939>
- Moreno A, Lelli D, De Sabato L, et al (2017) Detection and full genome characterization of two beta CoV viruses related to Middle East respiratory syndrome from bats in Italy. *Virol J* 14:1–11. <https://doi.org/10.1186/s12985-017-0907-1>
- Moussy C, Hosken DJ, Mathews F, et al (2013) Migration and dispersal patterns of bats and their influence on genetic structure. *Mamm Rev* 43:183–195. <https://doi.org/10.1111/J.1365-2907.2012.00218.X>
- Mühldorfer K, Speck S, Kurth A, et al (2011) Diseases and Causes of Death in European Bats: Dynamics in Disease Susceptibility and Infection Rates. *PLoS One* 6:e29773. <https://doi.org/10.1371/JOURNAL.PONE.0029773>
- Naguib MM, Li R, Ling J, et al (2021) Live and Wet Markets: Food Access versus the Risk of Disease Emergence. *Trends Microbiol* 29:573–581. <https://doi.org/10.1016/J.TIM.2021.02.007/ASSET/25E2E3F1-49B1-4501-87B4-09B52F7908FC/MAIN.ASSETS/GR1.JPG>
- Nahar N, Asaduzzaman M, Mandal UK, et al (2020) Hunting Bats for Human Consumption in Bangladesh. *Ecohealth* 17:139–151. <https://doi.org/10.1007/S10393-020-01468-X/TABLES/8>
- Nakagawa S, Miyazawa T (2020) Genome evolution of SARS-CoV-2 and its virological characteristics. *Inflamm Regen* 40:0–6. <https://doi.org/10.1186/s41232-020-00126-7>
- Nal B, Chan C, Kien F, et al (2005) Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. *Journal of General Virology* 86:1423–1434. <https://doi.org/10.1099/VIR.0.80671-0/CITE/REFWORKS>

- Neuman BW, Kiss G, Kunding AH, et al (2011) A structural analysis of M protein in coronavirus assembly and morphology. *J Struct Biol* 174:11–22. <https://doi.org/10.1016/J.JSB.2010.11.021>
- Niederwerder MC, Hesse RA (2018) Swine enteric coronavirus disease: A review of 4 years with porcine epidemic diarrhoea virus and porcine deltacoronavirus in the United States and Canada. *Transbound Emerg Dis* 65:660–675. <https://doi.org/10.1111/TBED.12823>
- Nieto-Torres JL, DeDiego ML, Verdía-Báguena C, et al (2014) Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. *PLoS Pathog* 10:e1004077. <https://doi.org/10.1371/JOURNAL.PPAT.1004077>
- Nojiri T, Wilson LAB, López-Aguirre C, et al (2021) Embryonic evidence uncovers convergent origins of laryngeal echolocation in bats. *Current Biology* 31:1353–1365.e3. <https://doi.org/10.1016/j.cub.2020.12.043>
- Nusová G, Uhrin M, Voigt CC, Kaňuch P (2020) Tracing the geographic origin of common pipistrelles (*Pipistrellus pipistrellus*) swarming at a mass hibernaculum. *Mammalian Biology* 100:601–610. <https://doi.org/10.1007/s42991-020-00057-3>
- Nyberg T, Ferguson NM, Nash SG, et al (2022) Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *The Lancet* 399:1303–1312. [https://doi.org/10.1016/S0140-6736\(22\)00462-7](https://doi.org/10.1016/S0140-6736(22)00462-7)
- Oedin M, Brescia F, Vidal E, Millon A (2022) Make flying-fox hunting sustainable again: Comparing expected demographic effectiveness and hunters' acceptance of more restrictive regulations. *Ambio* 51:1078–1089. <https://doi.org/10.1007/S13280-021-01630-X/FIGURES/6>
- Olival KJ, Cryan PM, Amman BR, et al (2020) Possibility for reverse zoonotic transmission of sars-cov-2 to free-ranging wildlife: A case study of bats. *PLoS Pathog* 16:1–19. <https://doi.org/10.1371/journal.ppat.1008758>
- Olsen SJ, Chang HL, Cheung TYY, et al (2003) Transmission of the Severe Acute Respiratory Syndrome on Aircraft. *New England Journal of Medicine* 349:2416–2422. <https://doi.org/10.1056/NEJMoa031349>
- Openshaw JJ, Hegde S, Sazzad HMS, et al (2017a) Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. *Transbound Emerg Dis* 64:1287–1293. <https://doi.org/10.1111/tbed.12505>

- Openshaw JJ, Hegde S, Sazzad HMS, et al (2017b) Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. *Transbound Emerg Dis* 64:1287–1293. <https://doi.org/10.1111/TBED.12505>
- O’Shea TJ, Cryan PM, Cunningham AA, et al (2014) Bat flight and zoonotic viruses. *Emerg Infect Dis* 20:741–745. <https://doi.org/10.3201/eid2005.130539>
- Page RA, Bernal XE (2020) The challenge of detecting prey: Private and social information use in predatory bats. *Funct Ecol* 34:344–363. <https://doi.org/10.1111/1365-2435.13439/SUPPINFO>
- Papineau A, Berhane Y, Wylie TN, et al (2019) Genome Organization of Canada Goose Coronavirus, A Novel Species Identified in a Mass Die-off of Canada Geese. *Sci Rep* 9:. <https://doi.org/10.1038/S41598-019-42355-Y>,
- Peiris JSM, Guan Y, Yuen KY (2004) Severe acute respiratory syndrome. *Nat Med* 10:S88–S97. <https://doi.org/10.1038/nm1143>
- Pensaert MB, de Bouck P (1978) A new coronavirus-like particle associated with diarrhea in swine. *Arch Virol* 58:243–247. <https://doi.org/10.1007/BF01317606>,
- Perry RW (2013) A review of factors affecting cave climates for hibernating bats in temperate North America. *Environmental Reviews* 21:28–39. <https://doi.org/10.1139/ER-2012-0042;PAGEGROUP:STRING:PUBLICATION>
- Phelps KL, Hamel L, Alhmoud N, et al (2019) Bat research networks and viral surveillance: Gaps and opportunities in western Asia. *Viruses* 11:. <https://doi.org/10.3390/v11030240>
- Phillips AM, Gonzalez LO, Nekongo EE, et al (2017) Host proteostasis modulates influenza evolution. *Elife* 6:. <https://doi.org/10.7554/ELIFE.28652>
- Platto S, Zhou J, Wang Y, et al (2021a) Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease. *Biochem Biophys Res Commun* 538:2–13. <https://doi.org/10.1016/j.bbrc.2020.10.028>
- Platto S, Zhou J, Wang Y, et al (2021b) Biodiversity loss and COVID-19 pandemic: The role of bats in the origin and the spreading of the disease. *Biochem Biophys Res Commun* 538:2–13. <https://doi.org/10.1016/j.bbrc.2020.10.028>
- Pratelli A, Tempesta M, Elia G, et al (2022) The knotty biology of canine coronavirus: A worrying model of coronaviruses’ danger. *Res Vet Sci* 144:190–195. <https://doi.org/10.1016/J.RVSC.2021.11.014>

- Prentice MB, Gilbertson MLJ, Storm DJ, et al (2024) Metagenomic sequencing sheds light on microbes putatively associated with pneumonia-related fatalities of white-tailed deer (*Odocoileus virginianus*). *Microb Genom* 10:. <https://doi.org/10.1099/mgen.0.001214>
- Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes De Novo Assembler. *Curr Protoc Bioinformatics* 70:1–29. <https://doi.org/10.1002/cpbi.102>
- Pujol FH, Branda F, Ceccarelli G, et al (2025) Nipah Virus: A Zoonotic Threat Re-Emerging in the Wake of Global Public Health Challenges. *Microorganisms* 2025, Vol 13, Page 124 13:124. <https://doi.org/10.3390/MICROORGANISMS13010124>
- Pyankov O V, Bodnev SA, Pyankova OG, Agranovski IE (2018a) Survival of aerosolized coronavirus in the ambient air. *J Aerosol Sci* 115:158–163. <https://doi.org/10.1016/j.jaerosci.2017.09.009>
- Pyankov O V, Bodnev SA, Pyankova OG, Agranovski IE (2018b) Survival of aerosolized coronavirus in the ambient air. *J Aerosol Sci* 115:158–163. <https://doi.org/10.1016/j.jaerosci.2017.09.009>
- Racey PA (2015) The Uniqueness of Bats. *Bats and Viruses: A New Frontier of Emerging Infectious Diseases* 1–22. <https://doi.org/10.1002/9781118818824.CH1>
- Rainho A Summer foraging habitats of bats in a Mediterranean region of the Iberian Peninsula. <https://doi.org/10.3161/150811007781694408>
- Ramanathan K, Antognini D, Combes A, et al (2020) Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. *Journal of Hospital Infection* 92:235–250
- Ramírez-Fráncel LA, García-Herrera LV, Losada-Prado S, et al (2022) Bats and their vital ecosystem services: a global review. *Integr Zool* 17:2–23. <https://doi.org/10.1111/1749-4877.12552>
- Ravi V, Saxena S, Panda PS (2022) Basic virology of SARS-CoV 2. *Indian J Med Microbiol* 40:182–186. <https://doi.org/10.1016/J.IJMMB.2022.02.005>
- Reguera J, Santiago C, Mudgal G, et al (2012) Structural Bases of Coronavirus Attachment to Host Aminopeptidase N and Its Inhibition by Neutralizing Antibodies. *PLoS Pathog* 8:e1002859. <https://doi.org/10.1371/journal.ppat.1002859>
- Reusken CBEM, Lina PHC, Pielaat A, et al (2010) Circulation of group 2 coronaviruses in a bat species common to urban areas in Western Europe. *Vector Borne Zoonotic Dis* 10:785–791. <https://doi.org/10.1089/vbz.2009.0173>

- Reyes A, Semenkovich NP, Whiteson K, et al (2012) Going viral: Next-generation sequencing applied to phage populations in the human gut. *Nat Rev Microbiol* 10:607–617. <https://doi.org/10.1038/nrmicro2853>
- Reyes-del Valle J, Chávez-Salinas S, Medina F, del Angel RM (2005) Heat Shock Protein 90 and Heat Shock Protein 70 Are Components of Dengue Virus Receptor Complex in Human Cells. *J Virol* 79:4557–4567. <https://doi.org/10.1128/JVI.79.8.4557-4567.2005/ASSET/D00B8FCF-B4BA-4E36-84D5-BAC18F19286A/ASSETS/GRAPHIC/ZJV0080560520008.JPEG>
- Richard M, Kok A, de Meulder D, et al (2020) SARS-CoV-2 is transmitted via contact and via the air between ferrets. *Nat Commun* 11:. <https://doi.org/10.1038/S41467-020-17367-2>,
- Riskin DK, Racey PA (2010) How do sucker-footed bats hold on, and why do they roost head-up? *Biological Journal of the Linnean Society* 99:233–240. <https://doi.org/10.1111/J.1095-8312.2009.01362.X>
- Rizzo F, Edenborough KM, Toffoli R, et al (2017) Coronavirus and paramyxovirus in bats from Northwest Italy. *BMC Vet Res* 13:1–11. <https://doi.org/10.1186/s12917-017-1307-x>
- Rodrigues L, Zahn A, Rainho A, Palmeirim JM (2003) Contrasting the roosting behaviour and phenology of an insectivorous bat (*Myotis myotis*) in its southern and northern distribution ranges. *Mammalia* 67:321–335. <https://doi.org/10.1515/MAMM.2003.67.3.321/MACHINEREADABLECITATION/RIS>
- Roffler AA, Maurer DP, Lunn TJ, et al (2024) Bat humoral immunity and its role in viral pathogenesis, transmission, and zoonosis. *Front Immunol* 15:1–12. <https://doi.org/10.3389/fimmu.2024.1269760>
- Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun* 109:102433. <https://doi.org/https://doi.org/10.1016/j.jaut.2020.102433>
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022a) Ecology, evolution and spillover of coronaviruses from bats. Springer US
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022b) Ecology, evolution and spillover of coronaviruses from bats. Springer US
- Ruiz-Aravena M, McKee C, Gamble A, et al (2022c) Ecology, evolution and spillover of coronaviruses from bats. Springer US

- Russo D (2023a) Chiroptera., 2023rd edn. Springer
- Russo D (2023b) Chiroptera., 2023rd edn. Springer
- Russo D, Cistrone L, Budinski I, et al (2017) Sociality influences thermoregulation and roost switching in a forest bat using ephemeral roosts. *Ecol Evol* 7:5310–5321. <https://doi.org/10.1002/ECE3.3111>
- Saeng-chuto K, Lorsirigool A, Temeeyasen G, et al (2017) Different Lineage of Porcine Deltacoronavirus in Thailand, Vietnam and Lao PDR in 2015. *Transbound Emerg Dis* 64:3–10. <https://doi.org/10.1111/TBED.12585>,
- Santana SE, Dial TO, Eiting TP, Alfaro ME (2011) Roosting Ecology and the Evolution of Pelage Markings in Bats. *PLoS One* 6:e25845. <https://doi.org/10.1371/JOURNAL.PONE.0025845>
- Schnitzler HU, Denzinger A (2024) Foraging strategies of echolocating bats. *A Natural History of Bat Foraging: Evolution, Physiology, Ecology, Behavior, and Conservation* 83–107. <https://doi.org/10.1016/B978-0-323-91820-6.16003-6>
- Schnitzler HU, Moss CF, Denzinger A (2003) From spatial orientation to food acquisition in echolocating bats. *Trends Ecol Evol* 18:386–394. [https://doi.org/10.1016/S0169-5347\(03\)00185-X/ASSET/ECEFFF0C-8B89-45C9-A7C1-D3C758339FC8/MAIN.ASSETS/GR5.GIF](https://doi.org/10.1016/S0169-5347(03)00185-X/ASSET/ECEFFF0C-8B89-45C9-A7C1-D3C758339FC8/MAIN.ASSETS/GR5.GIF)
- Serra-Cobo J, López-Roig M (2017) Bats and emerging infections: An ecological and virological puzzle. *Adv Exp Med Biol* 972:35–48. https://doi.org/10.1007/5584_2016_131
- Shen YY, Liang L, Zhu ZH, et al (2010) Adaptive evolution of energy metabolism genes and the origin of flight in bats. *Proc Natl Acad Sci U S A* 107:8666–8671. https://doi.org/10.1073/PNAS.0912613107/SUPPL_FILE/STXT01.PDF
- Shipley R, Wright E, Selden D, et al (2019) Bats and viruses: Emergence of novel lyssaviruses and association of bats with viral zoonoses in the EU. *Trop Med Infect Dis* 4:1–22. <https://doi.org/10.3390/tropicalmed4010031>
- Silva PG, Branco PTBS, Soares RRG, et al (2022a) SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* 32:e13083. <https://doi.org/https://doi.org/10.1111/ina.13083>
- Silva PG, Branco PTBS, Soares RRG, et al (2022b) SARS-CoV-2 air sampling: A systematic review on the methodologies for detection and infectivity. *Indoor Air* 32:e13083. <https://doi.org/https://doi.org/10.1111/ina.13083>

- Simonin Y (2023) Emerging Diseases/Viruses Prevention, Control, Surveillance, and One Health. *Trop Med Infect Dis* 8:257. <https://doi.org/10.3390/tropicalmed8050257>
- Singh R, Kølvrå S, Bross P, et al (2006) Heat-shock protein 70 genes and human longevity: A view from Denmark. *Ann N Y Acad Sci* 1067:301–308. <https://doi.org/10.1196/ANNALS.1354.040;PAGE:STRING:ARTICLE/CHAPTER>
- Smith I, Wang LF (2013) Bats and their virome: An important source of emerging viruses capable of infecting humans. *Curr Opin Virol* 3:84–91. <https://doi.org/10.1016/j.coviro.2012.11.006>
- Smith SH, Somsen GA, Van Rijn C, et al (2020) Aerosol persistence in relation to possible transmission of SARS-CoV-2. *Physics of Fluids* 32:. <https://doi.org/10.1063/5.0027844>,
- Soler-Tovar D, Escobar LE (2025) Rabies transmitted from vampires to cattle: An overview. *PLoS One* 20:e0317214. <https://doi.org/10.1371/JOURNAL.PONE.0317214>
- Srivastava P (2002) Roles of heat-shock proteins in innate and adaptive immunity. *Nat Rev Immunol* 2:185–194. <https://doi.org/10.1038/NRI749;KWRD=BIOMEDICINE>
- Stalin Raj V, Farag EABA, Reusken CBEM, et al (2014) Isolation of MERS coronavirus from dromedary camel, Qatar, 2014. *Emerg Infect Dis* 20:1339–1342. <https://doi.org/10.3201/EID2008.140663>,
- Stidsholt L (2025) Unlocking the mysteries of biosonar use and foraging behaviour in wild bats with on-board acoustic recording tags. *Journal of Experimental Biology* 228:. <https://doi.org/10.1242/JEB.250777/368992>
- Subudhi S, Rapin N, Misra V (2019) Immune System Modulation and Viral Persistence in Bats: Understanding Viral Spillover. *Viruses* 2019, Vol 11, Page 192 11:192. <https://doi.org/10.3390/V11020192>
- Teeling EC, Jones G, Rossiter SJ (2016) Phylogeny, Genes, and Hearing: Implications for the Evolution of Echolocation in Bats. 25–54. https://doi.org/10.1007/978-1-4939-3527-7_2
- Tellier R, Li Y, Cowling BJ, Tang JW (2019) Recognition of aerosol transmission of infectious agents: A commentary. *BMC Infect Dis* 19:1–9. <https://doi.org/10.1186/s12879-019-3707-y>
- Thomas SP, Suthers RA (1972) The Physiology and Energetics of Bat Flight. *Journal of Experimental Biology* 57:317–335. <https://doi.org/10.1242/JEB.57.2.317>

- Tisza M, Javornik Cregeen S, Avadhanula V, et al (2023) Wastewater sequencing reveals community and variant dynamics of the collective human virome. *Nat Commun* 14:1–10. <https://doi.org/10.1038/s41467-023-42064-1>
- Tiwari R, Dhama K, Sharun K, et al (2020) COVID-19: animals, veterinary and zoonotic links. *Veterinary Quarterly* 40:169–182. <https://doi.org/10.1080/01652176.2020.1766725>
- Torquetti CG, Guimarães ATB, Soto-Blanco B (2021) Exposure to pesticides in bats. *Science of The Total Environment* 755:142509. <https://doi.org/10.1016/j.scitotenv.2020.142509>
- T.S. YI, Yuguo L, Wai WT, et al (2024) Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus. *New England Journal of Medicine* 350:1731–1739. <https://doi.org/10.1056/NEJMoa032867>
- Vakulenko Y, Deviatkin A, Drexler JF, Lukashev A (2021) Modular evolution of coronavirus genomes. *Viruses* 13:1270. <https://doi.org/10.3390/V13071270/S1>
- Verreault D, Moineau S, Duchaine C (2008) Methods for Sampling of Airborne Viruses. *Microbiology and Molecular Biology Reviews* 72:413–444. <https://doi.org/10.1128/mmbr.00002-08>
- Vlasova AN, Halpin R, Wang S, et al (2011) Molecular characterization of a new species in the genus Alphacoronavirus associated with mink epizootic catarrhal gastroenteritis. *Journal of General Virology* 92:1369–1379. <https://doi.org/10.1099/VIR.0.025353-0>,
- Voigt CC, Kingston T (2015) *Bats in the anthropocene: Conservation of bats in a changing world*. Springer International Publishing
- Wang B, Zhang A, Sun JL, et al (2005) Study of SARS transmission via liquid droplets in air. *J Biomech Eng* 127:32–38. <https://doi.org/10.1115/1.1835350>
- Wang L, Zhang Y, Byrum B (2014) Complete genome sequence of porcine coronavirus HKU15 strain IN2847 from the United States. *Genome Announc* 2:. <https://doi.org/10.1128/GENOMEA.00291-14>,
- Wang LF, Anderson DE (2019) Viruses in bats and potential spillover to animals and humans. *Curr Opin Virol* 34:79–89. <https://doi.org/10.1016/j.coviro.2018.12.007>
- Wang W, Lin XD, Guo WP, et al (2015) Discovery, diversity and evolution of novel coronaviruses sampled from rodents in China. *Virology* 474:19–27. <https://doi.org/10.1016/j.virol.2014.10.017>

- Wang Z, Huang G, Huang M, et al (2023) Global patterns of phylogenetic diversity and transmission of bat coronavirus. *Sci China Life Sci* 66:861–874. <https://doi.org/10.1007/s11427-022-2221-5>
- Waterhouse A, Bertoni M, Bienert S, et al (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46:W296–W303. <https://doi.org/10.1093/nar/gky427>
- Whittaker GR, André NM, Millet JK (2018) Improving Virus Taxonomy by Recontextualizing Sequence-Based Classification with Biologically Relevant Data: the Case of the Alphacoronavirus 1 Species . *mSphere* 3:. <https://doi.org/10.1128/MSPHEREDIRECT.00463-17>,
- WHO (2021) Coronavirus disease (COVID-19): How is it transmitted?
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2021a) Analysis of partial sequences of the RNA-dependent RNA polymerase gene as a tool for genus and subgenus classification of coronaviruses. *Journal of General Virology* 101:1261–1269. <https://doi.org/10.1099/jgv.0.001494>
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2020) Partial RdRp sequences offer a robust method for Coronavirus subgenus classification. *bioRxiv* 2020.03.02.974311. <https://doi.org/10.1101/2020.03.02.974311>
- Wilkinson DA, Joffrin L, Lebarbenchon C, Mavingui P (2021b) Analysis of partial sequences of the RNA-dependent RNA polymerase gene as a tool for genus and subgenus classification of coronaviruses. *Journal of General Virology* 101:1261–1269. <https://doi.org/10.1099/JGV.0.001494/CITE/REFWORKS>
- Wong ACP, Lau SKP, Woo PCY (2021) Interspecies jumping of bat coronaviruses. *Viruses* 13:. <https://doi.org/10.3390/V13112188>,
- Wong S, Lau S, Woo P, Yuen KY (2007a) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91. <https://doi.org/10.1002/rmv.520>
- Wong S, Lau S, Woo P, Yuen KY (2007b) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91. <https://doi.org/10.1002/rmv.520>
- Woo PCY, De Groot RJ, Haagmans B, et al (2023a) ICTV Virus Taxonomy Profile: Coronaviridae 2023. *Journal of General Virology* 104:. <https://doi.org/10.1099/JGV.0.001843>
- Woo PCY, Groot RJ De, Haagmans B, et al (2023b) Family : Coronaviridae. 1–27
- Woo PCY, Lau SKP (2019) Viruses and bats. *Viruses* 11:2–5. <https://doi.org/10.3390/v11100884>

- Woo PCY, Lau SKP, Chu C, et al (2005) Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. *J Virol* 79:884–895. <https://doi.org/10.1128/JVI.79.2.884-895.2005>,
- Woo PCY, Lau SKP, Huang Y, Yuen KY (2009a) Coronavirus diversity, phylogeny and interspecies jumping. *Exp Biol Med* 234:1117–1127. <https://doi.org/10.3181/0903-MR-94>
- Woo PCY, Lau SKP, Lam CSF, et al (2012a) Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavi. *J Virol* 86:3995–4008. <https://doi.org/10.1128/jvi.06540-11>
- Woo PCY, Lau SKP, Lam CSF, et al (2014a) Discovery of a Novel Bottlenose Dolphin Coronavirus Reveals a Distinct Species of Marine Mammal Coronavirus in Gammacoronavirus. *J Virol* 88:1318–1331. <https://doi.org/10.1128/jvi.02351-13>
- Woo PCY, Lau SKP, Lam CSF, et al (2009b) Comparative Analysis of Complete Genome Sequences of Three Avian Coronaviruses Reveals a Novel Group 3c Coronavirus. *J Virol* 83:908–917. <https://doi.org/10.1128/JVI.01977-08>,
- Woo PCY, Lau SKP, Lam CSF, et al (2012b) Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavirus. *J Virol* 86:3995–4008. <https://doi.org/10.1128/JVI.06540-11>,
- Woo PCY, Lau SKP, Teng JLL, et al (2014b) New hepatitis E virus genotype in camels, the Middle East. *Emerg Infect Dis* 20:1044–1048. <https://doi.org/10.3201/eid2006.140140>
- Woo PCY, Lau SKP, Wernery U, et al (2014c) Novel betacoronavirus in dromedaries of the Middle East, 2013. *Emerg Infect Dis* 20:560–572. <https://doi.org/10.3201/EID2004.131769>,
- Woo PCY, Wang M, Lau SKP, et al (2007) Comparative Analysis of Twelve Genomes of Three Novel Group 2c and Group 2d Coronaviruses Reveals Unique Group and Subgroup Features. *J Virol* 81:1574–1585. <https://doi.org/10.1128/JVI.02182-06>,
- Wood JLN, Leach M, Waldman L, et al (2012) A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:2881–2892. <https://doi.org/10.1098/RSTB.2012.0228>

- Wu Z, Lu L, Du J, et al (2018) Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. *Microbiome* 6:. <https://doi.org/10.1186/S40168-018-0554-9>,
- Xiao W, Wang M, Wei W, et al (2004) [Detection of SARS-CoV and RNA on aerosol samples from SARS-patients admitted to hospital]. *Zhonghua Liu Xing Bing Xue Za Zhi* 25:882–885
- Xu Q, Wang F, Jiao W, et al (2023) Virtual Screening-Based Peptides Targeting Spike Protein to Inhibit Porcine Epidemic Diarrhea Virus (PEDV) Infection. *Viruses* 15:381. <https://doi.org/10.3390/v15020381>
- Yang D, Leibowitz JL (2015) The structure and functions of coronavirus genomic 3' and 5' ends. *Virus Res* 206:120–133. <https://doi.org/10.1016/J.VIRUSRES.2015.02.025>
- Yen HL, Sit THC, Brackman CJ, et al (2022) Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. *The Lancet* 399:1070–1078. [https://doi.org/10.1016/S0140-6736\(22\)00326-9](https://doi.org/10.1016/S0140-6736(22)00326-9)
- Yinda CK, Zell R, Deboutte W, et al (2017) Highly diverse population of Picornaviridae and other members of the Picornavirales, in Cameroonian fruit bats. *BMC Genomics* 18:249. <https://doi.org/10.1186/s12864-017-3632-7>
- Yuan Y, Cao D, Zhang Y, et al (2017) Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nature Communications* 2017 8:1 8:1–9. <https://doi.org/10.1038/ncomms15092>
- Zagmajster M (2019) Bats. *Encyclopedia of Caves*, Third Edition 94–101. <https://doi.org/10.1016/B978-0-12-814124-3.00013-3>
- Zaki AM, van Boheemen S, Bestebroer TM, et al (2012) Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *New England Journal of Medicine* 367:1814–1820. <https://doi.org/10.1056/NEJMOA1211721>,
- Zhao B, Chen C, Yang X, Li Y (2011) Role of two-way airflow owing to temperature difference in severe acute respiratory syndrome transmission: Revisiting the largest nosocomial severe acute respiratory syndrome outbreak in Hong Kong. *J R Soc Interface* 8:699–710. <https://doi.org/10.1098/rsif.2010.0486>
- Zhou P, Fan H, Lan T, et al (2018) Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* 556:255–259. <https://doi.org/10.1038/s41586-018-0010-9>

- Zhou P, Yang X Lou, Wang XG, et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579:270–273.
<https://doi.org/10.1038/s41586-020-2012-7>
- Zhu N, Zhang D, Wang W, et al (2020a) A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 382:727–733.
<https://doi.org/10.1056/NEJMOA2001017>,
- Zhu N, Zhang D, Wang W, et al (2020b) A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine* 382:727–733.
<https://doi.org/10.1056/NEJMOA2001017>,
- Zukal J, Pikula J, Bandouchova H (2015) Bats as bioindicators of heavy metal pollution: history and prospect. *Mammalian Biology* 80:220–227.
<https://doi.org/10.1016/j.mambio.2015.01.001>
- Zumla A, Hui DS, Perlman S (2015) Middle East respiratory syndrome. *The Lancet* 386:995–1007. [https://doi.org/10.1016/S0140-6736\(15\)60454-8](https://doi.org/10.1016/S0140-6736(15)60454-8)

Conclusions

The first practical chapter, reporting the circulation of AlphaCoVs in cavernicolous bats from Portugal, allowed the documentation of these viruses for the first time in the country, thereby expanding the known geographic distribution of these lineages. The predominance of detections in faecal samples, compared with anal and buccal swabs, indicates that the gastrointestinal tract represents a primary site of viral replication and shedding, supporting an enteric–oral transmission route consistent with previous reports of environmental dissemination via bat excreta. Moreover, AlphaCoVs were identified across multiple bat species, including *M. myotis*, *M. schreibersii*, *R. mehelyi*, and *R. ferrumequinum*, underscoring a broad host range within Chiroptera and suggesting active interspecies transmission dynamics. Phylogenetic analysis further revealed a high degree of genetic similarity (93–100% identity) between the Portuguese strains and those previously described in bats from Bulgaria, Italy, and Spain, highlighting regional viral connectivity and potential transboundary dissemination pathways. Despite the use of a Coriolis air sampler, no viral RNA was detected in air samples, a limitation likely linked to methodological factors such as suboptimal sampling duration or the intrinsic characteristics of the cyclone-based device, pointing to the need for refined approaches in future airborne viral surveillance. Collectively, these findings reinforce the ecological role of bats as reservoirs of CoVs, shaped by their unique physiological adaptations, social structures, and large colony sizes that favour viral maintenance and intra-colony propagation, while also emphasizing the importance of continued virological surveillance to better understand CoV evolution, transmission, and zoonotic risk. The second practical chapter, reporting the detection of Alphacoronaviruses in tree- and crevice-dwelling bats from Portugal, expanded the known ecological niche of CoV circulation in the region. Although the prevalence detected (1.15%) was considerably lower than that observed in cave-dwelling bats (8.87%), this finding demonstrates that CoV presence is not limited to cavernicolous species. The study reinforces the existence of a monophyletic clade of CoVs circulating specifically within *Pipistrellus* spp. across Europe, with the *P. pipistrellus* strain showing close genetic relatedness to *Pedacovirus*, a subgenus with documented pathogenic potential in various animal hosts, thereby suggesting a degree of host specificity within the genus. Phylogenetic analysis also revealed a clear genetic divergence between the *Pipistrellus*-associated CoVs and those previously detected in cave-dwelling bats in Portugal, implying both ecological and evolutionary partitioning among bat communities and their roles in CoV maintenance and transmission. The close relationship

of the detected strains to pathogenic *Pedacovirus* underscores their zoonotic relevance and highlights the necessity of continuous surveillance of tree- and crevice-dwelling bats as part of broader CoV monitoring efforts. Finally, the genetic exchange observed within *P. pipistrellus* colonies emphasizes the role of bat migration in viral ecology, suggesting that movement between colonies may facilitate the dissemination of CoVs across geographically distant regions, with significant implications for understanding viral evolution, ecology, and epidemiology. The third practical chapter successfully identified and sequenced two near-complete AlphaCoV genomes from Portuguese bats, representing the first comprehensive genomic characterization of AlphaCoV from these species in Portugal and providing key insights into their genetic composition and evolutionary relationships. Metagenomic analysis of faecal samples further revealed a diverse virome, including viruses from the Picornaviridae, Adenoviridae (*Mastadenovirus*), and Parvoviridae (*Dependoparvoviridae*) families, highlighting the rich viral diversity harbored by cave-dwelling bats and underscoring their role as reservoirs of multiple viral taxa. Notably, one sample (F45) that initially tested negative for CoV by conventional RT-PCR was subsequently detected as positive through metagenomic sequencing, a discrepancy attributed to primer mismatches and illustrating the superior sensitivity and unbiased nature of metagenomic approaches for detecting novel or divergent viral strains. In silico protein docking analyses predicted strong binding affinities between the newly identified bat CoV spike protein and aminopeptidase N (APN) receptors from bats, pigs, and humans, indicating a potential for cross-species transmission and highlighting a zoonotic risk that warrants further experimental validation. Collectively, these findings emphasize the importance of a One Health approach to viral surveillance, demonstrating that continuous monitoring of bat populations is essential for understanding viral ecology, anticipating potential spillover events, and informing proactive public health strategies to mitigate the emergence of novel infectious diseases.

In conclusion, this doctoral research significantly underscores the intricate and multifaceted relationship between bats and CoVs, particularly within the Portuguese context. Bats serve as crucial natural reservoirs for a diverse range of CoVs, including those with significant zoonotic potential. The prevalence and genetic diversity of these viruses are influenced by a complex interplay of factors, including bat species, habitat characteristics (cave-dwelling versus crevice- and tree-dwelling), geographical location, and social behaviors such as colony size and migration patterns. While the faecal-oral route appears to be a primary mode of CoV transmission among bats, as evidenced by the high detection

rates

in stool samples, the role of airborne transmission remains an area requiring further investigation.

Current limitations in air sampling methodologies and the potential for low viral concentrations in the environment necessitate the development of more sensitive and standardized sampling strategies. Understanding the dynamics of virus aerosolization in bat habitats is crucial for a comprehensive assessment of transmission risks. Genetic analyses consistently reveal close relationships between CoV strains circulating in European bats and, in some cases, with strains from other continents like Asia. This highlights the potential for shared ecological niches to facilitate global viral transmission and emphasizes that viral evolution and spread are not solely dictated by bat species but also by environmental factors and geographical connectivity. The observed genetic partitioning between CoVs in different bat species and habitats further illustrates the complex ecological dynamics within bat communities.

Human activities, including habitat destruction, urbanization, and direct interactions with bat populations, can significantly impact the ecology of bat-borne viruses and increase the risk of spillover events. Therefore, continuous and robust surveillance programs are paramount. These programs should ideally be longitudinal, cover diverse bat species and habitats, and integrate advanced molecular techniques like viral metagenomics to identify novel viruses and understand their evolutionary dynamics. Furthermore, addressing public misconceptions about bats and their role in disease transmission is essential to foster conservation efforts and prevent harmful actions that could inadvertently exacerbate viral spread. In essence, a holistic approach that combines ecological, genetic, and epidemiological research with public health initiatives and conservation strategies is vital to mitigate the risks associated with emerging zoonotic diseases. Continued investment in understanding bat viromes and their complex interactions with their hosts and the environment will be key to predicting, preventing, and responding to future viral threats.