BatID 2025

International Symposium on the Infectious Diseases of Bats

Abstract Program

July 9–11, 2025
University of Chicago
batid.org

Session I Abstracts:

Bat immunology and within-host dynamics

Session I Plenary Talk

Demographic circumstances necessary to explain unique bat (immune) adaptations

Liliana M. Dávalos[1]
[1]Department of Ecology and Evolution, Stony Brook University, NY, USA

Co-Authors:
Rayfield K[1], Delamonica B[2], D'Andrea R[1], Lauterbur E[3]
[2]Graduate Program in Applied Math and Statistics, Stony Brook University, NY, USA;
[3]Department of Biology, University of Vermont.

Abstract:
Unique adaptations, many of them involving immunity, characterize bat genomes and powered flight has been the leading explanation for the emergence and maintenance of these modifications. Flight is, however, at best a partial explanation for immune adaptations because it is compatible with increasing fitness via fecundity—favoring early reproductive success—, survival—favoring successive iterations that lead to longevity—, or some combination of the two. Here we use a combination of demographic simulations and comparative genomics to investigate the evolution of the peculiar bat immunogenome. While simulations of alleles favoring fecundity or longevity reveal the slow population growth and density dependence that characterizes bats is essential to the fixation of 'pro-longevity' alleles, genes inferred to have adapted in ancestral bats differ markedly from those in birds and small hyperactive mammals such as shrews, but not much from those in naked mole rats. By exploring the role of demographic structure in reshaping selection early during bat evolution, our analyses reveal the evolutionary basis for bat innovation and illuminate the seemingly inextricable connections between modified immunity and longevity.

Session I Short Talks

Bat-inspired new targets to fight infectious diseases and beyond

Author list:

Ahn M[1,2], Wang LF[1]

[1]Programme in Emerging Infectious Disease, Duke-NUS Medical School, Singapore; [2]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

Abstract:

Bats are attracting the highest attention as a putative origin of SARS-CoV-2 responsible for the COVID-19 pandemic. Although less known to the public, bats have several unique features with high value to human health. These include asymptomatically hosting many viruses lethal to humans and their exceptionally long lifespan (and healthspan). Our mission is to uncover what makes them special. One of the key lessons learnt is that they have naturally evolved to dampen inflammation and protect themselves from inflammatory diseases, via controlling the inflammasome pathway tightly (Nat Micro 2019, PNAS 2020, Nature 2021, Cell 2023). This work has led to patents that could lead to a new class of anti-inflammatory drugs and were licensed to a bat biotech company for commercialization in 2023. The in-depth research into the natural mechanisms of disease resistance and healthy longevity in bats can help us fight human diseases including infectious diseases and beyond. In this presentation, I will share some of the key lessons we have learned from the unique biology of bats and my approaches to uncovering more bat-inspired novel targets to fight human diseases using the established ‘disease-free’ bat models.

Humoral immune responses to Ebolavirus antigens in little epauletted fruit bats (*Epomophorus labiatus*) show evidence of disease tolerance towards RNA-virus sensing pathways

Author list:

Field KA[1], Viquez-R L[1], Ejotre I[2], Mazzarulli T[1], Anguyo D[2], Powers L[3], Adiga K[2], Alumai A[2], Andama M[2], Sak A[1], Sutton J[3], Steinberg I[3], Repke M[3], Reeder D[1]

[1]Department of Biology, Bucknell University, Lewisburg, PA, USA; [2]Muni University, Arua, Uganda; [3]Prior Affiliation: Department of Biology, Bucknell University, Lewisburg, PA, USA.

Abstract:

Background.
To better understand the unique immune responses of bats to viral pathogens, we sought to characterize the humoral immune response of an African fruit bat, *Epomophorus labiatus*, to an ebolavirus (EBOV) vaccine in the presence of various adjuvants. We hypothesized that the strength of the immune response depends on which immune pathways have undergone selection for tolerance in this bat species. **Methods.** In Arua, Uganda, 67 *E. labiatus* bats were captured and housed in flight cages. The animals were divided into 5 treatment groups, balanced for sex and age: sham treated, EBOV virus-like particles (eVLPs) alone, LPS+eVLP, Poly(I:C)+eVLP, and CGAMP+eVLP. Twenty one days after prime immunization, each bat received a boost of eVLP only, except the sham group. Plasma was collected from each bat on days 0, 1, 7, 14, 21, 22, 28, and 35. **Results.** We found moderate increases in anti-GP titers after primary immunization with eVLPs, that depended on the co-administered adjuvant. The highest pre-boost titers were in bats 21 days after immunization with LPS+eVLP, which was significantly greater than eVLP alone (3.4 ± 1.6-fold higher, padj = 0.0224). Responses with cGAMP+eVLP were also larger than eVLP (padj = 0.1091). The Poly(I:C)+eVLP group did not show significant differences from eVLP alone (padj = 0.9827). After the booster, all four groups generated robust anti-GP titers. **Conclusions.** These results support our hypothesis that this bat species has dampened immune responses to RNA viruses during its coevolution with filoviruses. Unexpectedly, the use of cGAMP as an adjuvant was as effective as LPS, showing that there are STING-independent pathways active for this cytosolic DNA-sensing pathway. This further supports our overarching hypothesis that coevolution has specifically affected responses to RNA viruses, at least in this lineage of bats.

Filovirus – bat compatibility is spectral

Author list:

van Tol S[1], Munster VJ[1]

[1]Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Abstract:

Background. Despite extensive evidence implicating the Egyptian rousette bat as Marburg virus' as reservoir host, the reservoir hosts of the pathogenic filoviruses, Bundibugyo, Ebola, Sudan, and Taï Forest, remain unknown. The inability to identify the reservoir host(s) hampers our ability to understand and mitigate spillover. We previously found that Jamaican fruit bats' (JFB) competence for Ebola virus but not Marburg virus is driven partially by intrinsic differences in viral entry and type I interferon (IFN-I) signaling antagonism. **Methods.** Here, we inoculated JFBs with six additional filoviruses, Bundibugyo, Bombali, Lloviu, Reston, Sudan, and Taï forest virus to compare virus dissemination and shedding, and we executed in vitro experiments to evaluate entry and IFN-I antagonism.

Results. In JFBs, Bundibugyo, Bombali, and Sudan disseminated, Reston and Taï Forest were restricted to the site of inoculation, and Lloviu did not replicate. Despite their dissemination, Bundibugyo and Bombali were not detected in oral swabs at the magnitude or frequency of Ebola and Sudan. To evaluate, we infected JFB cells with vesicular stomatitis virus pseudotyped with filovirus glycoproteins. Unlike for Marburg, none of the other filoviruses enter less efficiently than Ebola virus. To assess filoviral antagonism of IFN-I signaling, we infected JFB cells with all eight filoviruses species for five days to monitor IFN-I responses. Increased expression of antiviral IFN-stimulated genes was only detected in cells infected with Marburg and Taï Forest. **Conclusions.** The results from these studies demonstrate that filovirus species-specific compatibility with JFBs that exists on a gradient. Entry does not contribute to the attenuation of Lloviu, Reston, or Taï Forest, and Taï Forest virus' inability to antagonize JFB IFN-I signaling may contribute to its inability to disseminate. Studies are in progress to investigate the potential roles of IFN-I induction, replication, transcription, and/or budding in the incompatibility of Lloviu and Reston virus with JFBs.

This research was supported in part by the Intramural Research Program of the NIH, NIAID.

An insectivorous bat model to investigate Middle East respiratory syndrome coronavirus infection dynamics, tissue tropism, antiviral immunity and persistence

Author List:

Baid K[1], Shrivastava S[1,2], Asavajaru A[1], Bhuinya A[1,2], Hurtado A[1], Falzarano D[1,2,3], Faure P[4], and **Banerjee A**[1,2,5,6,7]

[1]Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, SK, Canada. [2]Department of Veterinary Microbiology, University of Saskatchewan, SK, Canada. [3]Department of Biochemistry, Microbiology, and Immunology, University of Saskatchewan, SK, Canada. [4]Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton,ON, Canada. [5]Department of Biology, University of Waterloo, ON, Canada. [6]Department of Laboratory Medicine and Pathobiology, University of Toronto, ON, Canada. [7]Department of Biochemistry and Molecular Biology, University of British Columbia, BC, Canada.

Abstract:

Background. Some bat species are reservoir hosts of multiple zoonotic viruses, including coronaviruses, filoviruses, and paramyxoviruses to name a few. These viruses can cause severe disease in humans and livestock, but bats that are naturally or experimentally infected with these viruses do not show apparent clinical signs of disease. Vesper bats harbor betacoronaviruses and are speculated to be the ancestral reservoirs of Middle East respiratory syndrome coronavirus (MERS-CoV). Despite the existence of MERS-CoV-related viruses in vesper bats, the dynamics of MERS-CoV infection in these bats remain unknown. Here, we experimentally infected *Eptesicus fuscus* bat with MERS-CoV and monitored virus replication, shedding, clinical signs of disease, pathological outcomes of infection, and the development of innate and adaptive antiviral immunity in infected bats. **Methods**. We mock infected or infected *E. fuscus* bats with 10^5TCID50/ml of MERS-CoV. We sampled bats at 2-, 4-, 7- and 14-days post infection. We monitored weight loss and body temperature in all bats. We observed gross pathology in lungs and performed tissue and cellular-level screening for infection-mediated responses. We assessed antiviral response by qPCR and bulk RNA-sequencing. We assessed the development of virus specific neutralizing antibodies by performing micro-neutralization assays. We further assessed antigen specific B cell responses against MERS-CoV in infected and control bats. **Results.** None of the control or infected bats lost weight or developed elevated body temperature during the study, despite detection of virus in samples from infected bats. Infected bats upregulated transcripts for interferon-related genes indicating the presence of an active antiviral response. Gross pathology of lung tissue did not indicate apparent tissue damage in infected bats. **Conclusions.** Our study suggests that MERS-CoV can infect *E. fuscus* bats and infected bats mount an antiviral response without developing apparent signs of disease. *E. fuscus* bats can be used as a model to study MERS-CoV.

Adaptations to the MHC-I Antigen Processing Pathway in Bats

Author list:

Cresswell P[1], Spencer M[1]

[1]Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA

Abstract:

Background. The immune system of many bat species allows viral infection with minimal associated morbidity, generally attributed to modifications of innate immune mechanisms that cause morbidity and even mortality in other mammals. Bat adaptive immune mechanisms have been less well studied. Here we examine bat Major Histocompatibility Complex Class I (MHC-I) molecules, which consist of highly polymorphic glycoproteins (heavy chains) non-covalently associated with Beta-2-microglobulin (B2m). MHC-I binds peptides derived from cytosolic proteins and expresses them on the cell surface for screening by cytotoxic T lymphocytes (CTL). MHC-I molecules associated with virus-encoded peptides are recognized by CTL that kill virus-infected cells. Peptide binding occurs in the endoplasmic reticulum (ER) in a coordinated process involving the Peptide Loading Complex (PLC) that includes the dimeric Transporter associated with Antigen Processing (TAP), which translocates peptides from the cytosol into the ER, and a transmembrane glycoprotein called tapasin. MHC-I-B2m dimers associate with the PLC via interactions with tapasin, a 'peptide editor' that optimizes high affinity peptide binding. The lectin chaperone calreticulin, normally involved in quality control of glycoprotein folding, also contributes to PLC association by interacting with a glycan on the MHC-I heavy chain and with ERp57, a disulfide isomerase covalently linked to tapasin. **Methods.** 35S-methionine labeling of *Pteropus alecto* cell lines combined with cell biological and protein chemistry approaches and CRISPR/reconstitution analysis. **Results.** We find that the system, best defined in humans, is largely conserved in bats, with the major differences being that bat B2m possesses an N-linked glycan and has a relatively low affinity for the heavy chain. Human B2m outcompetes bat B2m for MHC-I association when expressed in bat cells. **Conclusions.** B2m glycosylation provides an additional quality control step that regulates B2m association with MHC-I heavy chains. Its low affinity may reduce the range of peptides displayed, downregulating inflammatory cytokine release.

Entry, replication and innate immunity evasion of BANAL-236, a SARS-CoV-2-related bat virus, in Rhinolophus cells

Author list:

Gracias S[1], Vendramini L[2], Moundib A[1], Miorin L[3], Rutkowska M[3], Cupic A[3], Juste J[4], Temmam S[2], Donati F[5], Martinez-Romero C[3], Marin A[3], Morel N[6], Schwartz O[7], Batra J[8], Krogan NJ[8], Roingeard P[9], Munier S[10], Garcia-Sastre A[3], Caval V[1], **Jouvenet N**[1]

[1]Institut Pasteur, CNRS UMR 3569, Virus Sensing and Signaling Unit, Paris, France; [2]Institut Pasteur, Université Paris Cité, Pathogen Discovery Laboratory, Paris, France; [3]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [4]Estación Biológica de Doñana, Avda, Seville, and CIBER Epidemiology and Public Health (CIBERESP), Madrid, Spain; [5]Institut Pasteur, National Reference Center for Respiratory Viruses, Paris, France.

Abstract:

Background. Asian Rhinolophus bats are the likely reservoir hosts of an ancestral SARS-CoV-2. Our understanding of the biology of SARS-CoV-2-related viruses in bat cells is very limited. **Methods.** We studied the replication of BANAL-236, the only bat SARS-CoV-2 relative isolated so far, in Rhinolophus cells, using a panel of biochemical, virological and electron microscopic approaches, as well as entry assays using pseudoviruses expressing the viral spike proteins (S). **Results.** BANAL-236 did not replicate in wild-type Rhinolophus cell lines. Entry assays revealed that efficient S-mediated entry of SARS-CoV-2, BANAL-236, and BANAL-52 required the expression of hACE2 and hTMPRSS2 into human and Rhinolophus cells. Expression of the Rhinolophus entry factors, alone or in combination, did not allow SARS-CoV-2 nor BANAL-236 entry in human cells. This suggests that the S proteins of BANAL-236 interact more efficiently with hACE2 than with rACE2. BANAL-236 and SARS-CoV-2 replicated efficiently in one clone of Rhinolophus cells expressing high level of hACE2 and hTMPRSS2. Despite an efficient replication of BANAL-236 in Rhinolophus and in human cells, no induction of interferon-stimulated genes was detected. Since these cells are immunocompetent, this suggests that the virus has evolved potent mechanisms to evade the interferon response in cells derived from its host, as well as in human cells, which has implications for zoonosis. **Conclusion.** The Rhinolophus cellular model that we have generated has enabled the first characterization of the molecular interactions between a bat coronavirus and its reservoir species and will facilitate further investigations that could help understand its zoonotic potential.

From ancient duplication break to new functionality?

Author list:

Pursell T[1,2], Reers AB[3], Mikelov A[1], Kotagiri P[4], Lam B[1], Ellison JA[5], Boyd SB[1,6], **Frank HK[3]**

Affiliations: [1]Department of Pathology, Stanford University, Stanford, CA 94305, [2]Department of Microbiology & Immunology, Stanford University, Stanford, CA 94305, [3]School of Science & Engineering, Tulane University, New Orleans, LA 70118, [4]Department of Immunology and Pathology, Monash University, Melbourne, Australia, [5]Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA 30329, [6]Sean N. Parker Center for Allergy and Immunology Research, Stanford University, Stanford, CA 94305

Abstract:

Background. Bats are major reservoirs of viruses that can be transmitted to humans in zoonotic outbreaks. Antibody-mediated immunity plays an important role in shaping viral evolution and immune evasion but is understudied in bats. All known mammals have a single immunoglobulin heavy chain (IgH) gene locus, and up to two light chain loci. **Results.** We have identified dual, complete and functional IgH loci on separate chromosomes in 11 bat species, highlighting extreme variation of immunogenetic architecture in Order Chiroptera. Single-cell transcriptomes confirm functional rearrangement and expression of both loci, but with different mechanisms for generating antibody diversity and function. **Conclusions.** These results define a novel mammalian immune adaptation, providing a foundation for analysis of humoral immunity in bats.

Deciphering the role of the Dicer protein in bat antiviral immunity

Author list:

Gaucherand L[1], Marie H[1], Cremaschi J[1], Maesen S[2], Etienne L[2], Pfeffer S[1]

[1]Institut de Biologie Moléculaire et Cellulaire (IBMC), CNRS UPR9002, Université de Strasbourg, Strasbourg, France; [2]Centre International de Recherche en Infectiologie (CIRI), CNRS UMR5308 – Inserm U1111, ENS Lyon, Université Lyon 1, Lyon, France.

Abstract:

Background. Bats are reservoirs for many viruses that can cause severe pathologies in humans. Yet, they show minimal clinical symptoms, suggesting a unique immune system that allows them to tolerate viruses. The mechanism behind this tolerance is not fully understood. A study found that the Dicer protein plays an antiviral role in *Pteropus alecto* cells through its RNA interference activity. While Dicer is mostly known for maturing micro (mi)RNAs, we found that Dicer also modulates innate immunity in humans. Whether Dicer participates in bat viral tolerance through this immunomodulatory role is unknown. **Methods.** We started characterizing Dicer from the *Myotis myotis* species by immunofluorescence microscopy, co-immunoprecipitation, and by expressing it in a human cell line knock-out for Dicer (NoDice). Through inactivation assays, we are also investigating its activity against Sindbis virus (SINV). **Results.** Dicer is well conserved at the protein level across bats and mammals. Consistently, expressing *M. myotis* Dicer in NoDice cells rescued miRNA maturation. In *M. myotis* nasal epithelial cells, Dicer was diffuse in the cytoplasm in mock conditions but relocalized to large puncta upon SINV infection. Dicer also co-localized and co-immunoprecipitated with the stress granule marker G3BP1. Interestingly, *M. myotis* Dicer did not form these puncta when expressed in infected human NoDice cells, suggesting that specific factors of *M. myotis* cells are needed for relocalization. Finally, knocking down Dicer in *M. myotis* cells protects them from SINV infection, suggesting a pro-viral role. **Conclusion.** While the canonical roles of Dicer seem conserved in M. myotis cells, Dicer plays an additional pro-viral role, potentially through its immunomodulatory function. This function may be linked to its accumulation in large puncta during infection. We are currently investigating whether these puncta are stress granules or viral factories, what the factors for relocalization are, and the consequences of Dicer knock-down on innate immunity.

Interferon beta specificity in humans and bats contributes to differences in viral replication and tolerance

Author list:

Quintela-Tizon RM[1,2], Côté J[3], Gonzalez V[1,2], Lanke V[4], Wang J[1], Wang LF[5], Doxey AC[6], Gobeil SMC[3], Banerjee A[1,2,6,7,8]

[1]Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada; [2]Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada; [3]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC, Canada; [4]Department of Computer Science, University of Saskatchewan, Saskatoon, SK, Canada; [5]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore; [6]Department of Biology, University of Waterloo, Waterloo, ON, Canada; [7]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [8]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

Abstract:

Background. Bats are ancestral hosts of multiple zoonotic viruses, including betacoronaviruses (βCoVs) that cause severe disease and death in humans and livestock. Conversely, bats infected with these viruses do not show clinical signs of disease, making them a fascinating model to study the evolution of virus-host interactions. The innate immune response is the first line of defense against viral infections. Type I interferons (IFNs) are among the first cytokines to be released when an infection is detected in vertebrates. IFNs bind to the interferon-α/β receptor (IFNAR1/2) in host cells and induce the production of antiviral Interferon Stimulated Genes (ISGs). βCoVs have evolved to impair type I IFN activity in humans, increasing our vulnerability to infections. Little is known about how type I IFNs signal in bat cells and whether bats have evolved more efficient processes to better tolerate viral infections. **Methods.** We have identified that wildtype bat IFNβ do not protect human cells and vice versa, suggesting species-specific mechanisms. Based on computational modelling and positive selection analyses of IFNβ sequences from several mammals, we have produced mutant human and bat (*Eptesicus fuscus* and *Pteropus alecto*) recombinant IFNβ to test their antiviral potency against βCoVs. **Results.** Infection studies show that IFNβ with two specific point mutations have lower antiviral capacity compared to wildtype IFNβ in species-matched cells for both humans and bat species. Mutated IFNβ cannot effectively phosphorylate STAT transcription factors, suggesting a failure to prime IFNAR1/2. These results are supported by structural modelling showing significant changes on binding interface properties for mutated IFNβ-IFNAR1/2. **Conclusions.** The observed differential antiviral protection suggests that our identified amino acid residues are key determinants of IFNβ-mediated protection. Our study identifies remarkable species-specific adaptation of IFNβ and downstream antiviral processes which will inform basic and translational science for the development of IFNβ antiviral therapies for humans.

Viral relapse is driven by the immunological costs of spring migration in wild bats

Author list:

Becker DJ[1], Dyer KE[1], Allira M[1], Hightower MG[1], Olbrys BL[1], Demory B[1], Lock LR[1], Vicente-Santos A[1], Neely BA[2]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]Chemical Sciences Division, National Institute of Standards and Technology, Charleston, SC, USA.

Abstract:

Background. Efforts to understand the ecological drivers of bat–virus interactions have focused on birth pulses, metapopulation dynamics, and reproductive or nutritional stress. However, long-distance migration remains understudied as a mechanism of viral persistence. In taxa such as birds, seasonal migrations often carry energetic costs that suppress immunity in ways that increase susceptibility or allow chronic infections to reactivate. If and how such within-host processes operate in bats remains unknown. **Methods.** Mexican free-tailed bats often undertake migrations of over 1,000 kilometers between wintering grounds in Mexico and large maternity roosts in the southwestern USA. During 2022 and 2023, we sampled over 500 individuals from a fully migratory population in western Oklahoma from their spring arrival in March through fall departure in October. We used hematology and, for a subset of bats, plasma proteomics to quantify physiological stress and immune proteins, using generalized additive models (GAMs) to test for seasonal trends linked to migration and reproduction. We also used PCR and Sanger sequencing to characterize herpesvirus infections in oral swab samples, followed by GAMs and cross-correlation analyses to assess seasonality in viral prevalence and seasonal synchrony with physiological and immunological data. **Results.** We identified significant seasonality in neutrophil-to-lymphocyte ratios and plasma proteins that support high energetic costs and possible immunosuppression during spring but not fall migration. Herpesvirus prevalence was also greatest following spring migration, declining throughout the reproductive period and into fall migration. As a result, migratory bat physiology and immunology were highly synchronized with herpesvirus infection dynamics. **Conclusion.** Our findings suggest spring migration carries distinct physiological and immunological costs in bats, likely due to energetic expenses of long-distance movement coupled with early pregnancy. Further, our herpesvirus data support the idea of migration-induced relapse of chronic infections, which we suggest may be an important driver in many other bat–pathogen systems.

Testing of Bat STING Orthologs Reveals Species Specific Differences in STING Functionality

Author list:

Pierson L[1], Sriram A[1], Matreyek K[1], Bruchez A[1]

[1]Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

Abstract:

Background. Bat immunology research has grown exponentially since the COVID-19 pandemic, leading to increased genome sequencing and identification of bat orthologs for essential immune proteins. This expansion has outpaced our ability to functionally test these protein variants. Published studies suggest that bat STINGs (bSTING) have muted transcriptional response due to loss of a phosphosite, yet normal autophagy induction when compared to human STING (hSTING). Reversion of this mutation did not fully rescue all bSTINGs, suggesting other mutations modulate activity. Comprehensive studies into bSTINGs functionality are needed to better understand differences between species.

Methods. We have developed three assays that measure STING induced transcription, autophagosome formation, and cell death. Our assays use specially engineered HEK293T cells that integrate plasmids at the same site ensuring consistent expression of STING orthologs between cell lines. Transcriptional activation is measured with a luciferase based IRF3-dependent reporter. Autophagosome formation is quantified by measuring puncta in cells expressing GFP-LC3b. We measure cell death by assaying for reduced metabolism in the presence of increasing amounts of diABZI, a potent synthetic agonist. **Results.** Three bat orthologs, E. fuscus, P. alecto, and R. aegyptiacus, activated the IRF3-dependent reporter slightly less than WT hSTING and slightly more than phosphomutant hSTING despite having the phosphosite mutation. The bSTINGs activated autophagy at a lower rate compared to human STING and had a differential response depending on the agonist used. Induction of cell death only happened with P. alecto, which was less robust than hSTING. **Conclusion.** Our findings suggest bSTING orthologs have differential magnitudes of pathway activation that vary depending on agonist. To understand how bSTING orthologs functionality evolved between species we plan to test 23 orthologs. As our ability to scale protein variant screening increases, we will gain a deeper understanding of how bats evolved immune adaptations to allow for tolerance of infections.

Retroviral restriction in bats: characterization of a specific Pteropid post-entry restriction to primate lentiviruses and identification of *P. alecto* TRIM5 as a functional antiretroviral restriction factor

Author list:

Poeschla EM[1], Morrison JH[1]

[1]Division of Infectious Diseases, University of Colorado School of Medicine, Aurora, CO, USA.

Abstract:

Background. Bat genomes tell a tale of many past exogenous retroviral infections, and infectious gammaretroviruses are known to currently circulate in the Black Flying Fox *Pteropus alecto*. **Methods.** Lentiviral, foamy virus, and gammaretroviral life cycle assays in *P. alecto* and other mammalian cells, mutant virus/vector testing, cyclosporine A use, cloning of restriction factors, RNAi, immunofluorescence, *P. alecto* cDNA library construction and testing. **Results.** We have assembled evidence for a specific post-entry restriction in *P. alecto* that blocks the lentivirus genus of retroviruses. Primate lentiviruses including HIV-1 were potently blocked post-entry. This has a hallmark feature of species- and virus-specific restriction in that neither the non-primate lentiviruses nor foamy retroviruses were restricted. Interspecies heterokaryons revealed the block is dominant. Some aspects suggested potential TRIM5 or MX2 protein restriction: post-entry action, cyclosporine A sensitivity, reversal by HIV-1 capsid cyclophilin A (CypA) binding loop substitutions (HIV-1 CA P90A, G89A), partial reversal by HIV-1 CA N74D. Viral nuclear import was significantly reduced, which was substantially rescued by cyclosporine A treatment. Saturation with HIV-1 virus-like particles (VLPs) did not relieve the restriction at all, which contrasts with known TRIM5alpha or TRIMCyp restrictions. Consistent with that, *P. alecto* TRIM5 was inactive against HIV-1. It is, however, a functional antiretroviral restriction factor as it specifically blocked the gammaretrovirus N-MLV (and did not block B-MLV). *P. alecto* MX2 had anti-HIV activity despite major divergence in a N-terminal motif. However, MX2 did not quantitatively account for the restriction and was independent of and synergistic with an additional CypA-dependent restriction. We have gone on to construct a *P. alecto* cDNA library, introduced it into non-restricting cells, and sorted for resistance to HIV-1. Responsible genes are being characterized. **Conclusions.** These results show specific restriction to primate lentiviruses in the Pteropodidae and conservation of TRIM5 antiretroviral activity in Chiroptera.

Mapping SARS-CoV-2 Host Networks: A Comparative Analysis in Bats and Humans

Author list:

Batra J[1,2,3], Rutkowska M[4], Adavikolanu R[1,2,3], Zhou Y[1,2,3], Malpotra S[1,2,3], Anand D[1,2,3], Moen J[1,2,3], Ye C[5], Martinez-Sobrido L[5], Miorin L[4], Garcia-Sastre A[4], Krogan NJ[1,2,3]

[1]The J David Gladstone Institutes, San Francisco, CA, USA; [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA; [3]Quantitative Biosciences Institute, University of California, San Francisco, CA, USA; [4]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [5]Texas Biomedical Research Institute, San Antonio, TX, USA.

Abstract:

Background. Bats are considered the likely natural reservoir for coronaviruses, including SARS-CoV-2, which has caused severe disease in humans. While these viruses can be lethal to humans, bats appear to tolerate infection without severe disease, though the underlying mechanisms remain poorly understood. Close relatives of SARS-CoV-2, such as BatCoV RaTG13, have been identified in Rhinolophus bats, with RaTG13 sharing 96.2% genome sequence similarity to SARS-CoV-2. We hypothesize that differences in host-specific protein interactions and signaling pathways contribute to the contrasting infection outcomes between bats and humans. **Methods.** To investigate these differences, we employed affinity purification mass spectrometry (AP-MS) to identify viral protein interactions in bat and human cells. **Results.** Our findings revealed both species-specific and conserved interactions, suggesting critical roles for these host proteins in virus infection. Our recent findings showed that SARS-CoV-2 Orf9b is implicated in innate immune evasion by targeting mitochondrial protein Tom70. Notably, RaTG13 Orf9b demonstrated weaker binding to Tom70 and stronger binding to MTARC2, indicating potential differences in immune evasion strategies. Structural and mutational analyses further identified residue 72 as a key determinant of these interactions, providing insights into the mechanisms of immune regulation and virus-host adaptation. Additionally, we identified several novel interactions unique to either bat or human cells, revealing previously unrecognized mechanisms of viral manipulation and host response. **Conclusion.** These insights will be instrumental in enhancing our understanding of antiviral mechanisms in bats and the processes underlying cross-species transmission, ultimately guiding the development of targeted antiviral strategies.

Session 1 Lightning Talks

Exploring the proteomic landscape of *Eptesicus fuscus* cells following Rio Bravo Virus infection

Author list:

Donaire-Carpio S[1], Bonaventure B[2], Johnson J[2], Caval V[1], Jouvenet N[1]

[1]Department of Virology, Institut Pasteur, Paris, France; [2]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Abstract:

Background. Bats are important reservoirs for zoonotic viruses, as they possess unique, yet poorly understood, mechanisms for tolerating infections. To explore the molecular interactions between bat-borne viruses and their hosts, we studied the proteomic landscape of *Eptesicus fuscus* cells after infection with Rio Bravo Virus (RBV), a bat-borne Orthoflavivirus with zoonotic potential. This model is valuable as it closely mirrors natural virus-host systems. **Methods.** We optimized conditions to infect *Eptesicus fuscus* kidney cells (Efk3b) with RBV at different time points, using flow-cytometry to detect the viral envelope. Cells were collected at early and late time points, and protein content was analyzed by mass spectrometry. Data were analyzed to identify dysregulated cellular pathways and potential antiviral effectors. Additionally, we produced lentiviral vectors encoding all RBV protein sequences with a C-terminal 3X-FLAG tag in an inducible system, which were then used to transduce Efk3b cells. Upon doxycycline induction, RBV protein expression was validated, and FLAG-tag affinity purification was performed. Significantly enriched proteins in immunoprecipitates were identified by mass spectrometry in combination with the MiST and SAINTexpress algorithms to construct a protein-protein interaction network between RBV and its host. **Results.** This study aims to enhance understanding of virus-bat interactions by combining two mass spectrometry approaches. Global proteomics at various infection stages helped assess how RBV affects the *E. fuscus* proteome through the upregulation of host factors, and the degradation of proteins, potentially induced by RBV. Additionally, the RBV protein-protein interaction network identified bat cellular partners that we are currently characterizing, offering insights into the viral-bat protein interplay. A comparison with human cells may reveal specific bat factors involved in viral infections. **Conclusions.** Uncovering the unique features of bat antiviral responses will improve understanding of bat-virus interactions and provide insights into the innate immune mechanisms of these important viral reservoirs.

MENTOR - Multiplex Embedding of Networks for Team-Based Omics Research analysis identifies potential novel mechanisms for regulating anti-viral immunity in the cave nectar bat

Author list:

Carr W[1], Townsend A[2], Garcia A[1], Jacobson D[2]

[1]Department of Biology, Medgar Evers College, City University of New York, Brooklyn, NY, USA; [2]Computational and Predictive Biology, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Abstract:

Background. Bats exhibit unique variants of innate immune genes, yet the mechanisms underlying their distinct responses to viral infections remain unclear. In Immunity (2022), Gamage et al. conducted a single-cell transcriptomic analysis of leukocytes in Eonycteris spelaea, identifying differentially expressed (DE) genes linked to antiviral responses and immune activation. We extended their findings using MENTOR (Multiplex Embedding of Networks for Team-Based Omics Research) to identify functionally interconnected gene clades, revealing conserved and cell-type-specific immune signatures. **Methods.** MENTOR systematically partitions gene sets into functionally interconnected clades using a Random Walk with Restart (RWR) approach on multiplex networks that integrate multiple layers of biological evidence. From the 13,111 DE genes identified by Gamage et al., we applied a log2(fold-change) threshold of 0.5 and adjusted p-value ≤ 0.01, refining the dataset to 1,134 unique DE genes. These genes were mapped to human orthologs and analyzed using a multiplex network incorporating HumanNetv3 and iRF-LOOP-derived GTEx lung single-cell RNA sequencing data, enhancing translational relevance. A dendrogram visualization delineated functionally interconnected clades, while a heatmap revealed both conserved and cell-type-specific expression patterns. **Results.** MENTOR identified 77 functionally interconnected clades within the 1,134 DE genes. Seventeen clades confirmed known innate immune activation and antiviral response signatures. Notably, novel apoptosis signatures implicated BCL3 inhibition of NF-κB, with classical monocytes and neutrophils exhibiting anti-apoptotic profiles. ISGylation and autophagy of pattern recognition receptors emerged as regulators of inflammation, and differential expression of bat APOBEC and HLA-C orthologs suggested viral immune evasion strategies. **Conclusions.** This approach generated testable hypotheses regarding BCL3-driven apoptosis and ISGylation-mediated autophagy as potential mechanisms underlying viral tolerance in bats. These findings highlight new pathways in immune modulation, inflammation control, and viral immune evasion, offering insights relevant to cross-species immune adaptations.

Early innate immune response and evolution of a SARS-CoV-2 furin cleavage site inactive variant in bat cells

Author list:

Baid K[1], Shrivastava S[1,2], Luc J[3], Richard D[4], Aguiar JA[3], Machado Y[5,6,7], Aicher SM[8], Siwak KC[9], LeBlanc EV[9], Khatooni Z[1], Lokugamage KG[10,11,12], Vu MN[101112#] , Morgan A[10,11,12], Bhuinya A[1,2], Chiok KR[1], Nguyen HT[1], Stacey HD[13,14,15], Scruten E[1], Prysliak T[1], Yim W[16,17], McArthur AG[13,15], Miller MS[13,14,15], Wilson HL[12], Capellini T[4], Faure PA[18], Liu Q[1,2], Mubareka S[16,17], Menachery VD[10,11,12#], Mossman K[13,14,19], Müller MA[20,21], Drosten C[20,21], Colpitts CC[9], Jouvenet N[8], Overall CM[5,6,7], Doxey AC[3,19,*], Banerjee A[1,2,3,18,22,*]

[1]Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada; [2]Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; [3]Department of Biology, University of Waterloo, Waterloo, ON, Canada; [4]Department of Human Evolutionary Biology, Harvard University, Cambridge, MA, USA; [5]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada; [6]Centre for Blood Research, Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada; [7]Department of Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia, Vancouver, BC, Canada; [8]Institut Pasteur, Université de Paris, CNRS UMR3569, Virus Sensing and Signaling Unit, Paris, France; [9]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; [10]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA; [11]Institute for Human Infection and Immunity, University of Texas Medical Branch, Galveston, TX, USA; [12]Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, TX, USA; [13]Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, ON, Canada; [14]McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada; [15]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada; [16]Sunnybrook Research Institute, Toronto, ON, Canada; [17]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [18]Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, ON, Canada; [19]Department of Medicine, McMaster University, Hamilton, ON, Canada; [20]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Virology, Berlin, Germany; [21]German Center for Infection Research (DZIF), partner site Charité, Berlin, Germany; [22]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

*Correspondence: acdoxey@uwaterloo.ca (A.C.D.) and arinjay.banerjee@usask.ca (A.B.)

#Current address: Emory University School of Medicine, Atlanta, GA

Abstract:

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the largest known coronavirus pandemic to date and is believed to have evolved in insectivorous bats, likely from the genus Rhinolophus. SARS-CoV-2-related viruses circulate widely in different bat species across South-East Asia with zoonotic potential. Yet, little is known about the ability of bats to tolerate viral infections that cause severe disease in spillover mammalian species, such as humans. **Methods.** We investigated SARS-CoV-2-host interactions using human lung and Eptesicus fuscus-derived cells, an insectivorous bat species. Using transcriptomic and proteomic assays, we elucidated global host responses in human and bat cells infected with SARS-CoV-2. Furthermore, through computational modelling and docking studies as well as biochemical assays, we characterized a novel R685P mutation within the furin cleavage site (FCS) of the SARS-CoV-2 spike protein that was naturally selected for in infected *E. fuscus* cells. **Results.** Our transcriptomic and proteomic data demonstrate that bat cells mount a more robust and early antiviral response but elicit a dampened pro-inflammatory response upon SARS-CoV-2 infection relative to human cells. Data from our computational modelling, docking, and mechanistic molecular studies indicate that the R685P mutation in SARS-CoV-2 spike abolished furin cleavage activity and enhanced the entry of R685P spike-pseudotyped lentiviruses via the TMPRSS2-independent endosomal route. Subsequently, we observed that SARS-CoV-2 R685P mutant virus replicated more efficiently in cells that have been shown to express low levels of TMPRSS2 compared to cells with higher TMPRSS2 expression. **Conclusion.** *E. fuscus* cells have evolved a differential antiviral immune response to SARS-CoV-2, likely to mitigate immunopathology that is observed in human patients with severe COVID-19. In addition, our study sheds further light on the evolution of sarbecoviruses in its reservoir host and extends molecular evidence to data from field studies where SARS-CoV-2-related viruses discovered in wild-caught bats lack an intact FCS.

Bat antiviral effectors: from comparative transcriptomic analysis to functional studies

Author list:

Caval V[1], Aicher S-M[2], Donaire Carpio S[1], Gracias S[1], Mougari S[3], Kornobis E[4], Jouvenet N[1]

[1]Institut Pasteur, CNRS UMR3569, Virus Sensing and Signaling Unit, Paris, France; [2]Faculty of Medicine, University of Toronto, Department of Laboratory Medicine and Pathobiology, Mubareka Lab, Toronto, Canada; [3]Institut Pasteur, Lyssavirus Epidemiology and Neuropathology Unit, Paris, France; [4]Institut Pasteur, CNRS USR3756, Bioinformatics and Biostatistics HUB, Paris, France.

Abstract:

Background. Bats are known to harbor many viruses without exhibiting symptoms of illness. This exceptional immune tolerance is largely attributed to their specialized immune system, which combines robust antiviral defenses with mechanisms preventing excessive inflammation. In this study, we chose to functionally investigate bat antiviral effectors, identified through transcriptomic analysis on a large collection of bat cell lines. **Methods**. Six bat cell lines (derived from *Myotis myotis*, *Eptesicus fuscus*, *Nyctalus noctula*, *Eidolon helvum*, *Rhinolophus ferrumequinum*, and *Rhinolophus alcyone*) were transfected with poly-I:C, a synthetic RNA that mimics viral RNA, and differentially expressed genes (DEG) were mapped using RNA sequencing. Comparative analysis of DEGs across bat cell lines allowed the identification of bat-specific and species-specific DEGs. Fifteen *Rhinolophus* genes were cloned into a lentiviral vector to derive *Rhinolophus ferrumequinum* (RhiFlu) and human (Caco-2) cells overexpressing *R. ferrumequinum* putative antiviral effectors. Those cells were infected with viruses known to circulate in *Rhinolophus* bats: European Bat 1 Lyssavirus (EBLV-1), Japanese Encephalitis Virus (JEV), and SARS-Cov2-related BANAL-236 Sarbecovirus. Viral replication was evaluated using staining of viral proteins by flow cytometry analysis and titration. **Results.** Functional studies identified bat antiviral effectors with broad antiviral activities, such as the RNA endonuclease ENDOV (rENDOV) or the transcription factor FoxS1 (rFoxS1). The E3 ubiquitin ligase rRNF4 also exhibited a potent antiviral activity against BANAL-236, which was dependent on its enzymatic function. Further mechanistics studies are ongoing to decipher RNF4 antiviral activity, including its ability to degrade viral proteins. **Conclusions.** Uncovering unique features of bat antiviral program will provide a better understanding of the molecular interplays between bats and viruses, as well as insights into the innate immune responses of these important viral reservoirs.

IGH reference sequences enable investigations and reveal insights into bat-specific immunity

Author list:

Reers AB[1], Zhan S[2], Pursell T[3–4], Reasoner C[2], Hodges N[2], Lama TM[5], Schountz T[2], Frank HK[1]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA; [2]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA; [3]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA; [4]Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA, USA; [5]Department of Biological Sciences, Smith College, Northampton, MA, USA.

Abstract:

Background. Recent comparative genomic, transcriptomic, and serological studies have made clear that bats mount a humoral response to infection involving the production of antibodies by B cells; however, the specifics of these responses remain poorly understood. Furthermore, a lack of basic knowledge of the composition of the germline immunoglobulin (Ig) loci has hampered more targeted studies of humoral immunity. **Methods.** To better understand the genetic basis of B cell responses in bats and provide a critical resource for the continued study of bat humoral immunity, we generated a highly-contiguous Jamaican fruit bat (Artibeus jamaicensis) genome assembly that captured the difficult-to-sequence Ig heavy chain (IGH) locus on one contig and the Ig light chain (IGL) locus on two contigs, allowing for detailed annotation of Ig genes and associated regulatory regions. With the IGH reference, we assessed baseline characteristics of the expressed B cell receptor repertoire in A. jamaicensis. **Results.** The bat germline shared many structures and features described in human Ig loci. However, some features were unique to A. jamaicensis, including an expansion of cysteine-rich IGHV genes. In the expressed BCR repertoire, we found an enrichment in the usage of IGHV3 and IGHV4 genes. Somatic hypermutation was increased in bats infected with rabies compared to uninfected bats, however overall levels were lower than those observed in humans. Additionally, compared to humans, A. jamaicensis had shorter CDRH3s. **Conclusions.** Our results demonstrate that while germline immunoglobulin loci are largely conserved between bats and humans, distinct differences exist in the bat germline, highlighting the need for more detailed genetic characterization of these mammals.

Ecological and evolutionary predictors of bat immunity

Author list:

Vicente-Santos A[1], Cummings CA[1], Czirják GÁ[2], Ledezma-Campos P[3], Jordán L[2], Lock LR[1], Carrera JE[4–5], Dyer KE[1], Altizer S[6–7], Streicker DG[8–9], Fenton MB[10], Simmons NB[11], Gillespie TR[12–14], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, USA; [2]Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany; [3]School of Biology, University of Costa Rica, San José, Costa Rica; [4]Departamento de Mastozoología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; [5]Programa de Conservación de Murciélagos de Perú, Lima, Peru; [6]Odum School of Ecology, University of Georgia, Athens, GA, USA; [7]Center for the Ecology of Infectious Diseases, University of Georgia, Athens, GA, USA; [8]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom; [9]Medical Research Council–University of Glasgow Centre for Virus Research, Glasgow, United Kingdom; [10]Department of Biology, University of Western Ontario, London, Ontario, Canada; [11]Division of Vertebrate Zoology (Mammalogy), American Museum of Natural History, New York, USA; [12]Program in Population Biology, Ecology, and Evolution, Emory University, Atlanta, USA; [13]Department of Environmental Sciences, Emory University, Atlanta, USA; [14]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, USA

Abstract:

Background. Bat immunology is an emerging field critical for understanding zoonotic spillover risks. However, comparative studies are limited by bats' exceptional ecological diversity and deep evolutionary history, making generalizations difficult. There is a pressing need to identify the ecological and evolutionary factors shaping bat immune diversity to better predict zoonotic reservoirs and pathogen dynamics. **Methods**. We analyzed immune data from 2,883 individual bats representing 46 species and six families sampled across the USA, Belize, Costa Rica, and Peru between 2014 and 2024. Immune metrics included total white blood cell counts (n=2,850), immunoglobulin G (n=1,272) and lysozyme concentrations (n=1,254), and bacterial killing ability (n=375). We compiled ecological traits (diet, roosting behavior, distribution, migration, co-roosting) from PanTHERIA, COMBINE, and published sources, and we quantified reported pathogen exposure using viral richness data from the VIRION database. Phylogenetic generalized linear models assessed the relative importance of evolutionary history and ecology in predicting immune variation. Causal mediation analyses evaluated whether pathogen exposure mediates ecological effects on immunity. **Results**. We found strong phylogenetic signal across all immune markers, indicating evolutionary history is a major driver of bat immune variation. Ecological traits were significant predictors only for cellular immunity: migration distance, habitat breadth, and co-roosting occurrence were positively associated with lymphocyte proportions, and

wider species distributions correlated with increased total white blood cell counts. Causal mediation analysis showed pathogen exposure explained up to 28% of the distribution range effect on total white blood cells. **Conclusions.** Bat immune responses are largely shaped by phylogeny, but ecological factors, likely linked to pathogen exposure risk, also influence cellular immunity. We are expanding species and geographic representation by harvesting additional immunological data from the literature, with updated analyses planned. Broader sampling will further clarify bat immune diversity and its role in zoonotic risk.

Session 2 Abstracts:

Bat Pathogen Evolution

Session 2 Plenary Talk

Paramyxoviruses from bats: receptor tropism and pathogenesis

Benhur Lee[1]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA

Abstract:
Bats host diverse paramyxoviruses from at least five major genera. Successful spillover into humans requires compatibility between viral attachment proteins and their cognate receptors. We evaluate receptor usage in the zoonotic potential of bat-borne henipaviruses, morbilliviruses, orthorubulaviruses, and pararubulaviruses, while emphasizing the importance of postentry restrictions in preventing spillover. Characterization of newly identified paramyxoviruses has improved our understanding of spillover determinants, enabling better forecasts of which bat-borne viruses pose the greatest risk for cross-species transmission.

Session 2 Short Talks

Ecological drivers of cross-species coronavirus transmission in bats

Author list:

Cords O[1], Wells H[2], Anthony S[2], Evans T[1,3], PREDICT Consortium[4], Johnson C[1]

[1]Epicenter for Disease Dynamics, One Health Institute, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [2]Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [3]Department of Integrative Biology, University of California, Berkeley, Berkeley, CA, USA, [4]PREDICT Consortium (https://ohi.vetmed.ucdavis.edu/programs-projects/predict-project/authorship)

Abstract:

Background. A number of coronaviruses with bats as suspected or established evolutionary hosts have crossed species barriers and established endemic transmission cycles in humans. Evidence suggests that cross-species transmission events are important in shaping coronavirus adaptation, host range, and ecological niche, yet these evolutionary processes remain poorly understood. **Methods.** Using phylogeographic and epidemiologic methods, we examined host ecological, phylogenetic, and geographic factors that facilitated associations between coronaviruses and their bat hosts by assessing 72 coronaviruses from three different continents. For each virus, we inferred the evolutionary history of cross-species transmission events using a discrete Bayesian phylogeographic approach. Bayes factor support values were calculated to quantify the rate of cross-species transmission for each species pair in the tree. Generalized linear mixed models were then used to test associations between cross-species transmission and ecological host traits, geographic range size and overlap, and host phylogenetic relatedness. **Results.** 32/72 (44.4%) bat-borne coronaviruses were shared between at least two bat species and 20/32 (62.5%) of these were found in more than two genera. We performed Bayesian phylogeographic and epidemiological analysis for a subset of 10 alpha- and 10 beta-coronaviruses found in at least three species and find broad evidence that cross-species transmission of coronaviruses is more likely to have occurred between closely related species (log pairwise identity OR = 2.77, 95% CI = 1.28-17.29, P = 0.05). Cross-species transmission was also positively associated with species that have been described to be co-roosting (OR = 8.24, 95% CI = 1.75-49.40, P = 0.01). **Conclusions.** We find viral sharing to be relatively common among bat species for the coronaviruses examined. Host genetic relatedness and ecological roosting behavior facilitate cross-species transmission. These findings can inform predictive modeling efforts aimed at assessing transmission dynamics in bat hosts and increase our understanding of evolutionary and ecological processes underpinning spillover risk.

Despite the Increasing Number of Studies, Bat Diversity is Underrepresented in Viral Experimental Infection Studies

Author list:

Viquez-R L[1], Mazzarulli T[1], Sak A[1], Field K[1], Reeder D[1]

[1]Department of Biology, Bucknell University, Lewisburg, USA

Abstract:

Background. Experimental infection studies (EIS) are crucial for understanding viral pathogenic potential and public health impact. Bats are important study species for EIS due to their unique immune responses and reservoir status. However, EIS face significant challenges, including ethical considerations and the complexities of bat physiology. We conducted a meta-analysis of EIS to highlight the need for new bat cell lines and specific target species in future studies. **Methods.** We collated data from peer-reviewed primary research articles published through January 2025 for live bat or bat-derived cell lines (BdCL) that were viral EIS, using several combinations of the terms: “Chiroptera”, “bats”, “virus”, “infection” and “experimental” in a Web of Science all database search. We then manually inspected all papers and processed the results according to species, bat family, bats vr. BdCL, virus species and viral family. **Results**. We found 502 papers, with 127 pertinent to our analysis that included 313 different bat-virus pairings. Of these studies 132 (42.2%) were performed on live animals and 181 (57.8%) were performed on BdCL. EIS have only been conducted in species from 7 of the 21 bat families, with Pteropodidae (41.5%) and Vespertilionidae (22.3%) being the most trialed. Of the 1487 bat species, 48 have been used for EIS with 64 different viruses. Rousettus aegytiacus (47, 15%) was the most studied species, followed by Tadarida brasiliensis (35, 11.2%) and Eptesicus fuscus (30, 9.6%). For viruses, Influenza A (26, 8.3%), Rabies (RABV) (26, 8.3%) and Marburg (19, 6.1%) are the most frequently tested. **Conclusions**. This analysis reveals significant gaps in virulence research involving bats. Greater taxonomic breadth and additional consideration of bat-virus experimental combinations in both BdCL and live bat studies are needed to enhance our understanding of bat-borne viruses and their public health implications.

MERS-CoV passaged on Jamaican fruit bat cells selects a variant with mutations in Orf5 gene with enhanced infectivity and immunogenicity in bats

Author list:

Charley PA[1], Zhan S[1], and Schountz T[1]

[1]Center for Vector-borne Infectious Diseases, Department of Microbiology, Immunology, and Pathology; Colorado State University, Fort Collins CO, USA

Abstract:

Background. Middle East respiratory syndrome coronavirus (MERS-CoV) is a merbecovirus (genus *Betacoronavirus*, subgenus Merbecovirus) that causes Middle East respiratory syndrome in humans. MERS-CoV first emerged in 2012 in Saudi Arabia. Dromedary camels (*Camelus dromedarius*) have been shown to be a secondary reservoir host, but its ancestral virus likely originated in insectivorous bats because they host many merbecoviruses. Furthermore, thousands of coronavirus sequences have been identified in bat species spanning Asia, Africa, the Americas, and Europe. However, there are no data on the interactions between reservoir bats and MERS-CoV, although experimental infection of Jamaican fruit bats (*Artibeus jamaicensis*) found limited viral infection without visible signs of disease. Previous studies have used human cells or transgenic mouse models to study this virus, but they do not give insight into a natural reservoir interaction. **Methods/ Results.** This study aimed to determine if MERS-CoV can be adapted for more efficient infection in the Jamaican fruit bat model. We determined primary Jamaican fruit bat kidney (AJK) cell lines are permissive for MERS-CoV, causing substantial cytopathic effect (CPE) and cell death. Ten passages of the virus (p10) led to enhanced CPE and sequencing identified eight nonsynonymous mutations in the Orf5 gene. This variant was subsequently determined to be at very low levels in our viral seed stock, indicating that strong selection occurred that favored these residues upon passage in the AJK cells. Bats challenged with the MERS-CoV p10 had more oral and rectal positives swabs but had lower amounts of measurable virus compared to the wild-type MERS-CoV EMC strain. Interestingly, bats euthanized on 21-day post infection all seroconverted. **Conclusion.** Overall, we show MERS-CoV ability to adapt to a New World bat species with enhanced immunogenicity that may be caused by Orf5.

Patterns and Drivers of Hemispheric Differences in Global Bat-Viral Diversity

Author list:

Eiseman HJ[1], Becker DJ[2], Betke BA[2], Frank HK[1]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA, [2]Department of Biology, University of Oklahoma, Norman, OK, USA

Abstract:

Background. In recent years, the spillover of zoonotic viruses from bats into novel populations has been of growing interest globally. These zoonoses are caused by diverse viruses from diverse species, and they spillover into humans through a variety of different mechanisms. However, despite this diversity, there are global patterns to bat-associated zoonotic-spillover. Notably, the majority of the high profile, bat-associated spillover events have been documented occurring in the Old World compared to the New World. In this work, we tested hypotheses regarding the drivers of these hemispheric differences in bat-associated viral spillover. **Methods.** We used the Global Virome in One Network (VIRION), a database of vertebrate-viral associations, combined with mammal trait-based databases (PanTHERIA, COMBINE) to model drivers of bat-viral diversity. Using phylogenetic generalized linear mixed models (PGLMMs) to control for phylogenetic relatedness among bat species and differences in sampling effort, we tested the impact of the following characteristics on bat-viral patterns across hemispheres: geography (e.g. latitudinal and longitudinal differences), viral traits (e.g. viral species and type), and host traits (e.g. roost type, diet, and life history characteristics). Models were run first with all bat-associated viruses and were then restricted to only zoonotic viruses. **Results.** Preliminary results (using 340 bat species and 2,029 viral species) from this research show evidence that bat species which occupy a wider variety of habitats are infected with more viral species than environmentally restricted taxa. We expect that the full models will reveal a complex mix of host traits, viral traits, and geographic differences that drive these patterns. **Conclusions.** Examining long term patterns of bat-viral associations, especially within the context of the geographic regions where they co-evolved is an important consideration in understanding spillover holistically. These large statistical models can reveal characteristics that predict viral spillover, explain current patterns, and indicate risk factors for future spillover.

Session 2 Lightning Talks

Ebolavirus evolution and emergence are associated with land use change

Author list:

Lange CE[1,2,3], Barnum TR[4], McIver DJ[3,5], LeBreton M[6], Saylors K[2], Kumakamba C[3], Lowes S[7], Montero E[8], **Cohen RL**[9]

[1]Biology Department, Kwantlen Polytechnic University, Surrey, Canada, [2]Labyrinth Global Health Inc., Washington, DC, USA, [3]Metabiota Inc., San Francisco, CA, USA, [4]Office of Research and Development, United States Environmental Protection Agency, Washington, DC, USA, [5]Institute for Global Health Sciences, University of California, San Francisco, San Francisco, CA, USA, [6]Mosaic, Yaoundé, Cameroon, [7]Department of Economics, University of California, San Diego, La Jolla, CA, USA, [8]Harris School of Public Policy, University of Chicago, Chicago, IL, USA, [9]Uniformed Services University of Health Sciences, Bethesda, MD, USA

Abstract:

Background. Anthropogenic land use change facilitates disease emergence by altering the interface between humans and pathogen reservoirs, and is hypothesized to drive pathogen evolution. We investigate the origins of Zaire ebolavirus (EBOV) and Sudan ebolavirus (SUDV), which diverged over 4,000 years ago yet were each first detected in 1976, having caused near-simultaneous outbreaks among villagers of the northern Congo forest and cotton factory workers in the southern Sudanese savanna, respectively. **Methods.** We update the phylogeographies of EBOV and SUDV, and use the associated estimates and decadal land use data to evaluate the hypothesis that habitat fragmentation is associated with accelerated Ebolavirus evolution, and relatedly with the emergence of Ebolavirus variants. We triangulate these findings with historical accounts, including survey data of village elders, to groundtruth the plausibility of the detected associations. **Results.** Land use change is associated with faster evolution and dispersal of EBOV and SUDV. The most recent common ancestor (MRCA) of EBOV was circulating around 1960 in the forests of northwestern DR Congo, while the MRCA of SUDV was circulating around 1958 in the southern Sudanese savanna. Both landscapes underwent significant anthropogenic fragmentation between 1940 and 1960, associated with specific colonial “schemes,” which substantially altered local human settlement and agricultural practices for intensive cash crop production. Since these disturbances, landscape fragmentation was spatiotemporally associated with the divergence and dispersal of new variants of both viruses into new ecoregions of Africa. These variants segregated geographically along ecoregion boundaries, resembling a pattern observable for other bat-borne viruses. The amino acid changes which characterized each variant disproportionately involved glycosylation-sensitive amino acids in the surface glycoprotein domain responsible for immune evasion and attachment to host cells, suggesting adaptation to new hosts amidst changing landscapes. **Conclusions.** Ebolaviruses demonstrate how some bat-borne viruses may evolve, spread, and emerge as human pathogens amidst habitat fragmentation.

Session 3 Abstracts:

Bat pathogen persistence and transmission dynamics

Session 3 Plenary Talk

Interventions for primary prevention of viral spillover from bats

Daniel G. Streicker[1]
[1]University of Glasgow, Glasgow, Scotland.

Abstract:
Most current management of zoonoses aspires to reduce the health and economic burden of pathogens after they are introduced into human or domestic animal populations. By design, these mitigations cannot interrupt transmission within or from natural reservoirs, leading to sustained burdens from neglected, re-emerging zoonoses and unconstrained opportunities for novel epidemics. Reservoir-targeted actions including landscape alteration, culling and vaccination are proposed to reduce spillover risk by reducing the incidence or intensity of pathogen circulation within reservoir hosts but are frustrated by weak understanding of host and pathogen responses to human interventions. Focusing on 3 viruses transmitted by common vampire bats (rabies, H18 influenza, betaherpesvirus), I aim to show how longitudinal monitoring, viral genomics and field ecology can reveal the epidemiological consequences of ongoing interventions, identify the reservoirs and transmission dynamics of newly identified viruses, and anticipate the outcomes of future interventions.

Session 3 Short Talks

The dynamics of henipavirus circulation and persistence in wild, Madagascar fruit bats

Author list:

Horigan S[1]*, Kettenburg G[1]*, Ruhs EC[1]*, Ranaivoson HC[1,2,3], Andrianiaina A[2,3], Andry S[2,5], Héraud JM[6], Tso M[4], Sterling SL[4], Yan L[4], Broder CC, Laing ED[4], **Brook CE[1,7]**

[1]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA; [2]Association Ekipa Fanihy, Antananarivo, Madagascar; [3]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar; [4]Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [5]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar; [6]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar; [7]Department of Integrative Biology, University of California, Berkeley, Berkeley, CA, USA.

**indicates co-first authorship*

Abstract:

Background. Understanding the mechanisms which underpin population cycles for host-parasite systems – and the factors that maintain persistence of acute infections in wild animal hosts – remains a central goal in the ecology of infectious disease. Bats host acute RNA viruses that cause higher case fatality rates upon spillover to humans than those derived from any other mammal, including Marburg filovirus, Hendra and Nipah henipaviruses, and SARS and MERS coronaviruses. Many of these pathogens exhibit strong seasonal dynamics, which also drive seasonality in zoonotic emergence. Nonetheless, the dynamics by which these virulent pathogens are maintained in wild bat hosts are poorly understood. **Methods.** Here, we fit epidemiological models to age-structured time series data collected from our longterm fruit bat field study in Madagascar, which combines serological and molecular surveillance to study the circulation and persistence of novel henipaviruses in wild *Eidolon dupreanum* fruit bats. **Results.** Longitudinal data from recaptured individual bats demonstrates signatures of rapidly waning antibodies but sustained immunity in wild fruit bat hosts, with some evidence of weakened immunity in the face of nutritional success. We find support for mechanisms that incorporate multiple infection cycles in individual bats with seasonal peaks in infection driven by both primary infection in naïve juveniles and recrudescent infections in immunocompromised adults. **Conclusion.** Both within-host immunological dynamics and regional dispersal within the greater Madagascar fruit bat metapopulation appear to play a role in henipavirus maintenance in this system.

Periodic shifts in viral load increase risk of spillover from bats

Author list:

Lunn TJ[1,2], Borremans B[3,4], Jones DN[5], Kessler MK[6], Dale AS[7], Yinda CK[8], Ruiz-Aravena M[9], Falvo CA[10], Crowley DE[10], Lloyd-Smith JO[11], Munster VJ[8], Eby P[12,13], McCallum H[12], Hudson P[14], Restif O[15], McGuire LP[16], Smith IL[17], Bat One Health Team, Plowright RK[10], Peel AJ[12,18]

[1]Odum School of Ecology, University of Georgia, Athens, GA, USA; [2]Center for the Ecology of Infectious Diseases, University of Georgia, Athens, GA, USA; [3]Wildlife Health Ecology Research Organization, San Diego, CA, USA; [4]Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium; [5]Department of Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA; [6]Department of Ecology, Montana State University, Bozeman, MT, USA; [7]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA; [8]Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA; [9]Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, Starkville, MS, USA; [10]Department of Public and Ecosystem Health, Cornell University, Ithaca, NY, USA; [11]Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, CA, USA; [12]Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia; [13]School of Biological Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia; [14]Center for Infectious Disease Dynamics, Pennsylvania State University, State College, PA, USA; [15]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom; [16]Department of Biology, University of Waterloo, Waterloo, ON, Canada; [17]Health and Biosecurity Business Unit, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra, ACT, Australia; [18]Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia.

Abstract:

Background. Prediction and management of zoonotic spillover requires an understanding of infection dynamics within reservoir host populations. Transmission risk is often assessed using prevalence of infected hosts, with infection status reflecting the presence of genomic material. However, detection of viral genomic material alone does not necessarily indicate presence of infectious virus, or sufficient viral load for transmission, which could decouple prevalence from transmission risk. **Methods.** We undertook a comprehensive and multi-faceted investigation of Hendra virus shedding in Pteropus bats (colloquially flying-foxes, the main reservoir hosts), including Hendra virus qRT-PCR of 6,151 urine samples collected from five sites over three years. We assessed longitudinal associations between viral prevalence, viral load proxies (Ct value and genome copies), and equine spillover using generalized additive models and a permutation analysis. **Results.** In addition to seasonal and interannual fluctuation in prevalence, we found evidence for periodic shifts in the distribution of viral loads. The proportion of bats shedding high viral loads was higher during peak prevalence periods

with spillover events, and lower during peak and non-peak prevalence periods when there were no spillovers. **Conclusions.** We suggest that prolonged periods of low viral load and low prevalence reflect prolonged shedding of non-infectious RNA, or viral loads that are insufficient or unlikely to overcome dose barriers to spillover. These findings show that incorporating viral load (or proxies) into longitudinal studies of virus excretion from bats will better inform predictions of spillover risk than prevalence alone. Our study provides key insights into the processes that facilitate spillover and a basis for further experimental studies to explore interacting mechanisms that drive high viral shedding in bats.

Metagenomics reveals recurrent coronavirus shedding pulses in a Serotine Bat maternity colony are not driven by variant introductions or immune escape mutations

Author list:

Mols VC[1], Nieuwenhuijse D[1], van den Boom SC[1], Boter M[1], Jeucken JLM[2], Koopmans MPG[1], Kuiken T[1], Oude Munnink BB[1], Begeman L[1]

[1]Department of Viroscience, Erasmus University Medical Center, Rotterdam, The Netherlands; [2]Stichting de Laatvlieger, Horst, The Netherlands.

Abstract:

Background. Virus prevalence in reservoir hosts is an important determinant of cross-species transmission risk. Bats sustain strikingly high coronavirus prevalence compared to other mammals, often reaching 30-40%. However, mechanisms maintaining such levels of circulation remain poorly characterized. Longitudinal studies reveal substantial fluctuations in virus prevalence that are often associated with synchronized parturition pulses. In an ongoing longitudinal study of a well-monitored Serotine Bat (*Eptesicus serotinus*) colony in Castenray, The Netherlands, RT-qPCR analysis of 3,654 fecal droppings revealed similarly high prevalences of co-circulating alphacoronavirus (ES-αCoV) and betacoronavirus (ES-ßCoV). Three shedding pulses of both viruses occurred at 8–9-week intervals, with the first peaks coinciding with the birthing pulse. Pulses largely overlapped, as ES-αCoV peaks shortly following those of ES-ßCoV. ES-αCoV prevalence ranged from 2.6% to 84.0% and ES-ßCoV from 0% to 98.4%. Notably, RNA of both viruses co-occurred in 947 (25.9%) samples. The factors sustaining these rapid, recurrent peaks in virus prevalence and enabling persistent circulation remain unclear. We hypothesized that rapid and recurrent peaks in virus prevalence are driven by introduction of novel viral variants or by mutations enabling immune escape and reinfection. **Methods.** To explore potential novel virus introductions and mutations, we selected three coronavirus-positive fecal samples per week for metagenomic sequencing. **Results and Conclusions.** This resulted in recovery of 51 ES-αCoV and 21 ES-ßCoV genomes. Time-resolved phylogenetic analysis revealed no evidence of novel introductions or selective variant sweeps between shedding peaks, suggesting that factors beyond virus genetic changes, such as transient immunity and environmental influences, sustain coronavirus persistence and drive shedding pulses.
Future work will integrate virological findings with ecological data on population demographics, behavior, food availability, and climate to further investigate mechanisms underlying high virus prevalence and short-interval shedding pulses. These data will inform predictive models to identify periods of increased shedding, enabling targeted surveillance and interventions to reduce spillover risk.

Exposure as a prerequisite to spillover: understanding human-bat interactions in houses of rural Kenya

Author list:

Forbes KM[1], Lunn TJ[1 2], Jackson RT[1 3], Sironen T[4], Ogola JG[5], Webala PW[6]

[1]Department of Biological Sciences, University of Arkansas, USA; [2]Odum School of Ecology, University of Georgia, USA; [3]Arizona Game and Fish Department, USA; [4]Department of Virology & Department of Veterinary Biosciences, University of Helsinki, Finland; [5]Department of Medical Microbiology, University of Nairobi, Kenya; [6]Department of Forestry and Wildlife Management, Maasai Mara University, Kenya

Abstract:

Background. Exposure to wildlife pathogens is the obligatory first step in pathogen spillover and the emergence of new zoonotic diseases. Identifying and characterizing exposure interfaces is therefore a priority for pandemic prevention and one of our strongest intervention points. **Methods.** We have conducted a series of studies to understand bat roosting in human houses in rural Kenya and potential implications for public health. This includes geographic surveys and individual inspections to quantify the proportion of houses that are bat roosts, comparison of structural characteristics between houses occupied and unoccupied by bats, and interviews and surveys with people who live in houses with bats. **Results.** We found that human-bat cohabitation in buildings is common in rural Kenya (around 10% of houses have evidence of bats) and that people who live in these houses have frequent direct and indirect contact with bats and their excreta. Interestingly, relatively recent changes in building practices from traditional houses with mud walls and thatched roofs to modern style buildings with concrete walls, metal roofs, and structural beams have increased bat habitation of houses and exacerbated this exposure interface. Certain features of houses such as height and humidity make them more or less likely to be used as bat roosts and offer potential opportunities for building designs that discourage bats. **Conclusions.** Together this research establishes shared houses between bats and people in rural Africa as a high-risk setting for human exposure to bat pathogens and offers potential remedial solutions through building design to mitigate risk in line with One Health philosophies.

Co-circulation dynamics of henipaviruses, filoviruses and rubulaviruses in South Asian bat populations

Author list:

Ross N[1,2], Islam A[1,3], Kilpatrick AM[4], Hayes S[5], Olival KJ[1], Gurley ES[6], Hossain MJ[7], Field HE[1,8], Crameri G[9], Wang L-F[9,10], Luby SP[11], Daszak P[1], **Epstein JH**[1,12]

[1]EcoHealth Alliance, New York, NY, USA; [2]rOpenSci, Austin, TX, USA; [3]Charles Sturt University, Wagga Wagga, NSW, Australia; [4]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, USA; [5]Imperial College, London, UK; [6]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA; [7]Medical Research Council Unit, The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, The Gambia; [8]School of Veterinary Science, The University of Queensland, Gatton, Australia; [9]CSIRO Australian Centre for Disease Prevention, Geelong, VIC, Australia; [10]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore; [11]Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA; [12]One Health Science, Mt Kisco, NY, USA

Abstract:

Background. *Pteropus medius* fruit bats live in close association with humans across South Asia and are a known reservoir of Nipah virus and other viruses of unknown pathogenicity. Here we analyze serodynamics for henipaviruses, rubulaviruses, and filoviruses in *P. medius* in Bangladesh between 2007 and 2012. **Methods.** We conducted a five-year longitudinal study sampling 100 *P. medius* bats quarterly in Faridpur and two one-year studies in Ramnagar and Chakhoria, sampling 40 bats monthly from each roost complex, which were approximately 225km apart, between April 2010 and May 2011. Monthly bat sampling in each location was performed to obtain data at a finer temporal scale. We used a sphere-based multiplexed immunoassay to detect IgG antibodies against Nipah, Ebola and Menangle virus glycoproteins. **Results.** Antibody dynamics indicated regular, simultaneous circulation of all three viral groups. Filovirus seroprevalence exhibited intense and nearly saturating annual cycles in juveniles, moderate annual fluctuations amongst adults, and fitted mathematical models suggested annual cycles of transmission in both age groups. Rubulavirus serodynamics also included intense annual cycles in juveniles but transmission was delayed compared to filoviruses, and antibody persistence was longer resulting in higher adult seroprevalence and limited transmission. Henipavirus serodynamics were less seasonal than rubulaviruses and filoviruses and seroprevalence peaks occurred approximately every two-three years in adults. **Conclusions.** This study provides the first evidence of filovirus transmission in *P. medius*. The use of a broad-based screening tool such as a multiplexed immunoassay allows for efficient study of multiple viruses within a single host and broadens our insight into the potential for spillover of more than one virus into humans via well-established mechanisms (e.g., contamination of date palm sap) and further underscores the value of interventions that limit exposure of humans to bat excreta.

Longitudinal serosurveys reveal bat-virus dynamics in northern Republic of Congo

Author list:

Seifert SN[1], Olson S[2], Bushmaker T[3], Ondzie AI[4], Schulz JE[5], Becker D[6], Letko M[1], Nkoua CB[7], Bounga G[4], Akongo MJ[4], Fischer RJ[5], Sterling SL[8], Yan L[8], Walzer C[2 9], Niama FR[7], Laing E[8], Munster VJ[5]

[1]Paul G. Allen School for Global Health, College of Veterinary Medicine, Washington State University, Pullman, WA, USA; [2]Health Program, Wildlife Conservation Society, New York, NY, USA; [3]Laboratory of Virology, Division of Intramural Research, NIAID, NIH, Hamilton, MT, USA; [4]Wildlife Health Program, Wildlife Conservation Society, Brazzaville, Republic of the Congo; [5]Virus Ecology Section, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT, USA; [6]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [7]Département de la Recherche et de la Production, Laboratoire National de Santé Publique, Brazzaville, Republic of the Congo; [8]Uniformed Services University, Bethesda, MD, USA; [9]Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria

Abstract:

Background. Zoonotic viruses which spillover from wildlife reservoirs are challenging to study and, in turn, challenging to predict viral emergence in time and space. Serological surveillance studies often yield more data than virus detection efforts due to the extended timeframe for detectable antibodies following infection relative to viral shedding. Apart from the detection of Ebola virus RNA in thirteen fruit bat samples from central Africa in 2005, the only published data on this filovirus come from serological surveillance efforts. We focus on monitoring seroprevalence over time in the hammer-headed bat (Hypsignathus monstrosus), which is one of only three bat species from which Ebola virus RNA has been sequenced and made publicly available. **Methods.** We conducted longitudinal serological surveillance using a multiplexed assay to detect exposure to filoviruses and henipaviruses in a hammer headed bat lek site in northern Republic of Congo from 2012-2018. To understand the factors influencing henipavirus and filovirus exposure rates across different bat demographics and time periods, we employed a generalized linear mixed model with predictors including age class, sex, season, and year with assay plate as a random effect. We then modified a mechanistic Susceptible-Infected-Recovered (mSIR) modeling framework to predict epidemic peaks in our hammer-headed bat population from serological surveillance data. **Results.** We show that hammer-headed fruit bats at this lek support circulation of Orthoebolavirus bundibugyoense (BDBV) and Henipavirus ghanaense (GhV), or antigenically related viruses. We find some evidence for demographic patterns of associations for GhV dynamics over time, but less so for circulation of BDBV in the population. Our mSIR model predicts transition period between seasons as the highest probability for detection of infectious BDBV. **Conclusions.** Our study underscores the utility of

serological surveillance in understanding the epidemiology of filoviruses and henipaviruses in bat populations to better forecast periods of increased zoonotic transmission risk.

Reconstructing prevalence dynamics from pooled and individual samples

Author list:

Borremans B[1], Falvo CA[2], Crowley DE[2], Hoegh A[3], Lloyd-Smith JO[4], Peel AJ[5], Restif O[6], Ruiz-Aravena M[7], Plowright RK[2]

[1]Wildlife Health Ecology Research Organization, San Diego, CA, USA; [2]Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA; [3]Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA; [4]Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, CA, USA; [5]Sydney School of Veterinary Science, The University of Sydney, Sydney, Australia; [6]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom; [7]Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, Starkville, MS, USA

Abstract:

Background. Sample collection for pathogen transmission studies in wildlife can be challenging and costly. A useful strategy can be to collect pooled samples, but this introduces challenges for estimating prevalence. This is because pooling can introduce a dilution effect where pathogen concentration is lowered by the inclusion of negative or lower-concentration samples, while at the same time a pooled sample can test positive even when some of the contributing samples are negative. **Methods.** We present a Bayesian multilevel model that reconstructs prevalence over time using pooled and individual samples. The model explicitly accounts for the complete mixing process that determines pooled sample concentration, enabling accurate prevalence estimation even from pooled samples only. The model allows the incorporation of individual-level samples so that covariate effects such as age, sex, or immune markers can be estimated. Crucially, when individual samples can test false negative, a potentially strong bias is introduced that results in incorrect estimates of regression coefficients. The model, however, can account for this by leveraging the combination of pooled and individual samples. Last, the model allows estimation of extrinsic environmental effects on prevalence dynamics. **Results.** Using a simulated dataset inspired by virus transmission in flying foxes, we show that the model can accurately estimate prevalence dynamics, false negative rate, and covariate effects. We test model performance for different sampling scenarios and find that while it is generally robust, there are a number of factors that should be considered in order to maximize performance. **Conclusion.** The model presents a useful opportunity to use pooled samples for estimating prevalence dynamics. It can be used with any biomarker of infection (Ct values, antibody levels, other infection biomarkers) and can be applied to a wide range of host-pathogen systems.

Session 3 Lightning Talks

Bats as Bacterial Reservoirs - Unravelling Staphylococcus aureus dynamics in Rousettus fruit bats

Author list:

Weinberg M[1], Weinert L[1], Zou G[1], Blum SE[3]

[1]Department of Veterinary Medicine, Cambridge Veterinary School, University of Cambridge, Cambridge, UK; [2]School of Life Sciences, University of Essex, Colchester, UK; [3]Department of Bacteriology, Kimron Veterinary Institute, Bet-Dagan, Israel

Abstract:

Bats are recognized as major carriers of zoonotic viruses, but their role in harboring bacteria, especially those with pathogenic potential, remains underexplored. Initial findings suggest that Rousettus fruit bats experience recurrent infections from S. aureus. This bacterium can cause severe and often antibiotic-resistant human infections, and the emergence of potentially new S. aureus strains from bats might pose a potential threat to public health. We hypothesize that bats tolerate bacterial infections, potentially serving as reservoirs for bacterial strains that could infect humans. To explore this, we collected 232 samples over nearly 15 years from both healthy and sick Rousettus bats across varied geographic locations. (I began collecting these strains as a bat veterinarian long before pursuing a Ph.D. on this topic). Using whole genome sequences from these isolates, we characterize virulence and resistance traits, examine transmission dynamics, identify bat-specific bacterial adaptations, and investigate how bats manage to tolerate S. aureus infections.The predominant sequence types (STs) identified include ST15, ST88, ST12, ST8, ST80, and ST30, and also 13 previously unreported STs, four of which form a new clonal complex unique to bats, representing a distinct bat-specific lineage. Preliminary findings suggest that several of these STs carry novel plasmids that are exclusive to bat populations. Additionally, ST15 isolates from bats were found to harbor human immune evasion genes (scn and chp).

We successfully traced these specific lineages back to the mid-1960s in Israel, aligning with the major immigration waves of the 1950s and the cessation of cave fumigations targeting fruit bats, which began in the same decade as part of the Ministry of Agriculture's policy. Recently, we have begun documenting cases of S. aureus isolation from healthy humans who are regularly exposed to bats. Our findings suggest the presence of bat-adapted S. aureus lineages that may have the potential to cause human infections.

Seasonal infection dynamics and anthropogenic landscape change drive henipavirus spillover risk from a Malagasy fruit bat

Author list:

Horigan S[1], Andry S[2], Andrianiaina A[3], Roland M[1], Ranaivoson HC[13], Brook CE[1]

[1]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA;
[2]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar;
[3]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar

Abstract:

Background. Fruit bats (Pteropodidae) in Madagascar are known hosts for potentially zoonotic viruses (e.g. henipaviruses, filoviruses, coronaviruses). Widespread anthropogenic modification of fruit bat habitat and high human-bat contact due to legally sanctioned hunting combine to present risks for zoonotic spillover of fruit bat viruses in this system. Here, we leverage both biological and landscape drivers to generate seasonal zoonotic risk maps for henipaviruses circulating in the Malagasy fruit bat, Eidolon dupreanum.
Methods. We developed spatial transmission models informed by field data to forecast seasonally varying viral shedding from bats. We use niche modeling approaches to map this seasonally varying viral shedding in space. We combine this output with human population and landscape modification layers to develop an index of seasonally-varying zoonotic risk across Madagascar. We compare these risk maps to the timing of the legal hunting season to evaluate their overlap. **Results.** We detect seasonal changes in bat movement, observing increased time and longer distances foraged during the dry, resource-limited season; enhanced bat presence on the landscape is likely correlated with increased duration of bat shedding of virus into the environment. Hotspot analyses reveal that the most significant landscape risk factors for Madagascar are agricultural and land and future landscape modification pressure. Our models reveal spatial and temporal variation in spillover risk and suggest the legal hunting season coincides with peak risk periods. **Conclusion.** This work offers a spatially and temporally explicit forecast of bat-borne zoonotic risk in Madagascar, providing clear guidelines for regions and seasons which could be targeted by public health interventions aimed at halting spillover prior to human emergence.

Anthropogenic Change, Bat Stress, and Ecotonal Shifts: Identifying High-Risk Nipah Virus Spillover Zones in Bangladesh

Author list:

Uelmen JU[1], Akter S[2], Islam N[3], Chowdhury S[4], Kemenesi G[5]

[1]Department of Population Health Sciences, University of Wisconsin, Madison, WI, USA; [2]Department of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh; [3]Department of Forest and Wildlife Ecology, University of Wisconsin, Madison, WI, USA; [4]Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh; [5]National Laboratory of Virology, University of Pecs, Pecs, Hungary.

Abstract:

Background. As human-driven land use change reshapes landscapes, flying foxes (Pteropus spp.), the primary Nipah virus (NiV) reservoir, face rising ecological stressors that may increase viral shedding and spillover risk. Habitat fragmentation, agricultural expansion, urbanization, and climate change-driven shifts alter bat foraging, social structures, and immune function. These stressors are particularly severe for gestating and nursing females, whose high energy demands, nutritional deficiencies, and hormonal dysregulation may increase viral loads. Roost disturbance, competition, and heat stress further suppress immunity, elevating transmission risks and altering bat-human interface dynamics.

Methods. This research follows two phases. Phase 1, now underway, integrates Landsat 8 imagery and machine learning to map high-risk ecotonal boundaries, where environmental and climatic shifts heighten bat-human interactions. These initial risk maps will be presented at BatID, guiding site selection for Phase 2, which will incorporate on-the-ground verification through bat population counts, pathogen sampling, and stress biomarkers (e.g., fecal cortisol, microbiome diversity, and nutritional indicators). **Results.** To improve real-time surveillance, we will deploy thermal-imaging drones aided by machine learning processes to detect individual bats and monitor nursing, grooming, and stress-induced behaviors—key factors in viral spread. Mobile biosafety labs will conduct on-site pathogen testing, linking viral shedding rates with identified stressors. **Conclusions.** This multi-scale approach advances Theme IV (Pathogen Persistence and Transmission Dynamics) by linking bat immunological stress to viral shedding across changing landscapes. By addressing conservation implications (Theme V), we also highlight the need for sustainable land-use strategies that balance bat ecological stability with zoonotic risk mitigation. Findings will refine disease risk models, feeding into a public-facing NiV risk dashboard to provide policymakers and public health officials with real-time data for targeted intervention strategies.

Session 4 Abstracts:

Bat pathogen discovery

Session 4 Plenary Talk

Building Predictive Intelligence for Pandemic Prevention

Simon J. Anthony [1]
[1]University of California, Davis

Abstract:
All pandemics begin with a single human infection. Yet that first case is only the visible endpoint of a much longer, largely unobserved chain of events. In the “pre-emergence” phase, viruses circulate, adapt, and diversify within animal hosts, their trajectories shaped by host ecology, viral evolution, and environmental change. Spillover is not a moment, but a process.

This presentation focuses on the pre-emergence phase, emphasizing the discovery and characterization of viruses in wildlife to understand their distribution, ecology, and evolutionary potential. These insights provide the foundational intelligence needed to inform prioritization strategies, whether for ecological interventions or medical countermeasures. Advancing our predictive understanding of the pre-emergence phase offers the greatest potential to reduce the frequency of future pandemics.

A cross-scale framework is presented, beginning with targeted viral discovery and extending through functional virology and evolutionary analysis to risk prioritization. Case studies include (i) Bombali virus, an ebolavirus detected in bats in Sierra Leone, (ii) PDF-2180, a MERS-like coronavirus found in bats in Uganda; and (iii) two related morbilliviruses from Myotis and phyllostomid bats in Brazil. These examples highlight how novel viruses can be systematically evaluated, supporting risk-ranking efforts tied to initiatives such as CEPI’s 100 Days Mission.

Finally, because the risk of spillover is dynamic, our framework incorporates evolutionary tipping points. Mechanisms like recombination in coronaviruses can generate phenotypic shifts that affect host range, virulence, and immune escape. These changes not only influence spillover risk but also challenge vaccine strategies, potentially altering antigenic targets faster than countermeasures can be deployed. Identifying regions, host species, or ecological contexts that promote accelerated evolution - such as high co-infection rates or the co-circulation of compatible viruses - can help define priority hotspots for surveillance and preparedness efforts.

Session 4 Lightning Talks

Building out our toolkit: environmental air sampling for wildlife viral surveillance

Author list:

Huntington C[1], Bonavita C[1], Tiemann J[1], Navarrete-Macias I[1], Anthony SJ[1]

[1]Department of Pathology, Microbiology, & Immunology, University of California - Davis, CA, USA

Abstract:

Background. Wildlife viral surveillance is essential for understanding the pre-emergence phase of zoonotic pandemics. However, traditional surveillance methods face major challenges due to the need for large-scale efforts to detect rare and intermittently shed viruses. New technologies are needed to improve the discovery and characterization of unknown viral diversity, as well as to monitor the dynamics of known zoonotic viruses. One emerging approach is environmental air sampling, which has a long history in detecting bioaerosols in indoor and agricultural settings but has yet to be systematically applied to wildlife viral surveillance. In this study, we explore the ability of environmental air sampling to detect viruses in a cave-roosting bat assemblage. **Methods.** During the summers of 2023 and 2024, we conducted 115 air sampling events alongside the collection of biological specimens from 1,948 bats representing six species in a cave system in Puerto Rico. Samples were screened for target viral families using a combination of next generation sequencing and degenerate PCR. **Results.** Air sampling successfully detected multiple mammalian viral families, including RNA viruses (Coronaviridae; Astroviridae) and DNA viruses (Parvoviridae; Herpesvirus). Two alpha- and betacoronaviruses identified in bat samples were also detected in air samples. Preliminary data suggest that air sampling can capture temporal dynamics in viral shedding, with periods of increased representation for certain viruses. Additionally, the presence of bat DNA in air samples highlights the potential for air sampling to complement host characterization. **Conclusions.** Environmental air sampling is a powerful addition to the wildlife viral surveillance toolkit, enabling the detection of zoonotic-relevant viral families in a cave-roosting bat population. Our findings suggest that air sampling can provide insights into viral shedding dynamics while also offering a non-invasive means of monitoring host presence and community composition. Future work will refine this approach to enhance viral discovery, transmission monitoring, and ecological surveillance.

Nanopore Adaptive Sampling as a Rapid Molecular Barcoding Tool for Identification of Host Species and their Metacommunities

Author list:

Frank LE[1], Kipp EJ[1], Faulk C[2], Larsen RJ[1], Lindsey LL[1], Milstein MS[1], Shaffer CA[3], Stone S[1], Wolf TM[4], Larsen PA[1]

[1]Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA; [2]Department of Animal Science, University of Minnesota, St. Paul, MN, USA; [3]Department of Anthropology, Grand Valley State University, Allendale, MI, USA; [4]Department of Population Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

Abstract:

Background. Species identification of pathogens and hosts is essential to disease ecology. Nevertheless, accurate identification is an obstacle, especially for cryptic and understudied hosts and rapidly evolving pathogens. Barcoding of genes, such as mitochondrial genes in hosts and 16S and RdRp genes in metacommunities, are standard for molecular identification but require time-consuming PCRs that necessitate a priori knowledge of targets. Advancements in next-generation sequencing produced tools capable of rapid identification and discovery. **Methods.** Nanopore adaptive sampling (NAS), a PCR-free method, enriches or depletes sequencing according to user-specified reference databases. In real-time, the first ~200-400 bases of DNA molecules are mapped to the database. 70% or greater matches are sequenced, while non-target molecules are ejected from the sequencing pore. Here, we utilized NAS to enrich sequencing of mitogenomes on MinION flow cells to identify host species in two ways (1, 2). Then, NAS was used to deplete host sequences and target sequencing of the bacterial metacommunity (3). **Results.** (1) Fecal DNA was sequenced from 11 mammals demonstrating utility for molecular barcoding of non-invasive and low DNA content samples. We putatively identify 10 of 11 species by mapping reads to reference genomes. (2) Liver tissue DNA from one marsupial, two rodents, and eight bats were sequenced and analyzed during fieldwork in Guyana, South America. Generated reads were compiled, creating consensus sequences for barcoding genes and phylogenetic trees. All mammals were identified to species, often in <36 hours. (3) Liver DNA from 5 Carollia sp. were sequenced, depleting for host DNA, to demonstrate using NAS for targeted bacterial sequencing. Bacterial reads were mapped to references and classified. **Conclusions.** This NAS approach offers a novel way to identify host species and their metacommunities. We provide a rapid molecular assessment of host species and the groundwork for applying this method for pathogen surveillance and discovery.

Model-guided paramyxovirus discovery in museum bat collections

Author list:

Juman MM[1], McDonough MM[2 3], Ferguson AW[3], Han BA[4], Becker DJ[5]

[1]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK; [2]Department of Biological Sciences, Chicago State University, Chicago, IL, USA; [3]Field Museum of Natural History, Chicago, IL, USA; [4]Cary Institute of Ecosystem Studies, Millbrook, NY, USA; [5]School of Biological Sciences, University of Oklahoma, Norman, OK, USA

Abstract:

Background. Several species of Old World fruit bats (family Pteropodidae) are known reservoirs of zoonotic paramyxoviruses, including the Hendra and Nipah henipaviruses. However, little is known about how many pteropodid species host paramyxoviruses and which traits are associated with host suitability. **Methods.** We compiled morphological, ecological, demographic, and evolutionary trait data for 194 pteropodid species from the literature as well as virus occurrence data through a systematic review. We then used boosted regression trees (BRT), a machine learning algorithm, to identify trait profiles of paramyxovirus-positive pteropodids and predict which additional unsampled or previously negative species are suitable hosts. We then empirically tested these predictions by screening specimens in natural history museum collections—a valuable and underutilized resource for viral discovery. We extracted RNA from frozen tissue samples of predicted host species housed at the Field Museum of Natural History, and then conducted RT-PCR targeting the paramyxovirus L gene, followed by Sanger sequencing. **Results.** Our BRT had high predictive capacity (mean test AUC = 97.7%) and suggested that species with larger range areas, greater body lengths, and high sympatry with other bats were more likely to be PCR positive for paramyxoviruses. Based on this trait profile, the top four predicted “novel” hosts are *Cynopterus sphinx, C. brachyotis, Epomops franqueti,* and *Rousettus madagascariensis*. We recovered three paramyxovirus sequences from museum specimens, including one from a previously unknown host predicted by our model, *E. franqueti*. Preliminary phylogenetic analyses show these novel sequences clustering with other bat paramyxoviruses, more closely related to henipaviruses than pararubulaviruses. **Conclusions.** Both our modeling and molecular results expand our library of known and suspected vertebrate–virus associations and inform future surveillance and spillover prevention efforts. More broadly, this case study lays out a promising framework for using modern methods such as machine learning to unlock pathogen data hidden in historical specimens.

Recurrent spillover of H5 avian influenza to vampire bats at the marine-terrestrial interface

Author list:

Tu IT[1,2], Lynggaard C[3], Walsh SK[2], Adams L[4], Raveendran S[2], Turnbull ML[2], Griffiths ME[1,2], Tello C[5], Valderrama Bazan W[5], Drexler JF[6], Faust CL[1], Cárdenas-Alayza S[7], Hutchinson EC[2], Murcia PR[2], Bohmann K[3], Harvey R[4], Streicker DG[1,2]

[1]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK; [2]MRC–University of Glasgow Centre for Virus Research, Glasgow, UK; [3]Globe Institute, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [4]Worldwide Influenza Centre, The Francis Crick Institute, London, UK; [5]Asociación para el Desarrollo y Conservación de los Recursos Naturales (Illariy), Lima, Perú; [6]Charité Universitätsmedizin, Berlin, Germany; [7]Centro para la Sostenibilidad Ambiental, Universidad Peruana Cayetano Heredia, Lima, Perú

Abstract:

Background. In late 2022, H5N1 avian influenza virus (AIV) caused mass mortality in seabirds and South American sea lions along the Peruvian coast. In these areas, common vampire bats (Desmodus rotundus) are suspected to feed on marine wildlife, potentially facilitating pathogen transmission at the marine-terrestrial interface. **Methods.** To investigate H5N1 AIV exposure in vampire bats, we collected 861 serum samples from 12 Peruvian sites (2011–2024) representing different foraging ecologies: coastal roosts with marine contact (<100 m from seafront, N=431), without marine contact (>5 km, N=373), and inland roosts (N=57). A subset of 2023 hair and rectal swab samples was analyzed for diet as a proxy for prey contact. Additionally, in-vitro experiments assessed the permissiveness of vampire bat cells to avian influenza viruses. **Results.** We detected 14 H5 seropositive vampire bats from marine-contact sites across multiple years. Isotopic analysis of seropositive bats' hair indicated a marine-derived diet (δ15N 23.75 ± 0.62‰), while seronegative bats from inland and non-marine sites had significantly lower nitrogen values (P=4.6e-12), suggesting livestock or mixed diets (δ15N 12.41 ± 1.84‰). DNA metabarcoding confirmed seropositive bats fed on species heavily impacted by Peru's H5N1 epizootic, including pelicans and sea lions. In vitro experiments demonstrated viral entry into vampire bat cells but limited replication. **Conclusions.** Low H5 seroprevalence in marine-contact sites suggests sporadic exposure from infected wildlife or contaminated environments. However, the permissiveness of bat cells to influenza and recurrent viral exposure provide opportunities for adaptation and reassortment with bat influenza viruses. This marine-terrestrial interface may facilitate pathogen spillover to other prey, particularly where bats feed on both marine and domestic animals. Our study provides the first evidence of recurring H5 exposure in vampire bats, highlighting the need for further research.

Session 5 Abstracts:

Reconciling bat infectious diseases and conservation

Session 5 Plenary Talk

Reconciling bat infectious disease research and conservation: One Health Action Plans for species at the human-bat interface

Tigga Kingston[1]
[1]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA

Abstract:
The actual and perceived roles of bats in the emergence of infectious disease challenge decades of progress in bat conservation. Yet, the very anthropogenic stressors that are accelerating spillover and disease emergence, such as climate change, habitat loss and degradation, disturbance and persecution, directly imperil bat populations, suggesting clear pathways for reconciling bat infectious diseases and conservation. That said, complex and sometimes antagonistic relationships between research and conservation communities may obfuscate this path, and poor communication with the public confound it still further. The IUCN SSC Bat Specialist Group's One Health Working Group (BSG OHWG) was established in response to COVID-19 and has been working to broker relationships between research and conservation communities. Efforts have focused on developing and disseminating global guidelines for working safely with bats, communicating about diseases, and sustainable guano harvesting. In a new initiative, over the next four years the BSG OHWG will return to the more explicit reconciliation of conservation and infectious disease, developing frameworks to support One Health Action Plans that prioritize securing sustainable bat populations while minimizing drivers that contribute to disease emergence and human risks. This will be a highly integrative approach incorporating diverse research perspectives e.g. ecology, social behavior and population dynamics, virology, physiology, genomics, and human dimensions of the human-bat interface. We begin by focusing on the Egyptian fruit bat, *Rousettus aegyptiacus*, a widespread species known for its role as a reservoir of Marburg virus. For decades, IUCN Action Plans have proven essential guides for the scientific and conservation communities. We invite the disease community to join us in this original endeavor, contributing their expertise and insights to develop comprehensive One Health Action Plans for species and the human-bat interface. Together, we can ensure the conservation of bat populations while safeguarding human health.

Session 5 Short Talks

Bats as Beneficial Animals: Perceptions and Public Health Implications in a Bat-Hunting Community of Bangladesh

Author list:

Khan AKM D[1 2], Begum F[2]

[1]Zoonotic Disease Research Program, Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, Bangladesh; [2]Department of Anthropology, University of Dhaka, Dhaka, Bangladesh

Abstract:

Background. Bats are natural reservoirs of many emerging infectious diseases that cause significant human morbidity and mortality. Bat hunting is a common practice in Bangladesh and poses a risk of deadly zoonotic diseases, such as Ebola and henipaviruses in humans. This research aimed to explore bat-hunting practices, including the associated socioeconomic factors, cultural beliefs, and health risks in a bat-hunting community in Bangladesh. **Methods.** We conducted a qualitative ethnographic study in a bat-hunting community in Bangladesh from May 2017 to April 2018, including observations of bat-hunting events, in-depth interviews with 4 bat-hunters, and 20 individuals involved in bat meat processing and selling. Collected data was analyzed using a thematic analysis approach. **Results.** The bat hunters were involved in hunting when there was no work to earn in winter which is an alternative source of income and lower-cost animal protein compared to domestic animal meats that they could not avail for the higher price. They believed that bats don't transmit infections but are used as beneficial animals for treating several illnesses like asthma, heart disease, and sexual vigor, by using bat meat and body parts. One individual exhibited symptoms of Nipah-like encephalitis and died before testing, leading to beliefs in supernatural causes among the community people. We observed that bat processing was placed at their homestead where family members including children were exposed to bat blood and raw meat and domestic animals like dogs and cats eat the offal of bats. **Conclusions.** The study findings highlight the statements of livelihood, cultural beliefs, and health risks associated with bat hunting and consumption. The study underscores the urgent need for a culture-sensitive intervention with educational outreach programs aimed at augmenting awareness and economic outcomes of these hunters to reduce the health risks of zoonotic diseases.

Protecting researchers, protecting bats—norms and control drive bat researchers' intent to adopt field hygiene practices

Author list:

Rutrough AL[5], Coleman JL[1,2,3], Macdonald EA[1,4], Straka TM[1,6], Little TD[7], Stickley Z[8], Kingston T[1,5]

[1]International Union for the Conservation of Nature (IUCN), Species Survival Commission (SSC), Bat Specialist Group (BSG), Human Dimensions Working Group, Gland, Switzerland; [2]Department of Biology, Queens College, City University of New York, Flushing, NY, USA; [3]Graduate Center, City University of New York, New York, NY, USA; [4]Worcester College, University of Oxford, Oxford, UK; [5]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA; [6]Freie Universität Berlin, Berlin, Germany; [7]Department of Educational Psychology, Leadership, & Counseling, Texas Tech University, Lubbock, TX, USA; [8]Yhat Enterprises, LLC., Lubbock, TX, USA

Abstract:

Infectious disease is a growing conservation threat to wildlife, with zoonotic transmission most likely at the human-wildlife interface. One underappreciated activity at this interface is fieldwork with wild animals, but associated risks can be mitigated through “field hygiene” (FH) practices such as using personal protective equipment and other appropriate behaviours. Following the dissemination of IUCN FH guidelines for bat researchers, we investigated factors that affect bat researchers’ intent to use FH practices under a Theory of Planned Behaviour (TPB) framework. Under the TPB, a person’s intent to perform a behaviour is influenced by their attitude toward, their subjective norms around and their perceived behavioural control (PBC) about the behaviour. We invited researchers who had recently conducted bat-related fieldwork to complete a qualitative questionnaire, generating data that we used to build a quantitative survey, which we disseminated widely to bat researchers. We analysed ~1 000 survey responses using structural equation modeling and assessed the role of career stage, research focus and socioeconomic status of the research location on intent. Bat researchers’ intent to adopt FH practices was high overall. For those who do not focus on disease projects, the subjective norm was a strong driver of intent, with mentors the most influential norm referents; authoritative bodies that set regulations and peers were influential too. The only modeled barrier to intent was PBC—with beliefs that FH practices are impractical or uncomfortable contributing most to PBC. We conclude that senior researchers should be encouraged to use FH practices and encourage their mentees to do likewise. Technical solutions and education to mitigate impracticality and discomfort issues should also be encouraged. Although we focused on bat researchers, all wildlife fieldwork entails pathogen transmission risks. To mitigate them, FH practices must become entrenched in the wildlife research community—achieving this goal requires both regulatory and social measures.

The Western Asia Bat Research Network (WABNet): First Regional Network to Integrate Bat Ecological and Virological Research

Author list:

Phelps KL[1], Sidamonidze K[2], Urushadze L[2], Alhmoud N[3], Alrwashdeh M[3], Bilgin R[4], Ali S[5], Attaullah[5], Spalton A[6], Ahmed Al-Abdulsalam Z[7], Ghazaryan A[8], Hasanov N[9], Bates P[10], Epstein J[1], Karesh W[1], Kingston T[11], Racey P[12], Hamel L[1], Olival KJ[1]

[1]EcoHealth Alliance, New York City, NY, USA; current affiliation of KLP: College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA; [2]Department of Virology, R. Lugar Center for Public Health Research, National Center for Disease Control & Public Health, Tbilisi, Georgia; [3]Bio-Safety & Bio-Security Centre, Royal Scientific Society, Amman, Jordan; [4]Institute of Environmental Sciences, Boğaziçi University, Istanbul, Türkiye; [5]Department of Wildlife & Ecology, University of Veterinary & Animal Sciences, Lahore, Pakistan; [6]Independent Wildlife Consultant, Muscat, Oman; [7]Ministry of the Environment, Muscat, Oman; [8]Faculty of Biology, Yerevan State University, Yerevan, Armenia; [9]Institute of Zoology, Azerbaijan National Academy of Sciences, Baku, Azerbaijan; [10]Harrison Institute, Kent, UK; [11]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA; [12]University of Exeter, Cornwall, UK

Abstract:

Background. Global bat research and bat-virus surveillance are geographically biased, with Western Asia understudied despite being a biogeographical 'mixing pot' of diverse bat species from Europe, Africa, and Asia. **Methods.** We established the Western Asia Bat Research Network (WABNet), a regional One Health network to integrate bat ecological and virological research. Bat researchers, virologists, public health experts, and policymakers networked via annual meetings and hands-on field and lab training. We conducted regional field surveillance from 2018-2022 in 7 WABNet countries (Armenia, Azerbaijan, Georgia, Jordan, Oman, Pakistan, Türkiye). We non-lethally sampled 4,273 bats of 41 species from 50 sites and screened rectal swabs/feces for coronaviruses at regional laboratories (R. Lugar Center, Georgia and Royal Scientific Society, Jordan). We used epidemiological analyses to determine bat species diversity and the correlation of individual-, community-, and site-level factors on coronavirus prevalence, spatial analyses to identify areas of greatest spillover risk, and phylogenetic analyses to identify known and/or novel bat-borne coronaviruses. **Results.** Coronavirus-positive bats were detected in all countries. Across bat species sampled, coronavirus prevalence was 9.9% but varied significantly among species and epidemiological factors. The geographic overlap of coronavirus-positive bat species and human population density indicates the Caucasus and southern coast of Türkiye have increased spillover risk. Altogether, 421 individual coronavirus sequences were identified, representing 31 unique lineages of alpha- and beta-coronaviruses (including SARS- and MERS-related viruses). Furthermore, we recorded and analyzed echolocation calls from 750 individuals of 31 bat species. **Conclusions.** Collaborations within WABNet are strengthened and continue to build capacity of multidisciplinary partners to conduct

integrative bat ecological and virological research in an understudied region. Working with country partners, we disseminated project outputs to diverse stakeholders, including contributions to publicly available databases (Chirovox, GBIF, NCBI), and created community outreach materials to reduce the threat of bat-virus emergence while simultaneously promoting bat conservation.

Targeting taste and leveraging leadership influence: a behavioral approach to reducing bat meat consumption and zoonotic disease risk

Author list:

Obitte BC[1,2], Kingston T[1]

[1]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA; [2]Small Mammal Conservation Organization, Benin City, Nigeria

Abstract:

Background. The Egyptian fruit bat (*Rousettus aegyptiacus*), a known reservoir for zoonotic viruses, including Marburg, is heavily hunted for food in Nigeria, posing significant public health risk. In rural communities in southern Nigeria, offtake levels can reach 4000 per hunting event to serve these huge markets. Beyond immediate population declines, hunting disturbs roosting bats and forces redistribution across landscapes, which may influence disease spillover dynamics. Here, we disentangle the behavioral drivers of bat meat consumption, and We used the identified drivers to develop effective interventions. **Methods.** To protect bats, and reduce the bat-human interface, we first conducted sociological surveys across two communities (n = 670) in southern Nigeria, using the Theory of Planned Behavior (TPB) to identify behavioral drivers of bat meat consumption. The TPB postulates that the intention to perform a behavior is a function of three behavioral antecedents - attitude, subjective norm and Perceived Behavioral control. We used Structural equation modeling to disentangle bat meat consumption behavior to inform the design of a targeted intervention to reduce this behavior and disease risk. **Results.** Attitude, specifically the belief that bat meat tastes good, strongly predicted consumption intentions in both communities. Subjective norm also significantly motivated intention to consume bat meat, with community leaders' opinions exerting the greatest normative influence. Based on these findings, we developed a livestock farming intervention designed to provide alternative meat source with similar taste profile to bat meat, which is now expanding to several communities. **Conclusions.** Reconciling bat conservation with disease risk reduction requires culturally sensitive interventions that address the root causes of risky behaviors. By leveraging community leadership and providing sustainable alternatives, we can reduce bat meat consumption, mitigate zoonotic disease risks, and promote bat-human coexistence. Our findings emphasize the importance of integrating human behavioral models into conservation and public health strategies.

Genomic insights into a Horseshoe bat (Rhinolophus fumigatus/eloquens) cryptic species complex

Author list:

Wickenkamp N[1], Lewis J[1], Kityo R[2], Lutwama J[3], Yiga F[3], Williams K[1], Siya A[2], Nassuna C[3], Nalukenge L[2], Nalikka B[2], Nakayiki T[3], Nabatanzi L[3], Mutebi J-M[2], Matovu B[2], Kayiwa J[3], Hartwick A[1], Harris E[1], Fagre A[1], Dewey T[4], Castle K[5], Azerigyik F[1], Kading RC[1]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA; [2]Department of Zoology, Entomology and Fisheries Sciences, Makerere University, Kampala, Uganda; [3]Uganda Virus Research Institute, Entebbe, Uganda; [4]Department of Biology, Colorado State University, Fort Collins, CO, USA; [5]Wildlife Veterinary Consulting LLC, Fort Collins, CO, USA

Abstract:

Background. Horseshoes bats (Genus *Rhinolophus*) are a species of insectivorous bat known to host a diversity of viruses, including coronaviruses. Despite their importance as viral reservoirs, horseshoe bats, particularly those native to sub-Saharan Africa, remain poorly described. Multiple cryptic species complexes in the genus have yet to be resolved. **Methods.** We collected morphometric and acoustic data from bats captured in the Mount Elgon region of Uganda from 2020-2023. DNA isolated from wing punches was assayed using an established microsatellite protocol prior to whole genome sequencing (WGS). **Results.** Two populations of *Rhinolophus* sp. bats captured in the area differed subtly in morphometric composition and acoustic calls. Principal Component Analysis of biometric data suggested two morphometrically distinct populations of horseshoe bats. Spectrograms of acoustic calls supported this distinction. When consensus trees constructed using existing *Rhinolophus* speciation assays targeting cytb and several nuclear introns failed to differentiate the two bat populations, we used WGS to provide further resolution. We propose the addition of an as-yet undescribed bat species that clusters phylogenetically within *Rhinolophus eloquens/fumigatus* species complex 1. **Conclusion.** We present evidence further resolving the Rhinolophus eloquens/fumigatus species complex using biometric, acoustic, and genomic data from horseshoe bats captured in the Mount Elgon region of Uganda, with implications on both viral surveillance and conservation. We also share information on how to best approach further molecular investigation into the species complex.

Poster Abstracts

Analyzing multiplex immunoassays with three-component Bayesian mixture models from bats in Cambodia.

Poster #1

Author list:

Hitch AT[1,2], Low DHW[3], Borthwick SA[3], Mendenhall IH[3], Smith GJD[3,4]

[1]Museum of Fish and Wildlife Biology, University of California at Davis, Davis CA, USA, [2]Department of Data Analytics, Harrisburg University of Science and Technology, Harrisburg, PA, USA, [3]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, 169857, Singapore, [4]Centre for Outbreak Preparedness, Duke-NUS Medical School, Singapore, 169857, Singapore

Abstract:

Background. A key objective arising from multi-pathogen serological studies is determining the probability of prevalence of each individual to the correct mixture component. **Methods.** We fit a three-component mixture model, with the three components being labeled as seronegative, antibody waning, and seropositive. The number of components was fixed at three because it is biologically of interest to classify the data into three groups and qualitatively the data support the assumption of a three-component mixture model. These components were defined as follows, seronegative was a truncated Gaussian distribution, antibody waning was represented by a Gaussian distribution, and the seronegative component was described as a skew normal distribution. We estimated five general parameters: mean and standard error of each component, the offset, the selection function (transition from one component to the next), and the simplex proportions of each of the components. We chose priors that would allow these parameters to vary at the plate level. The models incorporated antibody cross-reactivity based on genetic distances between antigens. The flexibility of this model allows choice of prior and component shape while accounting for between-plate variation in parameters. **Results.** Our multiplex assay data was comprised of filoviruses, paramyxoviruses, and coronaviruses from 2461 bats sampled from 234 sites across Cambodia. The models allow the calculation of the probability of seroprevalence for a genus or species of bat for any component instead of setting arbitrary cutoffs. This is important since in most cases the posterior densities of the mixture components overlap. Also, we were able to examine influences on seroprevalence based on spatial covariates such as urbanization, habitat type and species life history traits. **Conclusions.** Bayesian three component mixture models based on between-plate variation is a flexible robust analytical method to analyze multiplex immunoassay data. It also allows for the inclusion of spatial, environmental, and species covariates to inform disease surveillance.

Indirect Impacts of Ivermectin Cattle Treatments on Bat Ectoparasites

Poster #2

Author list:

Savage AR[1], Becker DJ[2], Simmons NB[3], Fenton MB[4], Speer KA[5]

[1]Department of Ecology and Evolutionary Biology, Ann Arbor, MI, USA, [2]School of Biological Sciences, University of Oklahoma, Norman, OK USA, [3]Department of Mammalogy, Richard Guilder Graduate School, Division of Vertebrate Zoology, American Museum of Natural History, New York, NY, USA, [4]Department of Biology, The University of Western Ontario, London, ON, CAN, [5]Department of Biological Sciences & Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA

Abstract:

Background. The Neotropics are considered a biodiversity hotspot, but the unintended consequences of large-scale agriculture on neotropical vectors and their hosts have not been well explored. Agricultural pesticides and anti-parasitic drugs can have lethal impacts on non-target organisms, including arthropod vectors of disease. Ivermectin, a commonly used antihelminthic drug for livestock, has high acute toxicity to invertebrates and can persist in the environment. The common vampire bat (*Desmodus rotundus*), which feeds on cattle, may be widely exposed to Ivermectin through contaminated blood meals. Bat flies and other arthropod ectoparasites of vampire bats play an important role in bat pathogen transmission. In this study, we examine the broader consequences of agricultural anti-parasitics on arthropod vectors in vampire bats. **Methods.** We used a combination of previously sampled vampire bat tissues (2016-2024) and samples collected from vampire bats specifically for this project. We confirmed local ivermectin use by collecting dung samples from surrounding cattle pastures and interviewing landowners. To test historical ivermectin prevalence, we used previously collected (2016) dried blood spot samples. All samples were analyzed using Liquid chromatography-tandem mass spectrometry (LC–MS/MS). **Results.** Here, we present data from our 2025 collecting trip. We use a tobit regression for left-censored data, with a lognormal distribution for concentration, to test for effects of distance to farm and concentration of ivermectin on the prevalence of ectoparasites. We anticipate that vampire bats roosting in sites closer to cattle ranch fields with heavy use of Ivermectin will have higher concentrations of ivermectin in their bodies and, as such, will have lower ectoparasite loads than bats with lower concentrations of ivermectin. **Conclusions.** The findings will provide insights into the indirect effects of agricultural practices on bat ectoparasite populations and their role as vectors for pathogens.

The Effect of Venipuncture Site on Hematology of Bats: Implications for Comparative Analyses

Poster #3

Author list:

Roistacher A[1], Demory B[1], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA

Abstract:

Background. Wildlife health comparisons within and across populations and species are essential for population assessment and surveillance of emerging infectious diseases. Due to low costs and high informational yield, hematology is commonly used in the fields of ecoimmunology and disease ecology, yet consistency and proper methods reporting are lacking. Previous investigations on various wildlife taxa have revealed noteworthy impacts of the vein used for blood collection on hematology measures. However, impacts of venipuncture site on bats, a taxon of increasing interest in ecoimmunology and disease ecology, have not yet been tested. **Methods.** Here, we use a long-term study system in western Oklahoma to test the effect of venipuncture site on hematology parameters of the Mexican free-tailed bat (*Tadarida brasiliensis*) and cave myotis (*Myotis velifer*), two abundant and representative bat species from the families Molossidae and Vespertilionidae. Between September 2023-October 2024, we collected paired peripheral blood from both the propatagial and intrafemoral veins in 25 individuals per species. We then measured total red and white blood cells, reticulocyte counts, and leukocyte differentials and used generalized linear mixed models to compare parameters among venipuncture sites within and between bat species. **Results.** Overall, venipuncture site had no effect on any hematology parameters assessed. However, we revealed small differences in neutrophil and lymphocyte proportions between veins among the species. By contrast, we detected significant species-level differences in most cell measurements. **Conclusions.** We propose observed species-level cell measurement differences could be explained by life-history strategy and phylogenetic differences. We emphasize the significance of thorough method reporting in publications to enable transparent comparisons and accounting for even small sampling-based artifacts. All future efforts are especially important for bats to improve conservation monitoring, ecosystem services estimations, and their association with emerging infectious diseases.

Regulatory role of alternative immune gene isoforms in bat immune responses

Poster #4

Author list:

Ordoñez AD[1], Allen H[1], Ray DA[2], Schountz T[3], Chuong EB[1]

[1]University of Colorado, Boulder, Boulder, Colorado, USA, [2]Texas Tech University, Lubbock, Texas, USA , [3]Colorado State University, Fort Collins, Colorado, USA

Abstract:

Background. Bats are notable zoonotic reservoirs due to their unique immune tolerance to viruses. Comparative genomic and molecular transcriptomic studies have identified bat-specific gene adaptations and expression patterns in regulators of inflammatory and antiviral responses. Studies across primates show that alternative isoform transcription plays a crucial role in shaping immune function across species. However, its contribution to bat immune regulation remains largely unexplored. **Methods and Results.** We generated long- and short-read RNA sequencing data from multiple tissues of *Artibeus jamaicensis* and *Eptesicus fuscus* and computationally identified and quantified transcript diversity. We found that immune-related genes are enriched for transcript isoforms. We compiled a list of candidates that contain truncations in key protein domains, suggesting regulatory functions. Further, we compared isoform structure and expression level of these candidates across multiple mammalian species. Some genes of interest with isoforms that may be functionally unique in bats are involved in promoting macrophage activity (CSF1R), regulating T cell immune response (IL4RA) and promoting inflammation (IL18R1). Interleukin-18 Receptor 1 (IL18R1), encodes the receptor for pro-inflammatory IL18. A truncated isoform, IL18R1-short, which lacks most of the intracellular signaling domain, is highly expressed across bat tissues. This isoform is minimally detected or absent in most mammalian species we have looked at, including humans. We have performed functional studies in a human cell line responsive to IL18 that reveal IL18R1-short over-expression dampens signaling. Interestingly, this isoform is also highly expressed in rodents, another group with notable viral tolerance. **Conclusions.** We propose that elevated expression of IL18R1-short in bats may contribute to their dampened inflammatory responses, acting as a molecular sponge to bind IL18 without activating signaling. Its conservation in rodents suggests a shared mechanism among viral reservoir species. Ongoing studies using bat and mouse models aim to further investigate the immunomodulatory role of IL18R1-short.

Seasonal Variation in Parasite Load of Cave-Dwelling Fruit Bats, *Eidolon dupreanum* and *Rousettus madagascariensis*, in Madagascar

Poster #5

Author list:

Andrianiaina AF[1, 2], Andry S[2, 3], Kettenburg G[4], Ranaivoson CH[1,2,4,5], Lacoste V[5], Dussart P[5], Heraud J-M[5], Laverty TM[6], Guth S[7], Young KI[8], Andrianarimisa A[1], Brook CE[4]

[1]Department of Zoology and Animal Biodiversity, University of Antananarivo, Madagascar, [2]Association Ekipa Fanihy, Ambohidrazana, Antananarivo, Madagascar, [3]Department of Entomology, University of Antananarivo, Madagascar , [4]Department of Ecology and Evolution, University of Chicago, IL, United States , [5]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar , [6]Department of Fish, Wildlife and Conservation Ecology, New Mexico State University, Las Cruces, NM, United States, [7]Department of Biology, Skyline College, San Bruno, CA, United States, [8]Department of Biological Sciences, El Paso, TX, United States

Abstract:

Background. Madagascar is home to three species of Old-World fruit bats which two of them are cave dwelling—*Eidolon dupreanum* and *Rousettus madagascariensis*. Over the last decade, knowledge about these bats as reservoirs of potentially zoonotic pathogens has increased. Bat ectoparasites, including mites, fleas, lice, ticks, and bat flies (Nycteribiidae and Streblidae), are known vectors of these pathogens. This study aimed to describe the diversity of ectoparasite infestation in both bat species through morphological observation and DNA barcoding using COI and 18S genes, and to elucidate ecological and climatic correlates of seasonal nycteribiid parasitism of these hosts. **Methods.** We captured alive bats monthly in northern and central-eastern Madagascar from 2013-2020 and collected all observed ectoparasites, counting them by groups and preserving them in 70% ethanol. We used generalized additive models to analyze seasonal variation in abundance and combined cross-correlation analysis with generalized linear models to examine the role of climate in seasonal patterns. **Results.** In total, we captured 873 E. dupreanum and 862 R. madagascariensis, of which 71.9% and 96.4% respectively were parasitized by ectoparasites. And a total of 8 principal types of ectoparasites were identified from these bats but they are commonly parasitized by the nycteribiids *Cyclopodia dubia* (42%) and *Eucampsipoda madagascariensis* (~74%) respectively. Additionally, *R. madagascariensis* was the only species parasitized by the streblid *Megastrebla wenzeli*. We observed significant seasonal variation in nycteribiid abundance on both hosts, which varied by bat sex and was positively correlated with lagged temperature, precipitation, and humidity variables. This seasonal variation correlates with resource availability. **Conclusions.** Understanding the diversity and seasonal ecology of these ectoparasites is important for identifying their potential role in pathogen transmission, considering their status as potential vectors of pathogens.

Bat-borne Issyk-Kul virus: production of monoclonal antibodies and recombinant Nucleoprotein for future diagnostic applications

Poster #6

Author list:

Castelli A[1], Corsa M[1], Soldati R[1], Facchini E[1], Maccarinelli F[1], Lelli D[1], Pezzoni G[1]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini" (IZSLER), Brescia, Italy

Abstract:

Background. Issyk-Kul Virus (ISKV) is a bat-borne neglected zoonotic agent recently detected in Italy from *Hypsugo savii*. Bats and ticks are both reservoirs of ISKV with transmission to humans being associated with tick bites and exposure to bat faeces and urine. Climate change is increasing the risk of arboviral zoonoses and timely diagnostics are essential for disease control and management. In the present study, we describe the production of the ISKV recombinant Nucleoprotein (rNP) and the characterization of monoclonal antibodies (mAbs) against ISKV produced using hybridoma technology. **Methods.** Two BALB/C mice were immunized with the purified inactivated ISKV. The mAbs-producing hybidomas were screened by indirect ELISA and Western Blotting (WB) using the purified virus and by immunoperoxidase (IP) assay on ISKV-infected cells. To further characterize the mAbs, the viral NP was produced in E.coli giving a yield of 2mg/L. The rNP and the mAbs were evaluated in WB and indirect ELISA. Several mAbs were tested with a serum from rabbit experimentally infected with ISKV in a cELISA with coated rNP. **Results.** A total of 69 hybridomas reacted against ISKV in IP and seven recognized a viral protein of 55 kDa, presumably corresponding to the viral NP in WB. The rNP was recognized in WB by all the seven mAbs that reacted with the viral NP antigen and five of them competed in cELISA. Further three mAbs recognized rNP in indirect ELISA, proving to react with conformational epitopes. **Conclusion.** The preliminary results are encouraging that the produced reagents can be used for the development of ISKV-specific assays. Since the NP is the primary target of the humoral immune response, the combination of mAbs and recombinant protein is a key factor in producing robust diagnostic assays and overcoming biosecurity issues due to the use of viral antigens.

Characterizing Egyptian rousette bat humoral responses to zoonotic viruses

Poster #7

Author list:

Roffler AA[1,2] , Glassey E [1], Forbes KM [3], Sironen T [4,5], Schmidt AG [1,6]

[1]Ragon Institute of Mass General, MIT, and Harvard, Cambridge, MA, USA, [2]Department of Medical Sciences, Harvard Medical School, Boston, MA, USA, [3]Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA, [4]Department of Virology, University of Helsinki, Helsinki, Finland, [5]Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland, [6]Department of Microbiology, Harvard Medical School, Boston, MA, United States

Abstract:

Bats are reservoirs for zoonotic viruses of pandemic potential (e.g., ebolaviruses, coronaviruses), yet they appear to tolerate these viruses without demonstrable immunological effects or succumbing to disease. Understanding how bats remain largely asymptomatic to potentially zoonotic viruses can provide insight into fundamental bat immunology and allow comparisons to human immunology. Previous studies in bats largely focused on characterizing their innate immune responses to viruses, but less is known about the role of their adaptive immunity. In humans, antibody-mediated neutralization is essential for protection against many viruses. Although bats can mount virus-specific serum antibody responses after a viral infection, there are limited tools to study these responses at the single B cell or monoclonal level; biochemical, biophysical, and functional characterization of the antibody repertoire is essential to understand the role, if any, of antibodies controlling viral pathogenesis. Here, we isolated total splenic RNA from wild-caught Egyptian rousette bats to construct a yeast-display library for cell-surface expression of bat antibodies. From this library, we can interrogate bat monoclonal antibodies to various surface-exposed glycoproteins from viruses of zoonotic potential. As a proof-of-principle, we used the influenza hemagglutinin from H9N2 virus and characterized the isolated monoclonal antibodies for their gene usage, degree of somatic hypermutation, binding affinities, and breadth. Collectively, these data show how such libraries can aid in our understanding of bat adaptive immunity and, more broadly, host-virus interactions.

Ecological and Behavioral Risk Factors for Nipah Virus Spillover in Bangladesh

Poster #8

Author list:

Islam A[1] , Choudhury DS[2] , Amin E[2] , Munro S[3] , Khan AKM[2] , Chowdhury NN[2], Islam M[2] , Khan A[2] , Mamun AI[2] , Kain M[3], Sultana S[2], Kaczmarek M[3] , Forwood JK[1] , Shirin T[2], Epstein JH[3]

[1]Gulbali Research Institute, Charles Sturt University, Wagga Wagga, NSW 2678, Australia , Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh , [3]EcoHealth Alliance, New York, NY, USA.

Abstract:

Background. Nipah virus (NiV) is an emerging bat-borne virus that has caused repeated outbreaks in Bangladesh and India with over 70% mortality. NiV outbreaks are spatially clustered in Bangladesh, predominantly linked to consumption of bat-contaminated raw date palm sap (RDPS) in the Western region (Nipah Belt). Nevertheless, NiV has been detected in bats across the country. It is therefore unclear what drives the pattern of NiV occurrence in human populations. Hence, this study aimed to determine how behavioral and ecological drivers contribute to spatial differences of NiV outbreaks in Bangladesh. **Methods.** Between 2022 and 2023, we designed the human study with the underlying goal of determining how behavioral and ecological drivers contribute to spatial differences in exposure and outbreak risk. We aimed to administer human behavioural and ecological questionnaires from 2400 individuals, comprising 400 per site among six sites. Sampling was conducted at the household level, and participating households were selected through a randomization process within communities. **Results.** The study revealed a significantly higher proportion of households in Nipah belt that consume raw date palm sap (RDPS) (55.5% vs 16.8%), harvesting RDPS (13.4% vs 1.6%), owning date palm trees (28.6% vs 7.2%), grazing domestic animals under bat roosts (43.5% vs 15.3%), and eat bat-bitten fruits (43.2% vs 29%) compared to households outside of the Nipah belt. Using a generalized linear mixed-effects model (GLM), we found that gender, education, income, harvesting sap, and owning a date palm tree significantly influenced the consumption of RDPS and were associated with NiV outbreaks in Bangladesh. **Conclusion.** The higher consumption of RDPS contaminated with bat excreta in western regions could conceivably drive a spatial discrepancy in NiV outbreaks. We recommend One Health surveillance and behavioral interventions to prevent bat-borne viral spillover from bats to humans and domestic animals in Bangladesh.

A shotgun pan-coronavirus library for entry receptor discovery and antibody specificity

Poster #9

Author list:

Taylor AL[1], Matson MJ[2], Starr TN[3]

[1]Department of Biochemistry, University of Utah, Salt Lake City, UT, USA, [2]Division of Infectious Diseases, University of Utah Health, Salt Lake City, UT, USA, [3]Department of Biochemistry, University of Utah, Salt Lake City, UT, USA

Abstract:

Background. Coronaviruses circulating in bats and other wildlife reservoirs continue to pose a spillover risk for humans. Entry receptors such as ACE2, APN, or DPP4 have been identified for viral lineages from which spillover has already occurred; however, receptors remain unknown for many coronaviruses, particularly lineages that have only been observed in bats and other wildlife thus far. Elucidation of these unknown protein receptors would enable characterization of zoonotic potential and therapeutic development for these understudied bat coronaviruses. **Methods.** We constructed a library containing >500 coronavirus spike receptor-binding domain (RBD) sequences spanning all known alpha-, beta-, gamma-, and delta-coronavirus subgenera available from NCBI. We cloned and expressed our RBD library in a yeast-surface display platform, allowing for multiplexed measurement of RBD protein expression, binding affinity to receptor proteins, and breadth of antibody cross-reactivity across all classes of coronaviruses. **Results.** We have conducted initial receptor-screening experiments to determine the range of coronavirus RBDs capable of binding ACE2 (human, cat), DPP4 (human), and APN (human, cat, dog, pig) receptor orthologs. Ongoing analysis of these datasets is validating the approach based on known receptor-specificities in the literature. New binding activities against these receptor orthologs are being validated via spike-pseudotyped lentiviral entry assays. **Conclusions.** Having validated the library and approach, we now have a high-throughput screening tool useful for assays such as receptor discovery and antibody specificity. Future work will screen additional host proteins and novel candidate receptors against this library to identify novel mechanisms of animal coronavirus entry.

Impacts of habitat fragmentation and host reproductive status on Bartonella infection in Neotropical fruit bats

Poster #10

Author list:

Ansil BR[1], Olbrys BL[1], Dyer KE[1], Lock LR[1], Vicente-Santos A[1], Simonis MC[2], Fenton MB[3], Simmons NB[4], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA, [2]College of Forestry, Wildlife and Environment, Auburn University, Auburn, AL, USA, [3]Department of Biology, Western University, London, ON, Canada, [4]Department of Mammalogy, Division of Vertebrate Zoology, American Museum of Natural History, New York, NY, USA

Abstract:

Background. Infection status is shaped by intrinsic and extrinsic factors, including life history stages and environmental conditions. Here, we sampled Neotropical frugivorous and nectarivorous bats with varying life history stages in a small and a large forest fragment embedded within a land-use matrix. We hypothesized infection risk would be greatest in small degraded forests, for reproductive bats, and in bats with poor body condition. **Methods.** We sampled select frugivorous (*Artibeus jamaicensis*, *Sturnira parvidens*, *Carollia sowelli*, and *Uroderma convexum*) and nectarivorous (*Glossophaga mutica*) bats from the Orange Walk District of Belize annually from 2021 to 2024. We collected blood on FTA cards, extracted DNA, and used nested PCR to target the Bartonella gltA gene. Positives were Sanger sequenced and analyzed to determine phylogenetic identities. We then tested the effects of site, year, age, sex, reproductive status, and body condition using generalized linear mixed models, accounting for species identity as a random effect. **Results.** We detected Bartonella in 154 of 293 (52.5%, 95% CI: 46.8–58.2%) samples across five bat species over four years. We did not observe differences in the odds of infection by site, age, sex, reproductive status, or body condition. However, risk of infection did significantly increase over time (odds ratio = 1.036, $P < 0.001$). Our phylogenetic analysis using a subset of gltA sequences (n=81) yielded 21 lineages with varying phylogenetic associations, suggesting considerable genetic diversity of bartonellae in these bats. **Conclusion.** Our preliminary results suggest Bartonella infection in Neotropical frugivorous and nectarivorous bats is not associated with energetic trade-offs or site-specific habitat quality. However, increasing prevalence over time could reflect the broader, increasing land conversion across the region or climatic effects. Ongoing work will investigate co-infection and physiological stress as potential factors shaping infection dynamics.

What are your bats really eating? Bioaccumulation of antiviral compounds and the future of viral resistance in bats

Poster #11

Author list:

Rosi E[1], Fick JB[2], Sindiku O[2], **Han BA**[1]

[1]Cary Institute of Ecosystem Studies, Millbrook, NY, USA, [2] Department of Chemistry, Umeå University, Umeå, Sweden

Abstract:

Background. Emerging viral diseases pose a significant threat to human health, prompting the rapid development and deployment of antiviral therapies. While antivirals and vaccines offer crucial protection, their widespread use has unintended environmental consequences. We investigated the potential for antiviral contamination of freshwater ecosystems and its impact on bat populations, which are known reservoirs for various zoonotic viruses. **Methods and Results.** Using SARS-CoV-2 as a case study, we identified a global overlap between bat habitats and elevated concentrations of pharmaceuticals in surface waters. Our analyses, placed in context with existing literature, suggest that antiviral contamination of freshwater can expose insectivorous bats through their consumption of emerging aquatic insects. This exposure could drive the evolution of antiviral-resistant viruses within bat populations, a phenomenon which has already been observed for waterfowl and antivirual-resistant strains of avian influenza. Despite evidence of such interactions, to date, no targeted sampling has been conducted for antivirals in bats. **Conclusions.** Understanding the ecological pathways of antiviral exposure and the potential for resistance development is critical. This includes investigating antiviral bioaccumulation in aquatic insects, the dietary habits of insectivorous bats, and the toxicokinetic properties of antivirals in these species. Addressing these urgent research frontiers is essential to ensure the long-term effectiveness of antiviral therapies and mitigate potential risks to public and environmental health. We are launching a fully funded surveillance campaign for antivirals in insectivorous bats and invite the BatID community to join us.

Functional and antigenic landscape of the Nipah virus entry proteins

Poster #12

Author list:

Larsen BB[1], McMahon T[1], Brown JT[2], Wang Z[2], Radford CE[1], Crowe JE[3], Veesler D[2,4], Bloom JD[1,4]

[1]Basic Sciences Division and Computational Biology Program, Fred Hutch Cancer Center, Seattle, WA, USA; [2]Department of Biochemistry, University of Washington, Seattle, WA, USA; [3]Department of Pathology, Microbiology and Immunology, The Vanderbilt Vaccine Center, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA; [4]Howard Hughes Medical Institute, Seattle, WA, USA

Abstract:

Background. Nipah virus is a deadly zoonotic virus that periodically spills over from bats into humans across SE Asia. Nipah virus expresses two different surface proteins, the Receptor Binding Protein (RBP, also called G), and the Fusion protein (F), which coordinate entry into cells. Understanding the phenotypic effect of mutations in these two proteins is critical for a better understanding of Nipah virus evolution and selection of effective monoclonal antibody cocktails and vaccines. **Methods.** We utilized deep mutational scanning based on pseudo typed lentivirus to safely measure the effects of all possible mutations (>9,000 mutations) in RBP and F for different phenotypes, including cell entry, receptor binding, and antibody escape. We subsequently validated the deep mutational scanning measurements across these different phenotypes. **Results.** We identified sites in both RBP and F that are highly mutationally constrained and likely indispensable for proper function. We also characterized specific mutations that differentially affect binding to two distinct host receptors, ephrin-B2 and ephrin-B3. Additionally, we obtained comprehensive measurements of all possible antibody escape mutations from a suite of monoclonal antibodies. **Conclusions.** Our findings provide critical insight into the potential functional and antigenic evolution of Nipah virus that can inform the development of effective antibody therapies and vaccines.

Integration of anthropogenic roosting ecology data in predictive models of viral outcomes in bats

Poster #13

Author list:

Betke BA[1], Gottdenker N[2], Meyers LA[3], Becker D[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]Department of Veterinary Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA; [3]Department of Integrative Biology, University of Texas at Austin, Austin, TX, USA

Abstract:

Background. The ability to live in anthropogenic structures (e.g., buildings, bridges, homes, tunnels) is observed widely across mammals, allowing potential points for bidirectional viral transmission at the human–wildlife interface. Anthropogenic roosting is especially relevant for bats in the context of viral spillover and spillback. However, the trait profile of bat species with the ability to live in these structures has not been characterized owing to a sparsity of standardized data, which in turn has hampered efforts to understand how this trait affects our ability to predict viral risks. Here, we summarize two projects to (1) describe what makes an anthropogenic roosting bat and (2) understand the importance of this phenotype in predicting viral outcomes in bats. **Methods.** We compiled a dataset of bat roosting ecology (anthropogenic vs. natural roosting) across 1279 bat species from existing literature. After harmonizing the roosting ecology data with standardized trait data, we applied machine learning and phylogenetic techniques to describe the trait profile of anthropogenic roosting. Subsequently, we evaluated how the importance of anthropogenic roosting ability in predicting viral outcomes (viral richness, zoonotic proportion, viral and zoonotic virus hosting) across bat species ranked among known predictors in the literature. **Results.** Our first analysis highlights that anthropogenic roosting can be predicted on the basis of ecological traits. Anthropogenic roosting is associated with larger geographic ranges, habitat generalism, temperate zone distributions, small litter and body size, and insectivory. Despite little improvement in classifier performance when including anthropogenic roosting in host prediction models, our second analysis showed that the inclusion of this trait lengthened the list of undetected hosts of zoonotic viruses. **Conclusions.** The results of this work could be useful for surveillance of zoonotic hosts specifically at the human–wildlife interface. Future directions include expanding these approaches to full link predictions between bat species and virus families.

Viral epidemic potential is not uniformly distributed across the bat phylogeny

Poster #14

Author list:

Cummings CA[1], Vicente-Santos A[1], Carlson CJ[2], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]Department of Epidemiology of Microbial Diseases, Yale University School of Public Health, New Haven, CT, USA.

Abstract:

Background. Characterizing host–virus associations is critical due to the rising frequency of emerging infectious diseases originating from wildlife. Past analyses have evaluated zoonotic risk as binary, but viral epidemic potential (i.e., virulence, transmissibility, and death burden) can vary dramatically. Recent work suggests bats harbor more viruses with high virulence in humans than other taxa. However, it remains unknown whether all bats harbor viruses of equal viral epidemic potential. **Methods.** We hypothesize that measures of viral epidemic potential cluster within clades of bats, based on the unique coevolutionary histories between different bats and viruses. We use a flexible graph-partitioning algorithm to identify clades of mammals with unusually high or low viral epidemic potential without the need to a priori assume a specific phylogenetic scale. Next, we mapped the geographic distributions of any identified bat clades with unusually high viral epidemic potential in tandem with anthropogenic footprint, visualizing hotspots of zoonotic risk. **Results.** We found that virulence, transmissibility, and death burden only cluster within specific bat clades, often composed largely of cosmopolitan families. Mapping the geographic distributions of these bat clades with anthropogenic footprint data suggests high zoonotic risk in coastal South America, Southeast Asia, and equatorial Africa. **Conclusions.** Contrary to prior analyses focusing on how bats differ from other mammals, our results find that viral epidemic potential is heterogeneous among bat species. The bat family Rhinolophidae exhibited unusually high death burden, corroborating previous analyses linking these bats to high-impact viruses. The cosmopolitan bat superfamilies Emballonuroidea and Vespertilionoidea exhibited high virulence despite low sampling effort, reinforcing the idea that zoonotic risk patterns are not merely outcomes of sampling bias but, instead, phenomena related to bat evolution and ecology. Our findings deepen our understanding of host–virus networks and identify clades for prioritizing viral surveillance and future studies characterizing mechanisms of viral tolerance.

The genetic basis for the convergent gain of human ACE2 binding in bat sarbecoviruses

Poster #15

Author list:

Craig CJ[1], Starr TN[1], Taylor AL[1]

[1]Department of Biochemistry, University of Utah, Salt Lake City, UT, USA

Abstract:

Background. Animal viruses pose a threat when they evolve traits that enable spillover into humans. The first step necessary for many viruses to cross species boundaries is to bind with high affinity to novel host receptors, allowing entry to initiate infection. I study the origin of a trait that underlies recent pandemic spillover events: the convergent evolution of human ACE2 usage in bat SARS-related coronaviruses (sarbecoviruses), which occurred twice independently during historical evolution from a bat-ACE2-specific ancestor – once on the trajectory to the common ancestor of SARS-CoV-1 and SARS-CoV-2, and on an independent trajectory to the European bat sarbecovirus Khosta-2. None of the substitutions along these branches are shared, suggesting a divergent genetic and functional basis for the convergent phenotypic gain of human ACE2 usage. **Methods.** To isolate the evolutionary interval when receptor usage changed in sarbecoviruses, we performed phylogenetic reconstructions of ancestral receptor-binding domains and characterized receptor-binding specificities. To identify the causal mutations that endowed human ACE2 binding along each evolutionary trajectory, we developed combinatorial libraries expressed in our yeast surface display platform and sorted cells for binding affinity using FACS. **Results.** We identify the evolutionary causal sequence substitutions that enabled the gain of human ACE2 binding in bat sarbecoviruses, including the role of epistasis in this historical evolutionary transition. We have identified an epistatic interaction between T498Y and a four amino acid insertion at residue 447 that enabled binding to human ACE2 (and ACE2 from many other species) on the trajectory to the common ancestor of SARS-CoV-2 and SARS-CoV-1. We compare this genetic and phenotypic solution to that utilized by Khosta-2, emphasizing the diverse mechanisms by which human receptor usage can evolve. **Conclusions.** This work identifies the molecular mechanisms of a dramatic change in protein function while informing efforts to predict spillover potential from this diverse viral lineage.

A minimum data standard for wildlife disease research, and the case for FAIR bat infection data

Poster #16

Author list:

Sánchez CA[1], Schwantes CJ[1], Carlson CJ[1]

[1]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA

Abstract:

Background. Wildlife infection data are shared with varying levels of detail, making it difficult for scientists to integrate data from multiple studies to understand broader infection patterns. For example, out of 110 studies included in a meta-analysis of coronavirus prevalence in bats, only 14 provided data at the individual (host) level, and just 3 reported both positive and negative results. Further, studies often differ in the data fields (i.e. columns) they collect and report, such as details related to host sampling, host identification and traits, and parasite detection and identification. **Methods.** To address these issues, we present a simple and flexible data standard for reporting wildlife disease data, which could be usefully applied to bat infection data. We developed the data standard through an iterative process using real-world data, informed by our experience conducting and publishing wildlife disease research. **Results.** The data standard is designed to be accessible to a range of users and to help researchers publish data that are Findable, Accessible, Interoperable, and Reusable (FAIR). We describe when and how to use the data standard, provide an example of its application on a survey for coronaviruses in vampire bats, and share thoughts on best practices for sharing data and publishing datasets. **Conclusions.** We hope researchers, especially those who study bats, will consider using this data standard to ensure their data are FAIR and meet journal and funder requirements for data sharing. Progress toward open science will make wildlife disease research a richer and more rigorous field, leading to better insights about emerging threats to human and animal health.

Reviewing experimental infection studies in the bat virus system

Poster #17

Author list:

Voirin CJ[1], Brook CE[1]

[1]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA

Abstract:

Introduction. Bats are important viral reservoirs, and understanding their within-host viral dynamics is essential for determining spillover risks. Long-term field projects provide essential information for understanding emerging zoonoses but are often challenged by limited temporal resolution, lack of controlled conditions, and the low probability of resampling the same hosts multiple times. These factors hinder efforts to identify causal features that underpin replication and transmission for zoonotic viruses. Experimental infections allow researchers to avoid these shortcomings and isolate causative mechanisms by which bats manage disease, through detailed tracking of immune responses and viral shedding trends to an extent not possible in a field setting. Here, we critically reviewed and compiled prior experimental infection studies in the bat virus system. **Methods.**

To identify studies, we conducted a keyword search in Web of Science™, limiting our review to viral infections in any species of bat. We collected all published studies that fit our criteria and recorded details such as bat species, virus, presence of viral shedding, assayed immune responses, and if there was any indication of infection-driven pathology. **Results.**

Experimental infections were performed across a variety of bat species, but *Rousettus aegyptiacus*, *Artibeus jamaicensis*, and members of the *Pteropus* genus are overrepresented taxa. As expected, filovirus, henipavirus, and lyssavirus experiments dominated the literature, though coronavirus increased in frequency following the COVID-19 pandemic. Studies tracked antibody responses more frequently than cell-mediated immunity (CMI), although very recent studies suggest this trend may be changing. Evidence of viral pathology was found in very few studies, excepting those documenting rabies infection.

Conclusions. Our work highlights the important insights generated by experimental infections in host-virus systems. In future experimental infections, researchers may find that attempting to measure CMI, implementing diet manipulations, and performing comparative studies between multiple species would help to elucidate the mechanisms behind bats' disease management.

Understanding the Association Between Introgression, Immunogenetic Variation, and Viral Diversity in Belizean Bats

Poster #18

Author list:

Graham CR[1], Roistacher A[2], Ladner J[3], Contreras P[1], Lasisi T[1], Becker D[2], Simmons N[4], Fenton MB[5], Speer K[3]

[1]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA; [2]Department of Anthropology, University of Michigan, Ann Arbor, MI, USA; [3]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA; [4]American Museum of Natural History, New York, NY, USA; [5]Department of Biology, Western University, London, ON, Canada

Abstract:

Background. The arms race between hosts and parasites shapes immune evolution, with introgression—gene transfer through hybridization—potentially facilitating the acquisition of beneficial immune traits. While bats are known for their remarkable immune tolerance, the role of introgression in shaping their immune responses and infection susceptibility remains underexplored. This study focuses on *Myotis elegans* and *M. pilosatibialis* from the Orange Walk District of Belize, insectivorous species with a history of ancestral introgression. We compare them to *Neoeptesicus furinalis*, another insectivorous species with a history of introgression, and to *Artibeus* (frugivorous) and *Saccopteryx bilineata* (insectivorous), which lack this history. Additionally, we explore how physiological stress influences pathogen and antibody diversity to assess potential trade-offs between stress and immune function. **Methods.** We are evaluating pathogen exposure, by using PepSeq, a multiplexed platform that detects antibodies against viral and other pathogen peptides. We also measured cortisol levels from fur samples to examine the relationship between physiological stress and immune function. **Results.** We anticipate *Myotis* bats to have lower antibody and pathogen diversity due to their history of ancestral introgression, which may contribute to enhanced immune tolerance. In contrast, we expect that bats with higher cortisol concentrations will exhibit lower antibody diversity due to stress-induced immune suppression, which can weaken the body's ability to fight infections. **Conclusion.** This study explores the intersection of introgression, physiological stress, and pathogen exposure in Belizean bats. Understanding how introgression shapes bat immunity will provide broader insights into host-parasite coevolution and reveal whether hybridization is an adaptive mechanism for disease tolerance. Ongoing immune gene expression analyses will further clarify links between introgression, stress, and pathogen diversity.

Eptesipox virus successfully replicates in cell lines from multiple species and inhibits host protein kinase R pathway in a species-specific manner

Poster #19

Author list:

Zhang C[1], Acharya S[1], Heye K[1], Brennan G[1], Tazi L[1], Rothenburg S[1]

[1]Department of Medical Microbiology and Immunology, School of Medicine, University of California - Davis, Davis, CA, USA

Abstract:

Background. Eptesipox virus (EPTV) is a poorly characterized poxvirus isolated from multiple big brown bats (*Eptesicus fucus*) who presented with swollen and contused joints due to necrosuppurative osteomyelitis. A key determinant of virus replication is its ability to evade the host immune response, including the protein kinase R (PKR) pathway, which is activated by viral double-stranded RNA (dsRNA). Most poxviruses encode two PKR inhibitors, called K3L and E3L in vaccinia virus. Uniquely among poxviruses, EPTV contains two E3L paralogs, in addition to a K3L ortholog. This study investigates the inhibition of bat PKRs by the three putative EPTV PKR inhibitors and tests the hypothesis that they target PKRs from different host species. **Methods.** We generated EGFP-expressing EPTV and infected 22 cell lines from 16 different bat and mammalian species to characterize its replication. To study the interaction of PKR with EPTV inhibitors, we are using a combination of in vitro luciferase-based reporter assays and virus replication assays in cell culture. We are also in the progress to knock out PKR inhibitors, individually or in combination to determine their roles in virus replication. **Results.** EPTV was able to replicate efficiently in 17 out of the 22 tested cell lines, including all tested bat cell lines. In reporter assays, EPTV PKR inhibitors exhibited species-specific activities against PKR from multiple bat species. EPTV E3L2 showed stronger PKR inhibition than E3L1, which might be due to specific mutations in the dsRNA-binding domain of E3L1. **Conclusions.** EPTV replicates in a broad range of cell lines and encodes PKR antagonists that exhibit host species-specific PKR inhibition. Our study lays the foundation for studying EPTV biology and pathobiology and expands our knowledge of viral proteins targeting the bat immune response.

Investigating pathogen-driven immune gene selection using de novo genome assemblies

Poster #20

Author list:

Whitehurst CH[1], Frank HK[1]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA

Abstract:

Background. Bats are important hosts for numerous pathogens affecting human health. However, bats rarely exhibit severe disease from these infections suggesting a long co-evolutionary relationship between bat hosts and their pathogens. Understanding how bats have evolved in response to their pathogens can provide insights into bat health and pathogen-tolerance mechanisms, with implications for human health and conservation. However, the diversity of bats complicates efforts to understand their unique immunology. Investigating positive selection in immune genes across species can garner insights into the diversity and commonalities of bat immunity that underlie their relationship with pathogens. **Methods.** We generated ~20X Illumina coverage of the whole genomes of 60 bat species across 5 bat families to explore the relationship between bat evolutionary history and pathogen pressure. De novo genome assemblies were generated using w2rap-contigger (https://github.com/bioinfologics/w2rap-contigger). From these genomes we are pulling immune-related genes of interest using BLAT (BLAST-like alignment tool) and inferring selection using the adaptive branch site-test of positive selection (aBSREL). **Results.** Thus far, we have generated de-novo gene assemblies for 29 species across 5 families. The assembled genomes have N50 values ranging from 1,343 to 106,958 (mean = 21,890, SD = 4,182). Initial results from a subset of pattern recognition receptors indicate that positive selection is more frequent in lineages living in areas associated with higher zoonotic risk due to spillovers with high human mortality. **Conclusions.** Efforts like Bat1K to sequence large numbers of bat genomes demonstrate the power of whole genomes to examine host-pathogen coevolution in these unique reservoirs. Our genomes will expand taxonomic coverage, bolstering tests of ecological and evolutionary hypotheses. Evidence of greater selection in higher-risk areas suggests that bats are not a monolith, and genomic analyses can reveal evolutionary drivers for current ecological patterns, as well as areas for further research.

Decrypting antiviral responses in Eptesicus bat cells

Poster #21

Author list:

Delon C[1,2], Gracias S[1], Banerjee A[3], Caval V[1], Jouvenet N[1]

[1]Virus Sensing and Signaling Unit, Department of Virology, Institut Pasteur, Université de Paris Cité, CNRS UMR3569, Paris, France; [2]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD, USA; [3]Vaccine and Infectious Diseases Organization, University of Saskatchewan, Saskatoon, SK, Canada

Abstract:

Background. Bats are resistant to pathogens, including viruses that cause severe diseases in humans, such as Ebola virus, Nipah virus, and Coronaviruses. Despite active viral replication and detection of high viral loads, bats rarely succumb to viral infection. Previous investigations have depicted multiple unique evolutionary adaptations in the bat innate immune pathways, rendering them able to tolerate viral infections. Our project aims at investigating bat innate immune responses, with a focus on Eptesicus genera, which is a reservoir for viruses known to cross species barrier. More precisely, we aim to identify and characterize interferon stimulated genes (ISGs) which have antiviral properties against a panel of viruses, including Eptesicus-borne viruses, such as the orthoflavivirus Rio Bravo Virus and the European bat lyssavirus 1. **Methods.** To identify ISGs expressed in cells from this bat genera, we performed an RNA sequencing analysis of five cell lines isolated from *Eptesicus serotinus* and *Eptesicus fuscus*, as well as two human cell lines. Cells were stimulated with universal Interferon type I to activate the antiviral state. **Results.** This comparative transcriptomics analysis revealed unique features of
Eptesicus cells comparatively to human cells. Amongst the differentially expressed genes, 34 genes were common to all cell lines and 41 specifically to all Eptesicus cells, including one non-coding RNA and 34 LOC genes that have no orthologs described as immune genes in other mammalian species. Amidst the latter, numerous duplications were identified,suggesting their positive selection over time. Functional characterization, using gain-of-function approaches, are on-going to assess the potential antiviral activities of these
Eptesicus-specific ISGs. **Conclusion.** Uncovering unique features of the Eptesicus antiviral program will provide a better understanding of the molecular interplays between bats and viruses, as well as insights into the innate immune responses of these important viral reservoirs.

Bat viromes from Peninsular and Northeastern India

Poster #22

Author list:

Darshan S[1], Ansil BR[1], Ramakrishnan U[1]

[1]National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru, KA, India

Abstract:

Background. Zoonotic viruses can overwhelm health systems and burden economies. Meta-analyses have predicted that mammalian diversity, human density and high land-use change are important correlates of zoonotic EIDs. This makes tropical countries including India hotspots for zoonoses. Yet our knowledge about animal-associated viral diversity, and their implications to human health remains poor in India. Among zoonotic reservoirs, bats exhibit remarkable ecological and physiological traits, making them key players in the emergence of infectious diseases. India in particular houses over 130 species of bats, yet only very few species have been sampled to characterize to understand their viral diversity. We are in the process of characterizing bat-borne viral diversity in India. We hope our work aids preparedness strategies for future bat-borne virus hazards. **Methods.** We sampled eight species of bats in India (2 from Northeastern India, which involved invasive samples and 6 from Southern India, which involved under the roost sampling of guano). We sequenced viruses by metatranscriptomics as well as capture probe-based methods. **Results.** From eight species sampled (*R. lescenualtii, E. spelaea, P. medius, C. sphinx, H. speoris, H. lankadiva, R. hardwickii* and *L. lyra*) overall we detected 63 bat-virus associations of which 48 were novel. Viruses we detected include coronaviruses, astroviruses etc.. **Conclusion.** Our preliminary results indicate previously undiscovered bat-virus associations are necessary to better understand viral diversity in bat communities. Expanding our work by sampling more species on a broader biogeographic scale would help our effort in alleviating the lack of knowledge on viral diversity in bats and facilitate further research in India.

Elucidating the evolutionary pressures underpinning sarbecovirus zoonosis

Poster #23

Author list:

Tafoya DJ[1], Starr TN[1]

[1]Department of Biochemistry, University of Utah, Salt Lake City, UT, USA

Abstract:

Background. RNA viruses contribute to a significant proportion of animal-human viral transmission events. SARS-CoV-1 and SARS-CoV-2 represent two RNA viruses that have reached epidemic and pandemic magnitude, respectively, in the past two decades. Though just these two sarbecoviruses have risen to epidemic levels, serological surveillance of individuals living at animal-human interfaces have shown that sarbecovirus transmission events occur more frequently than previously expected - suggesting that circulation of these viruses within their natural hosts fosters traits that mediate transmission into humans. The initial barrier to overcome in zoonosis is the acquisition of binding to the human ortholog of the viral entry receptor. Angiotensin-converting enzyme 2 (ACE2) is defined as the ancestrally shared entry receptor of sarbecoviruses, and its interaction with the receptor binding domain (RBD) of the viral spike glycoprotein initiates the viral lifecycle. The natural hosts of sarbecoviruses are Rhinolophus bats, a diverse, sympatric genus that exhibits high ACE2 diversity across and within species. Due to this ACE2 diversity and co-roosting of bats, sarbecoviruses have adapted a way to bind and infect a broad range of Rhinolophus species and we believe these pressures cause serendipitous binding to the ACE2 human ortholog (HsACE2). However, whether host plasticity is causative to zoonosis has not been experimentally tested. We hypothesize that promiscuous binding to a diverse population of ACE2 orthologs within Rhinolophus species drove the adventitious binding to HsACE2 (coined “promiscuous pressures” hypothesis). **Methods.** Combining yeast surface display and an in-vitro molecular evolution platform, we aim to model and define the evolutionary pressures that drove human ACE2 binding in the sarbecovirus lineage. **Conclusion.** Understanding the evolutionary pressures facilitating mutations that enable spillover will allow us to better understand how viruses overcome cross-species entry barriers.

Insight on virus discovery studies in bats in Italy

Poster #24

Author list:

Lelli D[1], Lavazza A[1], Trogu T[1], Sozzi E[1], Carrera M[1], Castelli A[1], Tolini C[1], Bertasio C[1], Moreno A[1]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Abstract:

Background. The diversity of viral agents found in bats worldwide is still largely unknown, and it is thought that many viruses are yet to be discovered and characterized. Herein, we report the most relevant results in terms of viral detection obtained within a survey implemented in Italy for discovering emerging viruses associated with bats. **Method.** The survey involved analyzing dead animals collected from bat rehabilitation centers since 2010. Fresh carcasses were necropsied, and tissue specimens were sampled and examined through a diagnostic protocol broadly targeting viral agents. This approach was set up using both "classical" virologic methods (virus isolation and negative staining electron microscopy) and molecular techniques (PCRs and NGS) in order to enhance the possibility of detecting newly emerging viruses. **Results.** Almost 1800 tissue samples from 19 different bat species (mostly *Pipistrellus kuhlii* and *Hypsugo savii*) were collected and analyzed. The survey provided evidence that insectivorous bats carry a variety of Mammalian orthoreoviruses (including new reassortant strains) and coronaviruses, both AlphaCoVs and BetaCoVs (MERS and SARS-related CoVs). A novel and previously unknown rhabdovirus belonging to the genus Ledantevirus was isolated from organs of a Pipistrellus kuhlii and named Vaprio virus. Adenovirus type 2, strongly related to a virus previously detected in bats from Germany in 2007, re-emerged in a new area in Northern Italy. Moreover, Hypsugopoxvirus (HYPV) was identified from *H.savii*, and full-genome sequencing clearly demonstrated that HYPV is a new virus that is distantly related to its closest known relative Eptesipoxvirus isolated from bats in USA. The bat-borne neglected zoonotic Issyk-kul virus (orthonairovirus genus) was also isolated and characterized. The most recent research developments for each of these new / emerging viruses will be presented. **Conclusions.** These findings provide new targets for the development of specific diagnostic and surveillance assays and open new perspectives for viral research in bats.

Interferon signaling in a Jamaican fruit bat organoid model of vesicular stomatitis virus infection

Poster #25

Author list:

Sebrell A[1], Madden S[1], Russel D[1], Quintela R[2], Cherne M[1], Hashimi M[1], Rynda-Apple A[1], Boulant S[3], Banerjee A[2], **Bimczok D[1]**

[1]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA; [2]Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK, Canada; [3]Department of Molecular Genetics and Microbiology, University of Florida, College of Medicine, Gainesville, FL, USA

Abstract:

Background. Compared to other mammals, bats appear to have an increased ability to tolerate viral infections without developing clinical disease. This increased viral tolerance is thought to be linked to unique properties of the bats' type I interferon (IFN) system. We recently showed that SARS-CoV-2 infection of intestinal organoids derived from Jamaican fruit bats (JFB, Artibeus jamaicensis) led to a strong type I interferon response, activation of regenerative pathways, but only limited viral replication. Here, we tested the hypothesis that the JFB intestinal epithelium generally mounts canonical type I IFN responses that promote epithelial regeneration. **Methods.** Intestinal organoids were generated from small intestinal tissues collected from adult JFBs. We also developed a viral infection model using a GFP-expressing vesicular-stomatitis virus (VSV-GFP). Type I interferon signaling was induced using a recombinant JFB interferon-β (rbIFN-β) and was measured by gene expression analysis and phosflow STAT-1 assay. Epithelial growth and regeneration were quantified using microscopy and wound healing assays. **Results.** VSV-GFP achieved robust infection of JFB organoids in 3-D and monolayer cultures and induced significant upregulation of the interferon response genes MX1, ISG15 and IRF7. Stimulation of the organoids with rbIFN-β led to rapid phosphorylation of STAT-1 and significantly increased expression of interferon response genes within 4 h. Importantly, pre-treatment of the organoids with rbIFN-β completely blocked subsequent infection with VSV-GFP. However, neither the rbIFN-β nor VSV infection had a significant impact on the formation and growth of 3-D organoids or on epithelial healing. **Conclusions.** We have established VSV infection of organoids as a tractable model to explore antiviral signaling mechanisms in JFBs. Our results indicate that JFB enterocytes mount a canonical response to type I IFN stimulation that leads to strong protection from viral infection. However, in contrast to SARS-CoV-2, VSV did not increase organoid growth and regeneration, pointing to virus-specific mechanisms.

Gut Microbiome of Ugandan Insectivorous Bats Carrying Coronaviruses

Poster #26

Author list:

Cordova EA[1], Williams KM[1], Wickenkamp NR[1], Harris EK[1], Matovu B[2], Nalikka B[2], Nalukenge L[2], Mutebi JM[2], Siya A[2], Rebecca L[5], Nassuna AC[5], Yiga F[5], Dewey TA[3], Castle K[4], Nakayiki T[5], Kityo RM[2], Kayiwa J[5], Lutwama JJ[5], Kading RC[1]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, USA; [2]Department of Zoology, Entomology, and Fisheries Science, Makerere University, Kampala, Uganda; [3]Department of Biology, Colorado State University, USA; [4]Wildlife Veterinary Consulting, Livermore, CO, USA; [5]Uganda Virus Research Institute, Entebbe, Uganda

Abstract:

Background. Many species of insectivorous bats are known to carry viruses, including those with potential human health concerns, such as coronaviruses. A link has been suggested between gut health and immune response regulation via the endogenous microbiota that drives the proliferation of B and T lymphocytes, particularly CD4+ T cells, suggesting a clear relationship between gut health and immune capacity. Thus, understanding the relationship between gut microbiome composition and viral infection status is critical but poorly understood. We hypothesize that bat species, cave, season, and coronavirus infection status, will impact the composition of the gut microbiome. **Methods.** Fecal samples (n=178) were collected from insectivorous bats (Hipposideros ruber, Nycteris thebaica, Rhinolophus spp., and Miniopterus fraterculus) from six cave systems from the Mount Elgon region of Eastern Uganda across both dry and wet seasons from 2022 to 2023. In addition to the fecal samples collected for this study, rectal and oral swabs were collected and tested for coronaviruses. Extracted DNA was PCR-amplified using 16S primer sets to amplify bacterial DNA. Purified amplicons were sent to Genewiz for amplicon sequencing. In this study we examine both alpha and beta diversity of the microbiome in relation to our four variables. These parameters are tested for statistical significance to determine which variables are primarily driving the changes observed in the gut microbiome. **Results.** The impact of coronavirus infection on gut microbiome composition and diversity will be presented. Additionally, we will determine which of our variables has the strongest correlation with gut microbiome composition. **Conclusion.** By understanding how the gut microbiome differs in bats carrying coronaviruses, we can begin to understand the relationship between the immune system and the microbiome. Furthermore, by examining the functional pathways associated with these bacteria, we can begin to see how they support the immune system metabolically during infection.

Susceptibility of Jamaican Fruit Bat Cell Lines to Sarbecoviruses

Poster #27

Author list:

Hossain F[1], Charley PA[1], Schountz T[1]

[1]Department of Microbiology, Immunology & Pathology, Colorado State University, CO, USA

Abstract:

Regardless of various reports of SARS-CoV-2-related viruses (sarbecoviruses) in horseshoe bats (*Rhinolophus* spp.), research on other sarbecoviruses from bats is limited. Many sarbecoviruses that have similar receptor binding domains (RBD) as SARS-CoV-2 that bind ACE2 have been found and tested against different cell lines. BANAL-52 and BANAL-236 are two sarbecoviruses from horseshoe bats that were found to be among the closest whose RBD differ from SARS-CoV-2 by only a few residues. We have determined that primary kidney epithelial cells (Ajk) from Jamaican fruit bats (*Artibeus jamaicensis*) are not susceptible to SARS-CoV-2 using a GFP-expressing infectious clone. A serotype 5 adenovirus vector, Ad5, that encodes human ACE2 transduces the cells but without virus replication was used to express human ACE2 in AJK cells. However, no change in viral titer was detected following infection, suggesting the cells are not permissive for SARS-CoV-2 replication. We are continuing to develop this model as a tool to examine other bat sarbecoviruses, including BANAL-52 and BANAL-236 with horseshoe bat ACE2 receptors to determine how these viruses may behave differently.

Detection of SARS-CoV-2 in Primates and Bat Populations in the Amazon Region

Poster #28

Author list:

Sales de Medeiros G[1,2,3], Oliveira do Nascimento F[1], Silva e Silva D[1], Contreras Mejia MDC[1], Alves do Nascimento V[1], Costa de Souza V[1], Bessa Luz SL[2], Gomes Naveca F[1], Dales Nava AF[2]

[1]Center for the Surveillance of Emerging, Re-emerging or Neglected Viruses, Laboratory for the Ecology of Communicable Diseases in the Amazon – EDTA, Instituto Leônidas e Maria Deane – ILMD/Fundação Oswaldo Cruz, Amazonas, AM, Brasil; [2]Center for Pathogens, Reservoirs and Vectors in the Amazon – PReV, Laboratory for the Ecology of Communicable Diseases in the Amazon – EDTA, Instituto Leônidas e Maria Deane – ILMD/Fundação Oswaldo Cruz, Amazonas, AM, Brasil; [3]Postgraduate Program in Pathogen-Host Interaction Biology – PPGBIO INTERACTION, Instituto Leônidas e Maria Deane – ILMD/Fundação Oswaldo Cruz, Amazonas, AM, Brasil

Abstract:

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which causes COVID-19, is an enveloped virus belonging to the subfamily Orthocoronavirinae, family Coronaviridae and order Nidovirales. Since its emergence, the number of cases has grown exponentially, quickly having its status classified by the WHO as a pandemic. It is speculated that SARS-CoV-2 evolved in bats, highlighting the ability of CoVs to jump between different species and cause serious illness. Given the occurrence of outbreaks of highly infectious epidemic zoonotic diseases such as SARS- CoV-2, studies on wild reservoirs are highly relevant, especially bats which have a history of co-evolution with viruses, and primates due to their phylogenetic proximity to humans. To detect the SARS-CoV-2 virus in primate and bat populations in the Amazon. Screening by real-time PCR (qPCR) to detect SARS-CoV-2 with the Biomanguinhos SARS-CoV-2 molecular kit (EDx) and to confirm positive samples, a second qPCR test with the 2019-nCoV RUO 500rxn kit. 334 pooled samples containing (oral and rectal swabs) from bats and (orotracheal, nasal and rectal swabs) from primates were processed. However, all the samples tested had negative final results. The study previously showed that the primates and bats sampled did not have the SARS-CoV-2 virus, which does not rule out the possibility that these animals have other CoV species circulating among these populations. The scarcity of studies focused on actively monitoring these two orders of public health importance involved in zoonotic outbreaks is still a reality.

Diversity of viral families in bats from forest fragments in the city of Manaus, Amazonas.

Poster #29

Author list:

Sales de Medeiros G[1,2,3], Costa da Silva V[2], Oliveira do Nascimento F[1], Silva e Silva D[1], Contreras Mejia MDC[1], Alves do Nascimento V[1], Costa de Souza V[1], Bessa Luz SL[2], Gomes Naveca F[1], Dales Nava AF[2]

[1]Center for Surveillance of Emerging, Reemerging or Neglected Viruses, Laboratory of Ecology of Communicable Diseases in the Amazon, Instituto Leonidas e Maria Deane – Fundação Oswaldo Cruz, Amazonas, AM, Brazil; [2]Center for Pathogens, Reservoirs and Vectors in the Amazon, Laboratory for the Ecology of Communicable Diseases in the Amazon, Instituto Leonidas e Maria Deane, Amazonas, AM, Brazil; [3]Postgraduate Program in Biology of Pathogen-Host Interaction, Instituto Leonidas e Maria Deane – Fundação Oswaldo Cruz, Amazonas, AM, Brazil

Abstract:

Measures to understand the diversity of pathogens present in wild animals are important, especially in mammals with higher susceptibility, such as bats. However, knowledge of infectious agents circulating in bats is still scarce, especially in the Amazon region, where 146 bat species have been described to date, representing about 80% of all species described in Brazil. To describe the diversity of viral families circulating in bats captured in forest fragments in the city of Manaus. Bat capture camps were carried out in Reserva Adolpho Ducke, Parque Zoológico do Centro de Instrução de Guerra na Selva - CIGS and Universidade Federal do Amazonas - UFAM. Sample pools were created using the criteria of species, location, collection period and sample type, and were extracted using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit. They were subjected to a targeted metagenomic approach with probes for the respiratory virus panel (VSP) and pandemic coronavirus (PAN-CoV), following the Illumina protocol and sequenced on the NextSeq 1000. 180 oral and rectal swab samples from 8 generalist bat species were processed and pooled into 21 pools. Metagenomic sequencing of the pools was used to build libraries and generate de novo contigs that matched 10 mammalian viral families, identifying DNA viruses (Adenovirus, Circovirus, Parvovirus, Poxvirus, Herpesvirus) and RNA (Astrovirus, Coronavirus, Flavivirus, Hepesvirus, Picobirnaviridae). Recent viral studies indicate that bats of the generalist species sampled in the study, such as Artibeus, Carollia, and Sturnira, are commonly infected with Flavivirus, Coronavirus, Adenovirus, Astrovirus, and Parvovirus, and studies in fragmented landscapes have demonstrated the presence of these viral families in generalist species, demonstrating that species with a greater ability to adapt to fragmented environments are more susceptible to viral infections than species found in more conserved regions.

Henipavirus, nobecovirus, and rabies-like lyssavirus seasonality in Malagasy fruit bats

Poster #30

Author list:

Kettenburg G[1], Ranaivoson HC[123], Andrianianina A[23], Andry S[24], Henry AR[5], Davis RL[5], Laboune F[5], Longtine ER[5], Godbole S[67], Raharinosy V[8], Randriambolamanantsoa TH[8], Lacoste V[8], Heraud JM[8], Dussart P[8], Douek DC[5], Brook CE[12]

[1]Department of Ecology and Evolution, University of Chicago, IL, USA; [2]Association Ekipa Fanihy, Antananarivo, Madagascar; [3]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar; [4]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar; [5]Chan Zuckerberg Biohub, San Francisco, USA; [6]Human Immunology Section, Vaccine Research Center, NIAID, NIH, Bethesda, USA; [7]PREMISE, Vaccine Research Center, NIAID, NIH, Bethesda, USA; [8]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar

Abstract:

Background. Endemic fruit bat species (*Eidolon dupreanum, Pteropus rufus,* and *Rousettus madagascariensis*) from Madagascar are hosts to divergent henipaviruses, nobecoviruses, and a rabies-like lyssavirus. Here, we used data from a longitudinal field study to decipher the dynamics of seasonal shedding for three prevalent viral clades circulating among the three fruit bat hosts. **Methods.** RNA was extracted from fecal (N=1952), urine (N=807), saliva (N=886), and tissue (N=225) samples collected from longitudinally sampled bats. We carried out family-level PCR for paramyxoviruses, coronaviruses, and lyssaviruses on resulting RNA, and positives were Sanger sequenced. A subset of samples was additionally subject to metagenomic Next Generation Sequencing (mNGS). Henipavirus and nobecovirus sequences recovered from mNGS were used to design primers for subsequent qPCR. We then used general additive models (GAMs) in the binomial family to evaluate correlates of negative/positive infection status by qPCR for henipaviruses and nobecoviruses and by conventional PCR for a rabies-like lyssavirus. We tested relationships between infection status and four predictor variables: sex, age class, mass:forearm ratio (a proxy for bat condition), and month of capture (a proxy for season). **Results.** Nobecovirus and RABV-like lyssavirus shedding was observed in all three species, with henipavirus shedding in *E. dupreanum* and *R. madagascariensis* only. Shedding peaked for all three viruses in the nutritionally-stressed winter season in male bats and energetically-demanding reproductive period in female bats. **Conclusions.** Longitudinal data are critical to decipher the stressors, which drive seasonal shedding of potentially zoonotic viruses from fruit bats, a key correlate of zoonotic spillover. Longitudinal viral shedding data can also be aired seroprevalence data to support the fitting of epidemiological transmission models that aim to explain viral maintenance and identify transmission bottlenecks on the pathway to cross-species emergence.

Herpesvirus Diversity and Patterns of Co-Infection in Neotropical Bat Species

Poster #31

Author list:

Malik H[1], Perez-Lazo J[1], Holmes A[1], Tello C[2], Da Silva Filipe A[1], Smollet K[1], Bergner L[3], Streicker D[1,3]

[1]MRC–University of Glasgow Centre for Virus Research, University of Glasgow, United Kingdom; [2]Association for the Conservation and Development of Natural Resources (ILLARIY), Peru; [3]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, United Kingdom

Abstract:

Background. Herpesviruses are ubiquitous across most animal classes, and have been identified extensively in several different bat species. However, due to the methods used, most studies have been unable to characterise the diversity of herpesviruses within individual bats; including the presence of co-infections with multiple herpesvirus families, and the intrahost diversity of these co-infecting families. In this study, we aimed to describe Herpesvirus diversity within populations of neoptropical bat species and identify co-infections within individual bats with strain-level resolution. **Methods.** Here, we developed a novel nanopore-based amplicon sequencing and bioinformatic approach, based on a universal DPOL herpesvirus PCR, to identify and sequence all Herpesviridae families in 110 saliva and faecal samples from neotropical bat species in Amazonas (Peru), permitting the identification of co-infections with strain-level resolution. **Results.** We found all three Herpesvirus families across the species tested. The Betaherpesvirinae and Gammaherpesvirinae were universally detected in all species, whereas the Alphaherpesvirinae were exclusively found in members of the Saccopteryx genus. We highlight the phylogenetic structure of these viruses to examine patterns of viral sharing between species. Additionally, we identified co-infections with multiple Herpesvirus families, and, more rarely, with all three. We also incorporated models to identify any biological factors associated with these co-infections. **Conclusion.** Here, we developed a novel sequencing and bioinformatic workflow for detecting Herpesvirus co-infections in bat samples with intrahost strain-level resolution. Using our results, we also highlight factors associated with herpesvirus co-infections, as well as the phylogenetic structure of all detected viruses. Whilst only tested on bat samples, this approach could also be used to characterise herpesvirus co-infections in multiple other mammalian species.

Barnyard bats: Do domestic animals bridge the viral gap?

Poster #32

Author list:

Karegi IA[1], Brown AJ[1], Rivero R[1], Seifert SN[1]

[1]Paul G. Allen School for Global Health, Washington State University, Pullman, WA, United States

Abstract:

Background. Understanding how bat-borne viruses cross species barriers requires examining host biology and ecology as key drivers of transmission. Bats harbor diverse viruses, some of which may be shared with domestic animals and humans due to conserved cellular receptors and biochemical pathways. However, the role of domestic animals in facilitating viral transmission from wildlife, including bats, remains unclear. Here, we investigate cross-species virus sharing among humans, domestic animals, wildlife, and bats by addressing two key questions: (1) What is the probability that a bat-associated virus is also found in domestic or wild hosts? (2) Do host and viral genomic features, such as codon usage bias, influence viral sharing? **Methods.** We identified unique host-virus associations, genome annotations, from 53 domestic or farmed host taxa from public datasets. Using probability analyses, network modeling, and machine learning, we evaluated viral transmission potential across host groups. **Results.** Our findings reveal that some bat-associated viral taxa are more likely to associate with domestic animals than humans. Conditional probability analyses show that bat-associated Togaviridae, Orthomyxoviridae, Phenuiviridae, Rhabdoviridae, and Filoviridae exhibit a higher likelihood of infecting domestic animals than humans. Conclusions. Our study provides insights into viral sharing patterns and identifies molecular signatures that may facilitate cross-species transmission. By integrating genomic data with host-virus associations, we offer predictive tools to assess spillover risks of bat-borne viruses. We also identify molecular signatures that may facilitate cross-species infections, enhancing our understanding of viral spillover risks.

Dynamics of bacterial pathogens in Kenyan bats and their ectoparasites

Poster #33

Author list:

DeAnglis ID[1], Lunn TJ[2], Jackson RT[3], Ogola JG[4], Webala PW[5], Sironen T[6], Becker DJ[7], Forbes KM[1]

[1]Department of Biological Sciences, University of Arkansas, Fayetteville, USA; [2]Odum School of Ecology, University of Georgia, Athens, USA; [3]Arizona Game and Fish Department, Phoenix, USA; [4]KAVI Institute of Clinical Research, University of Nairobi, Nairobi, Kenya; [5]School of Natural Resources, Environmental Studies and Agriculture, Maasai Mara University, Narok, Kenya; [6]Department of Virology, University of Helsinki, Helsinki, Finland; [7]School of Biological Sciences, University of Oklahoma, Norman, USA

Abstract:

Background. Identifying and characterizing zoonotic pathogens in wildlife is essential for understanding disease risk to humans. Bartonella spp. and hemotropic Mycoplasma spp. (hemoplasmas) are common vector-borne bacterial pathogens in bats and are known to spill over and cause infections in humans. Yet little is known about these bacteria in Afrotropical bat species. In sub-Saharan Africa, many people live with bats roosting in their houses and are likely exposed to their pathogens. To evaluate the disease risk to humans, we screened the three most common synanthropic bat species in Kenya, and their ectoparasites, for bartonellae and hemoplasmas to determine infection prevalence in these populations. We also assessed the relatedness of these bacteria to known species that are infectious to humans. **Methods.** We sampled little free-tailed (Mops pumilus), Angolan free-tailed (Mops condylurus), and heart-nosed (Cardioderma cor) bats from buildings in Taita-Tavetta county. We are conducting PCR and gel electrophoresis to screen blood samples for bartonellae and hemoplasmas and will be sequencing positive samples for phylogenetic characterization. We will also screen these bats' ectoparasites for the same bacteria to better understand their roles as vectors. **Results.** Initial results show that 23.7% of bats are positive for bartonellae (12.0% in M. pumilus; 25.1% in M. condylurus; 32.1% in C. cor). We have positive hemoplasma PCR results for a subset of our samples and are currently screening 850 samples to quantify infection prevalence in the three bat species. Next, we will perform a phylogenetic characterization of the bacteria. Our analysis will be completed prior to the conference. **Conclusions.** Our findings will determine how prevalent bartonellae and hemoplasmas are in these synanthropic bats and help assess the relatedness of these bacteria to known human pathogens. Due to the close contact between humans and synanthropic bats, this research is critical for mitigating pathogen spillover in high-risk communities.

Investigating Antiviral Immune Mechanisms in Bats

Poster #34

Author list:

Rosen JS[1], Frank HK[1], McLachlan JM[2]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA; [2]Department of Microbiology and Immunology, Tulane University, New Orleans, LA, USA

Abstract:

Bats (order Chiroptera) are known carriers of lethal human viruses yet rarely exhibit disease symptoms. Research suggests bats have evolved unique mechanisms to tolerate viruses, reducing disease severity through long-term co-evolution. Studying these adaptations could provide critical insights for developing antiviral therapies, which remain challenging due to viral diversity, rapid evolution, and host toxicity. Studies in the newly emerging field show that bats possess immune cell functions similar to those in humans and other mammals, alongside evidence of a dampened innate inflammatory response. However, how bats maintain immune tolerance while regulating virus replication remains poorly understood. Our research addresses this gap using in vitro models with the recombinantly recovered H18N11 influenza A virus in immortalized bat epithelial lung cells. We assess infection kinetics—including adhesion, entry, replication, and release—and compare them to human and mouse epithelial cells infected with species-specific influenza A viruses. Transcriptomics and RNAseq are employed to identify innate immune transcripts and differentially expressed proteins in response to infection. By isolating antiviral proteins and their effector mechanisms, we aim to uncover therapeutic insights inspired by bats' evolved immune responses. Leveraging bat's unique immune phenotype could lead to innovative antiviral strategies, strengthening pandemic preparedness.

The gut microbiome as a key player in viral shedding for MERS-CoV inoculated Jamaican fruit bats (*Artibeus jamaicensis*) with protein-restricted diets

Poster #35

Author list:

Williams KM[1], Priore M[1], Harris EK[1], Zhan E[1], Pulscher L[1], Fagre A[1], Schountz T[1], Kading RC[1]

[1]Microbiology, Colorado State University, Fort Collins, USA

Abstract:

Background. Bats are associated with many emerging zoonotic pathogens, including coronaviruses. Spillover of most of these viruses is new to the last several decades, which have also seen the loss of approximately 10% of Earth's primary forests. Loss of native forests results in loss of native fruits, which are often more rich in protein than cultivated or invasive fruits. Reliance on cultivated fruits often low in protein can lead to nutritional deficiencies in fruit bats that could change immune regulation, potentially via the gut microbiome. Previous studies on fruit bats have found specific bacteria groups in the gut to be predictors of the strength of the immune response. Thus, understanding the relationship between diet, gut microbiome, and outcome of viral infections is critical. **Methods.** To observe these interactions in a controlled setting, we inoculated Artibeus jamaicensis with Middle East respiratory syndrome coronavirus (MERS-CoV; Coronaviridae: Betacoronavirus) under both a normal diet and a protein-restricted diet. Rectal swabs were collected from dpi -14 to 21. DNA extracted from rectal swabs was PCR amplified in triplicate at the V4 region of 16S and Illumina 2x250 sequenced after purification. Pre-processing and analyses were performed using Qiime2. **Results.** The research presented will focus on the composition and diversity of the gut microbiome in relation to diet and viral shedding. These correlations will be examined statistically using Spearman and Mantel tests for viral shedding and Kruskal-Wallis and Permanova tests for diet. A linear mixed-effects model will be constructed to examine the impact of all variables. **Conclusions.** The field of gut microbiome-immune system interactions is still in its infancy. Our study aims to look at their relationship through the lens of one of the most critical factors amidst habitat loss, dietary health. The results of this study have implications for immunology as well as conservation strategies.

Life History, Flight, and Immune Adaptation: A Simulation Study in Bats and Alternative Taxa

Poster #36

Author list:

Rayfield KM[1], Delamonica B[2], D'Andrea R[1], Dávalos LM[1]

[1]Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA; [2]Graduate Program in Applied Math and Statistics, Stony Brook University, Stony Brook, NY, USA

Abstract:

Background. Bats are natural reservoir hosts for viruses that can cause severe diseases in other mammals but often show minimal symptoms, and many have argued this viral tolerance emerged because of molecular adaptations associated with powered flight. However, bats are not the only vertebrates to evolve powered flight; birds also fly yet they have not been identified as unusually tolerant of viruses, thus raising the question of precisely how flight may change selection in such a way that leads to immune adaptations. **Methods.** To better understand the connections between flight and modified immunity, we examine effects on selection for demographic structures in bats and other taxa exploring two axes: flying, non-flying, offspring carrying, and offspring non-carrying. **Results.** Using simulations, we find that flight alone does not provide a consistent fixation of alleles associated with greater survivorship (equivalent to immune modifications), and only does so when paired with limited annual fecundity. Conversely, limiting fecundity can lead to fixation of alleles for better survivorship, but only with tight limits on fecundity (as in bats), and not in scenarios with high adult mortality (i.e., without flight). **Conclusions.** Our simulation experiments point to life history changes in birds that can better mirror the unique life history, and perhaps the unique genomic adaptations of bats.

Seasonality of Ectoparasites in Migratory Bats: Implications for Host Health and Movement

Poster #37

Author list:

Dyer KE[1], Allira M[1], Demory BM[1], Hightower MG[1], Wingert JT[1], Ross JD[1], Castle KT[2], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]Wildlife Veterinary Consulting, Livermore, CO, USA

Abstract:

Background. Mexican free-tailed bats (Tadarida brasiliensis) migrate annually from Mexico to the southwestern U.S., forming maternity colonies of >20 million bats. These dense, temporary congregations provide ideal conditions for parasite spread. Blood-feeding ectoparasites, such as bat fleas and bat flies, can negatively impact host health through resource consumption and pathogen transmission, particularly during energetically demanding activities like migration and reproduction. However, the interplay between parasitism, bat health, and migration remains poorly understood. **Methods.** We assessed seasonal and site-specific parasite dynamics through monthly and bi-monthly bat sampling at Selman Cave, Oklahoma (n=523) and Bracken Cave, Texas (n=187) during 2023–2024. We evaluated ectoparasite presence by taxa, bat body condition via mass, and marked individuals with Passive Integrated Transponder (PIT) tags and radio-telemetry tags to track fall migration. Using generalized additive models, we tested seasonality in ectoparasite prevalence, whether ectoparasite presence predicted body condition, and whether body condition and parasitism affected fall migration timing. **Results.** We observed seasonal variation in ectoparasite prevalence, linked to bat life history. Flea prevalence peaked during pup birth, while bat flies were most common during fall migration. We PIT-tagged 789 bats at Selman Cave (76% detected via loop antenna) and 190 at Bracken Cave, and detected inter-cave movement of one individual. We radio-tagged 40 and 60 bats in fall 2023 and 2024, and tracked migration via Motus. Migration departure was driven by temperature ($p < 0.001$) but not body condition ($p = 0.09$) or ectoparasitism ($p = 0.7$). **Conclusion.** These findings suggest that while seasonal parasitism varies with bat life history, environmental factors such as temperature play a stronger role in migration timing. Seasonal peaks in ectoparasitism could contribute to long-term physiological stress, potentially affecting reproductive and migratory success. Understanding these interactions is crucial for wildlife conservation and managing the health of wild bat populations.

Determining the Impact of Cadmium on T Cell Immune Function and Viral Replication in Jamaican Fruit Bats (*Artibeus jamaicensis*)

Poster #38

Author list:

Pulscher LA[1], Charley PA[1], Zhan S[1], Reasoner C[1], Schountz T[1]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA

Abstract:

Background. Spillover of bat-borne viruses may be associated with periods of stress such as reproduction or diet. Other environmental stressors, including exposure to heavy metals such as cadmium (Cd), might similarly impair immune function and impact viral replication in bats but this has not been fully elucidated. The aim of this study was to understand the impacts of Cd exposure on T cell immune function and viral replication in Jamaican fruit bats (*Artibeus jamaicensis*). We hypothesized Cd would alter T cell function and increase viral replication in a dose-dependent manner. **Methods.** To determine the impact of Cd on T cell immune function, duplicate splenocyte cultures from eight Jamaican fruit bats were treated with 0, 1, and 10 μM $CdCl_2$ and stimulated with concanavalin A. RNA was extracted and a SYBR Green qPCR array was conducted. Within sample gene normalization was performed on the Rps18 and fold-change was calculated by comparing Cd treated to non-treated cells ($\Delta\Delta$Cq). To determine if Cd treatment increased viral replication, duplicate cultures of kidney cell clones were treated with $CdCl_2$ and infected with Cedar virus (CedV). Supernatants were collected over 3 days and viral titers were determined using a TCID50 assay. **Results.** Several genes in splenocyte cultures treated with $CdCl_2$ were upregulated, or trending upward, compared to non-treated cells. TGFβ and CD79 were significantly higher in splenocytes treated with 10 μM of $CdCl_2$. While not significant, Gata3 ($p=0.09$) was also increased in splenocytes treated with 10 μM of $CdCl_2$. Some kidney cell clones treated with low concentrations of $CdCl_2$ had 0.5-log higher CedV replication. **Conclusions.**
Results from this study suggest Cd alters Jamaican fruit bat T cell function and may increase viral replication. Further work is required to elucidate the impacts of Cd in vivo and determine if Cd exposure could impact viral replication or shedding.

Investigating Innate Immune Responses to Cytokine Treatment in *Artibeus jamaicensis* Cells

Poster #39

Author list:

Malsick LE[1], Mayton EH[1], Schountz T[1], Geiss B[1]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA

Abstract:

Background. Jamaican fruit bats (*Artibeus jamaicensis*) are frugivorous bats found throughout Central America that may serve as reservoirs for diverse viruses such as rabies virus, Tacaribe, and bat influenza viruses. How Jamaican fruit bats control viral infection has been hindered by limited understanding of their molecular innate immune responses to viral infections. To better define the innate immune responses in Jamaican fruit bats, our goal is to assess how their cytokines affect gene expression responses in primary kidney cells (AJ6 cells). **Methods.** In preliminary studies, we pretreated AJ6 cells with human universal interferon alpha (IFN-α) and examined resulting mRNA expression patterns at multiple times using RNA-seq analysis. Surprisingly, we found extremely limited upregulation of innate immune genes with IFN-α treatment, which was unexpected due to the significantly upregulated innate responses we observed flavivirus infection of AJ6 cells. Because little is known about the innate immune modulations of bats, we then explored how these primary cells respond to cytokine treatment. We tested commercially available Jamaican fruit bat recombinant cytokines (CCL5, CCL2, CXCL11, IFN-γ, and IL-1RA), Egyptian fruit bat (*Rousettus aegyptiacus*) recombinant cytokine (IL-6), vampire bat (*Desmodus rotundus*) recombinant cytokines (IL-22 and IFN-λ3), and human universal IFN-α on primary AJ6 cells. AJ6 cells were treated with each cytokine for up to 12 hours to observe the cellular responses at early timepoints and mRNA expression was assessed via RNA-seq to determine the innate responses of these cells to cytokine treatment. **Results/Conclusions.** We will discuss the gene expression patterns we are observing from treatment with these cytokines, which will improve our understanding of innate immune responses in Jamaican fruit bats. Understanding how cytokines influence innate immune responses in primary AJ6 cells will inform future studies using this species as a valuable model for viral infection in bats.

Investigation of Adipose Tissue as a Niche Reservoir for Ebola virus Infection

Poster #40

Author list:

Garnett L[1], Tran KN[1], Schiffman Z[1,2], Muise K[3], Fletcher Q[3], Dzal Y[3], Leung A[1], Deschambault Y[1], Warner B[1], Griffin B[1], Kobasa D[1,2], Willis C[3], Strong JE[1,2,4]

[1]Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, CAN; [2]Department of Medical Microbiology and Infectious Diseases, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, CAN; [3]Department of Biology and Centre for Forest Interdisciplinary Research, University of Winnipeg, Winnipeg, Manitoba, CAN; [4]Pediatrics & Child Health, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, CAN

Abstract:

Ebola virus (EBOV) is a zoonotic pathogen with a geographical range that overlaps with the habitat of diverse ecosystems, home to many potential reservoir species. While EBOV RNA and serological evidence have been found in rodents and bats, live virus has not been isolated from any species. While it is widely hypothesized that bats (or some other zoonotic species) serve as the EBOV viral reservoir, the mechanism by which infectious viruses may emerge and spillover has not been determined or sufficiently modelled. Predicting and preventing spillover events requires research on population distribution, ecology, host metabolism, and immunology. This study investigates adipose tissue as a potential site for prolonged EBOV infection, a role that has not yet been explored. Adipose Tissue is a dynamic endocrine organ coordinating homeostasis, energy metabolism, neuroendocrine, and immune functions. Through in vitro infection of human and bat (Eptesicus fuscus) brown adipose tissue cultures with wild-type EBOV, we observed high levels of viral replication for up to 28 days, with no qualitative indicators of cytopathic effects. Furthermore, alterations in adipocyte metabolism were observed, such as an increase in lipid droplet numbers and a decrease in droplet size, indicating potential lipolysis or adipocyte browning. In vivo, wild-type EBOV infection in Golden Syrian hamsters and Deer mice showed no clinical disease or detectable virus in some commonly sampled tissues, such as the liver, yet the virus persisted in adipose tissue depots for up to 128 days post-infection. These findings highlight the importance of adipose tissue in both long-term infection and its potential role in viral transmission between reservoir species and zoonotic spillover events.

Longitudinal Impacts of Habitat Fragmentation on Bartonella and Hemotropic Mycoplasma Dynamics in Vampire Bats

Poster #41

Author list:

Lock LR[1], Dyer KE[1], Yang A[2], Volokhov DV[3], Fenton MB[4], Simmons NB[5], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]Department of Geography and Environmental Sustainability, University of Oklahoma, Norman, OK, USA; [3]Center for Biologics Evaluation & Research, U.S. Food & Drug Administration, Silver Spring, MD, USA; [4]Department of Biology, Western University, London, ON, Canada; [5]Department of Mammalogy, American Museum of Natural History, New York City, NY, USA

Abstract:

Background. Habitat fragmentation can have negative impacts on bats, including increased risk of infectious disease. Habitat fragmentation rates in the Neotropics are among the highest in the world, driven largely by clearing for agriculture. To assess temporal changes in pathogen dynamics in bats impacted by habitat fragmentation, we compared the prevalence of two blood-borne pathogens and their genotypes between vampire bats (*Desmodus rotundus*) from one large and one small forest fragment in northern Belize across five years. **Methods.** We quantified forest loss within the matrix surrounding both sites using land cover data from the Sentinel-2 10-m land use/land cover time series of the world produced by Impact Observatory, Microsoft and Esri. DNA from 358 blood samples were screened for Bartonella and hemotropic Mycoplasma (hemoplasma) species through PCR amplification of the gltA and 16S rRNA genes, respectively. Positive amplicons were Sanger sequenced and genotyped based on phylogenetic similarity. We used general linear mixed models to test if the effects of regional forest loss per year or site-specific factors influenced the likelihood of infection. **Results.** Bartonella prevalence was positively influenced by forest loss, but only in the large fragment, whereas hemoplasma prevalence showed no response to forest loss. The effects of forest loss on infection likelihood varied by genotype for both Bartonella and hemoplasmas. We found that two Bartonella genotypes exhibited opposite responses to forest loss, as did two hemoplasma genotypes. Other genotypes of both pathogens were found to be unaffected by forest loss. **Conclusions.** Our work demonstrates that the effects of habitat fragmentation on infection prevalence depended on both the pathogen and specific lineage. Our findings complicate expectations of how habitat fragmentation affects infectious disease dynamics in bats. As such, management practices aimed at mitigating the impacts of infectious diseases in fragmented systems should be tailored to specific pathogens of concern.

Modelling the influence of spatial coupling on pathogen dynamics between natural and anthropogenic roosting molossid bats

Poster #42

Author list:

Sánchez-Trejo L[1], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA

Abstract:

Background. Among mammals with fission–fusion dynamics, bats have diverse roosting strategies. Different species show varying fidelity to specific roost types, and why they choose and change roosts is highly dependent on roost permanence, microclimate, social structure, and physiological requirements. Molossid bats show high plasticity to roost microclimate and structure, often using natural tree roosts and anthropogenic structures, which have highly contrasting attributes. While this can allow molossids to persist in heterogeneous environments, movement between natural and urban roost subpopulations (i.e., coupling) can influence pathogen dynamics and spillover events, as urban subpopulations have frequent human contact. **Methods.** We developed a susceptible–infected–recovered model, based on spatial coupling, to investigate how bat movement between tree and building roosts influences pathogen dynamics. Our model assumes different subpopulation sizes, driven by lower carrying capacity of tree roosts, and that time spent in the other subpopulation, as a function of movement rates between roost types, depends on bat density. We loosely parameterized our model around Molossus species that inhabit both roost types and assessed sensitivity to pathogen traits. **Results.** We predict that infrequent movement from building to tree roosts and higher occupancy in building roosts, possibly related to more favorable microclimate and larger roosting area, will have a positive effect on pathogen invasion. By contrast, while low occupancy and greater movement from tree roosts may reduce pathogen transmission within this subpopulation, we expect coupling to facilitate pathogen persistence. **Conclusions.** Our modeling framework, which accounts for movement between subpopulations with variable roosting attributes, may better reflect high–risk human–wildlife interfaces. Ongoing efforts to quantify roost preference in molossid bats and effects on pathogen dynamics are fundamental to validating our model predictions. Although movement between two bat roost types is a simplistic approach, it provides novel insights into understanding differential movement and pathogen dynamics in wild bats.

Bugs in the Blood: Exploring Trypanosoma Dynamics in Mexican Free-Tailed Bats

Poster #43

Author list:

Hightower M[1], Allira M[1], Dyer KE[1], Demory B[1], Lock LR[1], Olbrys BL[1], Vicente-Santos A[1], Verrett T[1], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA.

Abstract:

Background. Nearly all mammals are susceptible to infection with trypanosomes, a genus of vector-borne protozoan parasites, with *Trypanosoma cruzi* posing significant human health risks among endemic countries within the Western Hemisphere. While *T. cruzi* infections among mammals have recently been documented in Oklahoma, research concerning sylvatic transmission in the state is limited. Mexican free-tailed bats (*Tadarida brasiliensis*) encounter areas endemic to *T. cruzi* during their yearly migration from Mexico through the southwestern United States, where they form large congregations at maternity and bachelor roosts in the summer. These behaviors, along with their long lifespans and shared coevolutionary history with the parasite, implicate the bats as playing a key role in parasite transmission. **Methods.** During monthly sampling trips from 2022-2024, we collected blood and demographic data from over 700 Mexican free-tailed bats at two roosts in northwestern Oklahoma. We used PCR targeting the ssrRNA gene of mammalian trypanosomes and Sanger sequencing to identify infection. We further evaluated how infection risk varied by reproductive status, age, sex, year, and month using generalized additive models. **Results.** PCR detected a 5% infection prevalence, with sequencing identifying at least *Trypanosoma cruzi* and *T. dionisii*, which are closely related. Infection occurred more frequently in pregnant bats, and seasonality of infection and pregnancy were aligned in 2022 and 2023. Trypanosome prevalence corresponds with reproductive peaks during the early summer months, as females migrate to Oklahoma in the early stages of pregnancy. **Conclusions.** These results reflect the high physiological, energetic, and immune stressors associated with long-distance migration and pregnancy, which signifies the potential for vertical transmission. Mexican free-tailed bats contribute to trypanosome transmission cycles within the southern United States, and evaluating the drivers and diversity of trypanosome infection among these bats provides insight into host–parasite dynamics and parasite endemicity.

Investigating leukocyte morphology and response seasonality in Madagascar's Old-World Fruit Bats: *Rousettus madagascariensis*, *Eidolon dupreanum*, and *Pteropus rufus*

Poster #44

Author list:

Woodward M[14], Mendoza A[1], Ranaivoson HC[12], Andrianiaina A[2], Andry S[3], Cornelius Ruhs E[1], Brook CE[1]

[1]Department of Ecology and Evolution, University of Chicago, Chicago, USA; [2]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar; [3]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar; [4]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, USA

Abstract:

Background. Bats are reservoir hosts for a range of virulent zoonotic pathogens. Past research suggests that globally, bat borne pathogens emerge seasonally and exhibit pulses of viral shedding. Characterization of bat cellular immune function aids understanding of how bat physiology and environmental seasonality influence bat disease dynamics. The island of Madagascar is home to three endemic old-world fruit bat species: *Rousettus madagascariensis*, *Eidolon dupreanum*, and *Pteropus rufus*. We aim to describe the leukocyte morphology and response seasonality for all three bat species through analysis of blood smears from a longitudinal field study. **Methods.** Blood smears were collected from fruit bats sampled in Madagascar from 2020-2023. To examine leukocyte morphology, the blood smears were stained, examined, and photographed using light microscopy. For all three fruit bats species, the diameters of a subsample of leukocytes throughout each species' sampling period were measured. Total white blood cell counts and the neutrophil to lymphocyte ratios (N:L, a proxy for stress) per 10,000 red blood cells for every *R. madagascariensis* (N=304), *E. dupreanum* (N=244), and *P. rufus* (N=133) individual was estimated. The total white blood cell counts and N:L seasonalities were analyzed using generalized additive models. **Results.** Across the fruit bat species and sexes, the leukocyte diameter measurements varied for all leukocyte types. We observed significant seasonality for the total leukocyte counts for female P. rufus, which peaked during the lactation period, and male *R. madagascariensis* and *P. rufus*, which peaked during Madagascar's nutrient deficient dry season. Additionally, for the N:L we observed significant seasonality for female *R. madagascariensis* and *E. dupreanum*, which peaked at the transition between the gestation and lactation periods. **Conclusions.** Through characterizing Malagasy fruit-bat leukocyte morphology and response dynamics, we highlight the important relationship between bat cellular immune response and environmental seasonality.

Novel topical formulation for rabies vaccine delivery to vampire bats

Poster #45

Author list:

Knuese C[1], Cardenas-Canales EM[1], McDevitt-Galles T[2], Ramirez-Martinez MM[3], Limonta D[1], Powers LE[2], Walsh DP[4], Streicker DG[5,6], Osorio JE[1,7], Zamanian M[1], Rocke TE[2]

[1]School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA;[2]US Geological Survey National Wildlife Health Center, Madison, WI, USA. [3]Departamento de Ciencias de la Salud y Ecología Humana, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Autlán, JAL, México;[4]US Geological Survey, Montana Cooperative Wildlife Research Unit, Wildlife Biology Program, University of Montana, Missoula, MT, USA;[5]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK;[6]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK;[7]Global Health Institute, University of Wisconsin, Madison, WI, USA.

Abstract:

Background. Rabies vaccination of vampire bats (*Desmodus rotundus*) has been proposed as a superior control method to culling but has yet to be implemented. Success of rabies vaccination depends on a topical vehicle that spreads through a bat colony via allogrooming while additionally preserving vaccine immunogenicity. This work describes the *in vitro* and *in vivo* optimization of a new topical gel for rabies vaccine delivery to vampire bats. **Methods.** Transferability of our carboxymethyl cellulose (CMC) gel vaccine delivery formulation was tested in a microchipped vampire bat colony in rural Jalisco, Mexico. A proportion of captured bats were topically treated with 1 mL of CMC gel mixed with the fluorescent biomarker rhodamine B to trace intra-colony gel uptake. Rhodamine B was visualized in hair samples collected during subsequent captures 3- and 7-days post-treatment by fluorescence microscopy. The *in vitro* stability of our raccoon poxviral-vectored rabies vaccine candidate within CMC was measured by plaque assay at time points up to 3 months at 40 °C, 23 °C, and 4 °C. Furthermore, physical properties of our CMC formulation were compared to the previously used glycerin jelly at 40 °C, 20 °C, and 0 °C using rheological tests. **Results.** Application of topical treatment to ~20% of the colony resulted in estimated uptake in over 85% of the bats. In the stability assay, extended storage at 4 °C and short exposure at higher temperatures of potential vampire bat environments preserved vaccine titers within CMC. Lastly, rheological tests indicated that CMC exhibits optimal properties for topical application to bats even at extreme temperatures possible during field vaccination. **Conclusion.** This study advances our rabies vaccination strategy for vampire bats by providing a topical vehicle suitable for field application that may additionally be employed for other significant bat diseases.

Adaptive evolution in the bat immunometabolome

Poster #46

Author list:

Kaczmarek ME[1], Fontanez A[1], Tsang SM[2], Wiantoro S[3], Epstein JH[4], Vuong B[1]

[1]Department of Biology, The City College of New York, The City University of New York, New York, NY, USA; [2]Department of Mammalogy, American Museum of Natural History, New York, NY, USA; [3]Museum Zoologicum Bogoriense, Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Bogor, Indonesia; [4]One Health Science, New York, NY, USA

Abstract:

Background. Mammalian immune cells alter their metabolic profile or "immunometabolome" to mount effective immune responses. Despite recent discoveries on bat inflammation- and interferon-mediated responses to viral infections, virtually nothing is known about bat immunometabolomes and how they mobilize bat innate or adaptive immune responses. Here, we investigate whether selection has acted on immunometabolic enzymes in bats to promote antiviral immunity. **Methods.** We used evolutionary selection analysis to screen all enzymes in the glycolytic and glutaminolysis pathways in bats. Although previous work analyzed selection in glucose metabolism in nectivorous bats, we used an unbiased approach to determine if natural selection outside of this life trait would emerge. Specifically, we employed a selection test for recurrent positive selection and BUSTED-E with error correction to ensure appropriate homology prior to further testing. Subsequently, we assessed episodic selection (MEME) acting on specific codons and examined branch-level selection (aBSREL) in mammals to assess if selection targeted chiropterans. **Results.** Among 21 genes analyzed, only a single gene (hexokinase 3 (HK3)) was under positive selection in bats using our unbiased approach. There were multiple sites found under recurrent and episodic selection, all outside of the glucose binding cleft, in both the N and C domains of the protein. At the mammalian level, selection was not seen on the branch leading to chiropterans. **Conclusions.** Compared to the other hexokinases, HK3 is highly expressed in myeloid cell lineages, and has an auxiliary cytoprotective function. Selection acting on HK3, outside of the glucose binding domain, suggests an unaltered role for HK3 in glycolysis and a potential role for HK3 in myeloid cell function or viability. Further analysis will include cellular assays quantifying the impact of sites under positive selection on immune cell differentiation, maturation, and cyto-protection.

Seasonality and Sex Shape Malagasy Bat Movement Patterns in a High-Risk Spillover System

Poster #47

Author list:

Roland MC[1], Horigan S[1], Andry S[2], Andrianiana A[3], Ranaivoson HC[13], Brook CE[1]

[1]Department of Ecology and Evolution, University of Chicago, Chicago, USA; [2]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar; [3]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar

Abstract:

Background. Madagascar is home to three endemic fruit bat species that have been shown to host potentially zoonotic coronaviruses, filoviruses, and henipaviruses. A crucial component of understanding the persistence and circulation of these viruses is a thorough understanding of bat movement and population connectivity. We characterized the movement of the largest and most widely hunted fruit bat in Madagascar, *Pteropus rufus*, focusing on how seasonality, sex, and site differences affect movement patterns. **Methods.** We deployed GPS tags on 12 *Pteropus rufus* individuals from 5 sites across Madagascar. We used Minimum Convex Polygon (MCP) estimations as well as Kernel Density Estimations (KDE) from multiple roost sites across the island to assess variability in home-range size by season and sex. **Results.** Our analysis suggests seasonal changes in resource availability are correlated with shifts in range: bats expanded their MCP home-range by 13.9% during the dry season and contracted it in the rainy season when food was abundant. GPS-tracked females foraged much farther (average 22.7 km) than males (average 13.8km), a pattern potentially linked to increased energy needs during reproduction. Site-specific variation was pronounced, with bats at northern sites exhibiting more localized movement, whereas bats at central sites travelled much further to forage. **Conclusions.** We detected changes in *Pteropus rufus* movement by sex, season, and location. Notably, our detection of increased home range areas during the dry season may increase spillover risk, as bats are known to shed more virus during low-food, high-stress periods, and our work suggests that they spend increased amounts of time on the landscape as they do so. Altogether these differences in movement patterns likely contribute spatiotemporal variation in viral circulation among *Pteropus rufus* populations in Madagascar.

Annotation and Characterization of Bat Fc Receptors

Poster #48

Author list:

O'Shea MM[1], Reers AB[1], Frank HK[1]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA USA

Abstract:

Background. Beyond neutralization, antibodies induce a suite of effector functions through interactions between the Fc domain and Fc receptors (FcR) on immune cells. Some bat species fail to generate neutralizing antibodies during viral infection, suggesting the importance of non-neutralizing antibodies and Fc-FcR signaling to play a significant role in the antiviral immune response. Bats seem to contain canonical Fc genes (IgM, IgG, IgE, IgA, variably IgD) but their receptors remain largely uncharacterized. Here, I annotate the Fc receptors and Fc receptor-like proteins (FCRLs) in high-quality bat genomes. **Methods.**
Publicly available bat genomes were retrieved from NCBI. Mammalian FcR and FCRL sequences were pulled from NCBI using the E-utilities tool and mapped to each bat genome using the BLAST-like alignment tool (BLAT). FcR/FCRL sequences were then extracted, aligned, and screened for immunoreceptor tyrosine-based activation (ITAM) and inhibition (ITIM) motifs. **Results.** Early characterization of 8 bat species revealed differential presence and absence of FcR and FCRL genes. Several Fc gamma receptors seem to be absent among Hipposideridae, Phyllostomidae, and Vespertilionidae, but remain intact in pteropodid bats. Of particular interest is the high-affinity FCGR1, the only receptor capable of binding monomeric IgG in humans. The other, low-affinity Fc gamma receptors require multimeric IgG complexes for signaling, which may indicate that these bat species have a higher threshold for Fc-mediated activation. A similar pattern is seen for FCRL5, a receptor associated with autoimmunity and aging in humans. As taxonomic coverage is increased, this variation in Fc receptor composition across bat species is likely to expand. **Conclusions.** Bats and their immune systems are extremely diverse. Further biochemical, structural, and functional analysis of Fc-FcR interactions and how the Fc-mediated immune response varies across species will inform a more comprehensive understanding of bat antiviral immunity.

A multi-stressor approach to quantifying wildlife health: a pilot study with North American bats

Poster #49

Author list:

Simonis MC[1,2], Ciarrachi S[3], Dyer KE[1], Allira M[1], Demory B[1], Zubayr J[1], Van Parys D[4,5], Whitmore K[6], Chumchal MM[6], Haase CG[4,5], Foster JT[3], Becker DJ[1]

[1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]College of Forestry, Wildlife and Environment, and College of Veterinary Medicine, Auburn University, Auburn, AL, USA; [3]Department of Biological Sciences, Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA; [4]Department of Biology, Austin Peay State University, Clarksville, TN, USA; [5]Center for Excellence in Field Biology, Austin Peay State University, Clarksville, TN, USA; [6]Department of Biology, Texas Christian University, Fort Worth, TX, USA.

Abstract:

Background. Wildlife endure multiple intrinsic and extrinsic stressors, such as habitat loss, pathogen infections, and contaminant exposure, which can increase the energy needed to maintain optimal health and survival. Stressors such as reproduction or migration (intrinsic), and habitat loss, infection, and contaminant exposure (extrinsic), occur simultaneously throughout wildlife lifetimes. Identifying key determinants of wildlife immunity is important for assessing how multiple stressors impact wildlife health. **Methods.** To identify key intrinsic and extrinsic determinants of wildlife health, we piloted a longitudinal study of North American bats and their cellular immune phenotypes in summer 2023. From three sites in the US, we collected blood from each bat to characterize white blood cell differentials, micronuclei intensities, and bartonellae infection, and collected fur to quantify mercury concentrations. We also used literature-based data and the US Geological Survey's National Land Cover Database to quantify spatial features associated with each species per site. We compared 20 generalized linear models with varying combinations of intrinsic and extrinsic stressors for correlations with neutrophils to lymphocyte ratios, a measure of bat immune health. **Results.** The best fit models identified micronuclei intensities, reproductive stage, and land use proportions as key predictors of health. Thus, spatial features and contaminant exposure associated with bat foraging areas may impact bat health during intrinsically stressful life stages. **Conclusions.** As this project continues, we aim to gain more insights into how simultaneous stressors determine outcomes of cellular immune phenotypes. With these preliminary findings, we encourage other bat researchers to increase paired sampling of individual immune parameters with measurements of multiple stressors. Finally, we also solicit for growing collaborations to this broad, spatially coordinated approach for this ongoing study of North American bats.

Microbiome composition varies with infection status, sex and age in three Madagascan fruit bats

Poster #50

Author list:

Cortes-Delgado N[1], Kettenburg G[1], Kistler A[2], Ranaivoson HC[346], Andrianiaina A[36], Santino A[56], Tato CM[2], Brook CE[1]

[1]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA; [2]Chan Zuckerberg Biohub, San Francisco, CA, USA; [3]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar; [4]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar; [5]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar; [6]Association Ekipa Fanihy

Abstract:

Background. Pteropodids are fruit bats that are the reservoir hosts of a broad range of different viruses that can cause fatal diseases in humans including Ebola and Marburg viruses. Among the mechanisms that potentially contribute to bat virus tolerance, is the microbiome. Gut microbiomes influence the health and evolution of mammals and can have an important role in modulating virus dynamics, by competitively excluding pathogens or inhibiting their infectious potential. Factors such as diet, habitat and demographic status play a role in the gut microbiota. Available research on wild bat microbiomes is limited; however, there is evidence that age and reproductive status influence bat gut microbiome diversity and composition. The objective of this study was to characterize the gut microbiome of the Pteropodid bats *Eidolon dupreanum, Pteropus rufus* and *Rousettus madagascariensis*, and identify associations between demographic status, virus infection and microbial communities. **Methods.** We conducted metagenomic Next Generation Sequencing (mNGS) on RNA extracted from 255 fecal swabs. Alpha and beta diversity analyses, as well as differential abundance analyses were carried out on the resulting data using R statistical packages including ANCOM and phyloseq. In addition, we used mNGS data to assess the presence of Coronaviruses, Paramyxoviruses, Arenaviruses and Lyssaviruses in the samples analyzed. **Results.** Firmicutes and Proteobacteria were the dominant gut microbial phyla identified in the bat samples. The proportions of phyla and bacterial families recorded differed between bat species, which matched the observed differences in OTUs (Operational Taxonomic Units) abundances. Composition also changed between adults and juveniles, males and females, and between infected and non-infected individuals of the viruses examined. Alpha diversity metrics were significantly different between the bat species studied, with *R. madagascariensis* having a higher diversity and number of species. Rousettus adult bats also hold a more diverse microbiome in comparison to juveniles. **Conclusions.** Overall, the results obtained showed that bat microbiome diversity and composition change between species and it is influenced by age and health status, which could be

possibly related to genetics, diet differences, and microbiome responses to individual viruses.

Monitoring Nectar-Feeding Bat Communities and Their Exposure to Pathogens in the Ecuadorian Chocó

Poster #51

Author list:

Reuben PL[1], Frank HK[1]

[1]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA

Abstract:

Background. Nectarivorous bats are important pollinators of a wide range of agricultural, ornamental, and native tropical plants, maintaining broader ecosystem health and supporting local economies. Multiple individuals regularly visit the same flowers, potentially exposing them to pathogens from contaminated plant material. Still, little work has been done to validate this infection pathway, or to test the parameters required for contracting infections in this way. Further, nectarivorous bats are sensitive and can be challenging to assess using standard capture techniques. Environmental DNA (eDNA) monitoring provides a promising non-invasive alternative to sampling bats and their pathogens directly. While there is encouraging work on identifying bat visitation from eDNA on flowers, this technique has not been used in areas that contain multiple species, nor has it been evaluated in the context of viral surveillance. Here, I summarize early work to develop and optimize an eDNA monitoring protocol that is being used to track patterns of bat diversity and viral transmission between nectar feeding bats in the Chocó region of Ecuador. **Methods.** Flowers from two species receiving bat visitation were filmed for two hours after sunset using an infrared camera setup to confirm bat feeding. Flowers were either cut or swabbed and stored immediately in DNA/RNA shield solution. Metabarcoding was performed using pooled PCR amplicons of regions that identify bats, plants, and herpesviruses respectively. Local reference sequences were generated from wing punches collected during concurrent mist-net sampling. **Results.** Preliminary results are presented here for feedback. **Conclusions.** The use of eDNA monitoring offers an efficient and non-invasive method of monitoring bat diversity and community-level pathogen exposure. While successful detection provides valuable information, future efforts will include testing the degradation rates of both bat and viral genetic material to better understand time-sensitive transmission dynamics.

Ecological investigations of bat-associated viruses in Uganda

Poster #52

Author list:

Kading RC[1], Azerigyik F[1], Castle K[3], Dewey T[4], Fagre A[1], Harris E[1], Hartwick A[1], Kayiwa J[2], Matovu B[5], Mutebi J-M[5], Nabatanzi L[2], Nakayiki T[2], Nalikka B[5], Nalukenge L[5], Nassuna C[2], Siya A[5], Wickenkamp N[1], Williams K[1], Yiga F[2], Lutwama J[2], Kityo R[5]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA; [2]Uganda Virus Research Institute, Entebbe, Uganda; [3]Wildlife Veterinary Consulting LLC, Fort Collins, CO, USA; [4]Department of Biology, Colorado State University, Fort Collins, CO, USA; [5]Department of Zoology, Entomology and Fisheries Sciences, Makerere University, Kampala, Uganda.

Abstract:

Background. Caves in the Mt Elgon region of Eastern Uganda are commonly inhabited by bats in the genera *Rhinolophus, Hipposideros, Myonycteris, Rousettus, Miniopterus,* and *Nycteris*. Human encroachment into these caves for shelter, cultural purposes, hunting, mineral harvesting, and tourism pose a risk for exposure to infectious agents these bats may carry. Yet, little is known about the diversity of viruses present in these bats, and how bat movement amongst these disturbed caves may affect human and bat population health. **Methods.** Between 2021 - 2023, our collaborative team sampled cave-dwelling bats in this area during wet and dry seasons. In total, there were 1,091 bat captures from 845 individual bats from seven caves. Blood, saliva, feces, rectal swabs from 635 bats were molecularly screened for coronaviruses, paramyxoviruses, rhabdoviruses, flaviviruses, and filoviruses; samples positive for viral RNA were deep sequenced. Bats were also marked with Passive Integrated Transponder (PIT) tags and/or GPS transmitters to track their intercave and regional movement patterns. **Results.** Coronavirus nucleic acid was detected in oral and rectal swab samples from *Hipposideros ruber, Rhinolophus* spp., *Myonycteris angolensis*, and *Miniopterus fraterculus*. Four bats (2 *Hipposideros*, 1 *Myonycteris*, and 1 *Nycteris*) were positive for unique paramyxoviruses and two *Rhinolophus* spp. bats had distinct rhabdoviruses. Movement studies revealed that horseshoe bats flew westward from roost sites in forested mountain habitats to agricultural areas to forage. Angolan rousette bats repeatedly visited preferred fruiting trees in Mt Elgon National Park as well as in areas of dense human habitation, and were capable of flying hundreds of kilometers in a single night. **Conclusions.** This project has generated novel data on the association of bat species and different viral strains and movement patterns of these bats, advancing our knowledge of viral ecology and spillover risk at the human/bat interface.

Understanding How Interferon Regulatory Factor 3 Enhances the Antiviral Response in Bats

Poster #53

Author list:

Crake RM[1,3,4], Mossman KM[2,3,4]

[1]Department of Biochemistry and Biomedical Science, McMaster University, Hamilton, ON, Canada; [2]Department of Medicine, McMaster University, Hamilton, ON, Canada; [3]McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada; [4]Institute for Infectious Disease Research, McMaster University, Hamilton, ON, Canada.

Abstract:

Background. Bats are reservoirs for a diverse array of highly pathogenic viruses and experience limited clinical signs of disease, suggesting that bats may have evolved unique immune characteristics to combat viral infections. One such feature is the positive selection of a serine residue (S185) on interferon regulatory factor 3 (IRF3), an essential transcription factor for antiviral defence, which provides increased antiviral protection. In humans, IRF3 is critical for responding to viral infections, in part through the process of membrane perturbation, in an interferon-independent manner to circumvent proinflammatory cytokine production. Given the similarity of IRF3 in mammalian species, we hypothesize that bats can respond to membrane perturbation in a similar IRF3-dependent, IFN-independent manner that may be enhanced by the positively selected S185 residue in IRF3. **Methods.** HK-2 (human kidney), Paki T03 (P. Alecto) and EFK3B (E. fuscus) cells were infected with replication incompetent virus to assess membrane perturbation-induced antiviral responses. IRF3 knockout and IFNAR knockout cells were generated to determine if antiviral activity can be conferred in the absence of either protein. Antiviral activity was ascertained quantitatively through qPCR of interferon stimulated genes (ISGs) and qualitatively through visualising fluorescence of a GFP-tagged challenge virus.

To understand the function of S185 and its role in IRF3 activity, we performed targeted mass spectrometry to determine whether S185 is post-translationally modified (e.g., phosphorylated) under basal or stimulated conditions. Furthermore, native gels from basal and induced cells were used to assess nuclear translocation, dimerization, and phosphorylation of IRF3 as markers of activation. **Results.** Here, we will present results from our ongoing studies on bat IRF3 and the role of S185. **Conclusion.** This project helps elucidate the molecular mechanisms by which bat IRF3 modulates an antiviral response and provides insight into the unique aspects of bat immune signalling that allow them to serve as reservoirs.

Phylogenetics of non-bat fly ectoparasites in Malagasy fruit bats

Poster #54

Author list:

Andry S[1,4], Andrianiaina AF[2,4], Ranaivoson HC[2,3,4], Kettenburg G[3], Brook CE[3]

[1]Department of Entomology, Antananarivo, Madagascar; [2]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar; [3]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA; [4]Association Ekipa Fanihy, Antananarivo, Madagascar

Abstract:

Background. Malagasy fruit bats (family Pteropodidae) are known to host potentially zoonotic viruses, protozoa, and bacteria, as well as diverse ectoparasites (bat bugs, bat flies, mites, fleas and ticks). Nycteribiid and Streblid bat flies hosted by Eidolon dupreanum and Rousettus madagascariensis fruit bats are the best-described among this latter clade. In this present study, we focus on the diversity and phylogeny of non-bat fly ectoparasites. **Methods.** Mites, fleas, and ticks were collected using sterile forceps from fruit bats captured in a longitudinal Madagascar field study. Bat bugs were sampled from the surrounding cave walls of roosts for E. dupreanum. Ectoparasite specimens were stored in 90% ethanol, then subject to DNA extraction and subsequent PCR targeting the cytochrome C oxidase subunit I (COI), 18S, and 16S genes. PCR products were Sanger sequenced, and resulting sequences underwent BLAST analysis to confirm identity. Sequences from each taxonomic subset (mites, fleas, ticks, bat bugs) were aligned with related references from NCBI. We used RAxML-NG to construct discrete phylogenetic trees for each ectoparasite clade.

Results. Representative specimens from each clade were successfully sampled, barcoded, and deposited in GenBank. Resulting DNA barcodes represent multiple previously undescribed species, in addition to several previously unreported for Madagascar. We identified the first molecular barcodes for Thaumapsylla sp. and Echidnophaga sp. fleas from E. dupreanum hosts and Ischnopsyllidae sp. fleas from R. madagascariensis hosts. Meristapsis sp. mites and ticks in the family Argasidae identified from both bat hosts grouped into two monophyletic clades based on host species. Bat bugs collected were identified in the family Cimicidae, previously unreported in Madagascar. **Conclusions.** Madagascar fruit bats host diverse ectoparasites, many of which represent endemic species. Further research is needed to determine the roles that these parasites may play in the transmission and maintenance of microparasite infections in their fruit bat hosts.

The Quadripartite Framework in Bat Research: Enhancing Pathogen Risk Reduction and Data Quality

Poster #55

Author list:

Islam S[1,2], Kangoyé NM[3], Diallo AH[3,4], Katani R[5,6], Escobar LE[1,2,7]

[1]Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA, USA; [2]Global Change Center, Virginia Tech, Blacksburg, VA, USA; [3]Faculty of Earth and Life Sciences, Department of Animals Biology and Physiology, University Joseph Ki-Zerbo, Ouagadougou, Burkina Faso; [4]Department of Public Health, Centre Muraz Research Institute, Bobo-Dioulasso, Burkina Faso; [5]The Huck Institute of the Life Sciences, Pennsylvania State University, University Park, PA, USA; [6]Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania; [7]Center for Emerging Zoonotic and Arthropod-Borne Pathogens, Virginia Tech, Blacksburg, VA, USA

Abstract:

Background. As researchers increasingly interact with wildlife, the risk of pathogen transmission grows, necessitating stringent biosecurity and biosafety measures. Lapses in these practices, along with compromised sample quality, can pose threats to individuals, populations, species, and ecosystems. Understanding the current state of biosecurity and biosafety in bat research is crucial for mitigating these risks. This study aimed to assess biosecurity and biosafety practices in vampire bat research and introduce the concept of a quadripartite framework for improving research integrity and safety. **Methods.** We conducted a scoping review of published studies that involved direct capture and sampling of vampire bats. **Results.** Of 562 articles reviewed, 161 (28.65%) reported direct sample collection from vampire bats. However, only 4 (2.48%) explicitly mentioned biosecurity and biosafety measures or the protective tools used during sampling. Our findings highlight a critical gap—either in adherence to proper biosecurity and biosafety protocols or in the reporting of such practices. To address the biosecurity and biosafety practice limitations, we propose a holistic, interdisciplinary quadripartite framework that considers four key pillars: researcher safety, animal welfare, environmental health, and sample quality. Researcher safety emphasizes effective biosecurity and biosafety practices with regular monitoring and evaluation. Animal welfare follows the principles of replacement, reduction, and refinement. Environmental health focuses on proper cleaning, waste management, and disposal. Sample integrity ensures research-specific collection, secure transportation, and appropriate storage. **Conclusions.** This quadripartite framework will promote ethical research practices while ensuring transparent, high-quality data collection. The effective implementation of this framework will enhance research reliability and benefit human, bat, and environmental health alike.

Paramyxovirus prevalence in pteropodid fruit bats from the Philippines

Poster #56

Author list:

Borthwick SA[1], Low D[1], Café J[2], Nacion L[2], Sorino JCS[2], Tenoso JC[2], Hisuan JG[3], Garcia JJ[3], Taray K[3], Magsanoc S[3], Cruz H[3], Manzano D[3], Hayman DTS[4], Mendenhall IH[1], Laing ED[5], Dacuma MGB[3], Alviola PA[3], Demetria CS[2], Malbas FF[2], Smith GJD[1]

[1]Programme in Emerging Infectious Disease, Duke-NUS Medical School, Singapore; [2]Research Institute for Tropical Medicine, Philippines; [3]University of the Philippines Los Baños, Philippines; [4]School of Veterinary Science, Massey University, New Zealand; [5]Uniformed Services University of the Health Sciences, USA.

Abstract:

Background. Bats are the second most taxonomically diverse group of mammals, and are reservoirs for a multitude of zoonotic viruses including highly pathogenic paramyxoviruses, coronaviruses and filoviruses. There is cross-species transmission of bat-borne viruses to naïve hosts that can result in significant mortality and economic cost. The Philippines has experienced outbreaks of zoonotic viruses such as Nipah and Hendra virus, but there are still knowledge gaps in the circulation of these bat-borne paramyxoviruses. **Methods.** This project aims to identify temporal trends and the associated environmental risk factors that may influence viral load and persistence by surveying the Philippines bat population longitudinally across two years. **Results.** We have screened 7,043 rectal swabs collected from 10 fruit bat species across five sites in the Philippines between July 2023 to February 2025 for the presence of paramyxoviruses. Paramyxovirus prevalence was the highest in Desmalopex leucopterus and the 3 Pteropus species. Paramyxovirus sequences were obtained from 76 individual bats from several bat species collected during this period. **Conclusions.** Preliminary analysis suggest that the circulating paramyxoviruses in Pteropodid fruit bats from the Philippines belongs to the Henipavirus, Orthorubulavirus and Pararubulavirus genera with multiple other host species.

Functionally Distinct Duplicated Immunoglobulin Heavy Chain Loci in Bats

Poster #57

Author list:

Pursell T[1,2], Reers A[3], Mikelov A[1], Kotagiri P[4], Lam B[1], Ellison JA[5], Boyd SD[1,6], Frank HK[3]

[1]Department of Pathology, Stanford University, Stanford, CA, USA; [2]Department of Microbiology & Immunology, Stanford University, Stanford, CA, USA; [3]School of Science & Engineering, Tulane University, New Orleans, LA, USA; [4]Department of Immunology and Pathology, Monash University, Melbourne, Australia; [5]Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, CDC, Atlanta, GA, USA; [6]Sean N. Parker Center for Allergy and Immunology Research, Stanford University, Stanford, CA, USA

Abstract:

Background. Bats exhibit robust humoral immunity, as evidenced by serological studies. However, the genetic and molecular basis underpinning the formation and evolution of bat B cell receptor (BCR) repertoires, as well as the resulting antibodies, remain largely uncharacterized. The current mammalian immunological dogma states that the genes which generate the heavy chain of immunoglobulin (Ig) proteins are encoded by a single immunoglobulin heavy chain (IGH). Our analysis of recent high-quality, chromosome-level bat assemblies identified dual, complete, and functional IGH loci on separate chromosomes in eleven vespertilionid species. This genomic architecture is unprecedented among mammals. **Methods.** We utilized single-cell transcriptomics and bulk BCR repertoire data from Eptesicus fuscus to characterize these loci. This included characterizing functional rearrangements, diversity mechanisms, and antigen-driven selection in the loci. **Results.** Both IGH loci exhibit functional rearrangement and expression in all major B cell phenotypes. We observed distinct mechanisms of antibody diversity, with a strong bias toward the smaller, compact locus. Differential selection was evident in antigen-experienced B cells and plasma cells, suggesting unique functional roles for antibodies encoded by each locus. These findings imply that each loci contributes distinctly to the immune response. **Conclusions.** These findings reveal a unique adaptation in mammalian humoral immunity, offering new insights into the antibody responses in bats, and the functional specialization of their humoral immunity.

Comparative Analysis of Bat Immunoglobulin Constant Regions Identifies Adaptations of Bat Immunity

Poster #58

Author list:

Thavornwatanayong T[1,2], Wiantoro S[3], Kaczmarek ME[1], Kantorski M[1], Segovia E[1], Reyes K[1], Vuong BQ[1,2,5], Tsang SM[4]

[1]Department of Biology, The City College of New York, The City University of New York, New York, NY, USA; [2]PhD Program in Biology, CUNY Graduate Center, The City University of New York, New York, NY, USA; [3]Museum Zoologicum Bogoriense, Research Center for Biosystematics & Evolution, National Research and Innovation Agency, Bogor, Indonesia; [4]Department of Mammalogy, American Museum of Natural History, New York, NY, USA; [5]PhD Program in Biochemistry, CUNY Graduate Center, The City University of New York, New York, NY, USA

Abstract:

Background. Evidence suggests bat immune systems promote resistance or tolerance to viruses that cause diseases in other organisms. While many studies of bat immune responses have focused on innate immunity and immune signaling molecules such as interferons (IFNs), the role of adaptive immunity, including immunoglobulins (Igs), in viral resistance or tolerance in bats remains largely uncharacterized. Interestingly, positively selected immune genes in bats include complement C1 and TRIM21, which bind to Ig constant regions to facilitate adaptive immunity. We hypothesize that bat Ig constant regions evolved as a part of bat immunity adaptation. **Methods.** We reconstructed the phylogenies of bat IgM, IgA and IgG constant region genes and tested for signals of selection across phylogenies through Branch-Site Random Effects Likelihood (aBSREL). We further detected positive selection signal sites in each Ig constant region using Mixed Effects Model of Evolution (MEME). **Results.** Our Ig constant region gene trees broadly agreed with established evolutionary relationships between bat families and we found certain branches in each Ig constant regions are under positive selection. At the amino acid level, the sites where IgM and IgG interact with the complement C1q complex were positively selected. Additionally, the selected sites were clustered near the regions where the Ig constant regions interact with their respective Fc receptors: Fc alpha/mu Receptor (Fcα/μR) for IgM and IgA and neonatal Fc receptor (FcrRn) and Tripartite motif containing 21 (TRIM21) for IgG. **Conclusions.** Because the interactions of Ig constant regions with Fc receptors facilitate both innate and adaptive immunity, our data implicate a function for Ig in bat resistance or tolerance to viruses.

Bat-specific adaptations in interferon signaling and GBP1 contribute to enhanced antiviral capacity

Poster #59

Author list:

Gonzalez V[1,2], Lobb B[3], Cote J[4,5,6,7], Bhuinya A[1,2], Tubb AG[8], Nuthalapati SS[8], Asavajarau A[1], Zhou Y[1,2], Misra V[2], Falzarano D[1,2], Sweeney TR[8,9], Gobeil SMC[4,5,6,7], Wang L[10], Doxey A[3], Banerjee A[1,2,3,11,12]

[1]Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada; [2]Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada; [3]Department of Biology, University of Waterloo, ON, Canada; [4]Département de Biochimie, de Microbiologie et de Bio-informatique, Faculté des Sciences et de Génie, Université Laval, Québec, QC, Canada; [5]Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, QC, Canada; [6]PROTEO, Le Regroupement Québécois de Recherche sur la Fonction, L'Ingénierie et les Applications des Protéines, Université Laval, Québec, QC, Canada; [7]Centre de Recherche en Infectiologie de l'Université Laval, Université Laval, Québec, QC, Canada; [8]The Pirbright Institute, Guildford, Surrey, UK; [9]Department of Virology, University of Cambridge, Cambridge, UK; [10]Programme in Emerging Infectious Disease, Duke-NUS Medical School, Singapore; [11]Department of Laboratory Medicine and Pathobiology, University of Toronto, ON, Canada; [12]Department of Biochemistry and Molecular Biology, University of British Columbia, BC, Canada.

Abstract:

Background. Bats are reservoirs of emerging zoonotic viruses of concern that cause severe disease in humans and agricultural animals. However, it is poorly understood how bats are able to tolerate diverse viral infections, knowledge that could help pave the way for new therapeutic strategies. **Methods.** We investigated the type I IFN responses in bat cells through the synthesis of IFNβ from *Pteropus alecto*, *Eptesicus fucus*, and humans. *P. alecto* (PaKiT03), *E. fuscus* (Efk3B), and human (A549, RPTEC) cells were then treated with their species-matched IFNβ for various lengths to assess protection against vesicular stomatitis virus (VSV) and Middle East respiratory syndrome coronavirus (MERS-CoV). In addition, the IFNβ signaling pathway was profiled by RNA-sequencing. **Results.** We identified critical roles of STAT1 and STAT2 in IFNβ signaling, along with species-specific adaptations that collectively contribute towards a "steady and ready" antiviral state in bat cells. Unlike in humans, we find that bat interferon signaling processes resist the immune antagonistic properties of viruses like MERS-CoV which further explains the ability of bats to control coronavirus infections. Using transcriptomic analysis, we identified canonical and non-canonical interferon stimulated genes including two differentially expressed genes, IFIT1 and GBP1. Compared to their human orthologs, we show that bat IFIT1 and GBP1 exhibit enhanced antiviral activity against a wide range of RNA and DNA viruses, including coronaviruses and additional bat-derived Eptesipoxvirus. **Conclusion.** Using species-matched IFNβ, we observed a

potent response in bat cells that rapidly lead to protection against VSV and MERS-CoV infection. This may be due to the activity of non-canonical ISGs, such as GBP1, where we have identified a new functional (AV1) motif within *E. fuscus* GBP1 that enables it to restrict Eptesipoxvirus replication. Ultimately, our work provides important insights into the evolution of enhanced interferon-mediated antiviral responses in bats, contributing to their unique ability to resist viral diseases.

Loss of PYHIN Gene Family in Bats Reveals Unique Regulation of the AIM2 Inflammasome

Poster #60

Author list:

Wood G[1], Kiriakov S[1],Yang W[1], Randhawa R[1], Chiang SH[1], Hong L[1], Lim E[1], Loh A[1], Padmanabhan N[1], Kua L[1], Cockett M[1]

[1]Paratus Sciences, New York, NY, USA

Abstract:

Background. Bats display remarkable immune tolerance despite serving as reservoirs for zoonotic viruses. One molecular pathway activated in inflammation and viral response is the AIM2 inflammasome, composed of AIM2, PYCARD, and CASP1. The AIM2 sensor belongs to the PYHIN gene family, which senses DNA entering the cell through its HIN domain. Loss of this domain would predict reduced inflammasome activation in response to viral pathogens. Here, we investigate the status of PYHIN genes and AIM2 inflammasomes across publicly available bat genome alignment data (TOGA), showing how gene loss in bats affects their unique immunity. **Methods.** At Paratus Sciences, we are committed to the generation of high-quality bat data to share with researchers globally. We work with field experts to collect tissue samples and perform Hi-Fi and Hi-C sequencing in-house. Following sequencing, we work with collaborators to assemble and curate the genomes and then functionally annotate them internally. This generated data is then shared with the broader Bat1k community. Separately for this analysis, we retrieved publicly available PYHIN Gene Family and AIM2 Inflammasomes gene loss data from the TOGA (Tool to Infer Orthologs from Genome Alignments) browser. Patterns of gene loss and retention were assessed across all available bat species in the TOGA database. **Results.** All examined bat species showed loss of AIM2, IFI16, MNDA, and PYHIN1. The only gene that remained intact in the PYHIN gene family was POPDC3, which is consistent with the unique loss of the HIN domain in bats. Furthermore, the AIM2 Inflammasomes PYCARD and CASP1 remained intact in most species. **Conclusions.** Bats exhibit widespread loss or disruption of the PYHIN Gene Family, including AIM2, which is a key activator of the AIM2 inflammasome pathway. This gene loss contributes to bats' unique immune tolerance by dampening DNA-sensing inflammation and avoiding excessive immune responses to viral infection.