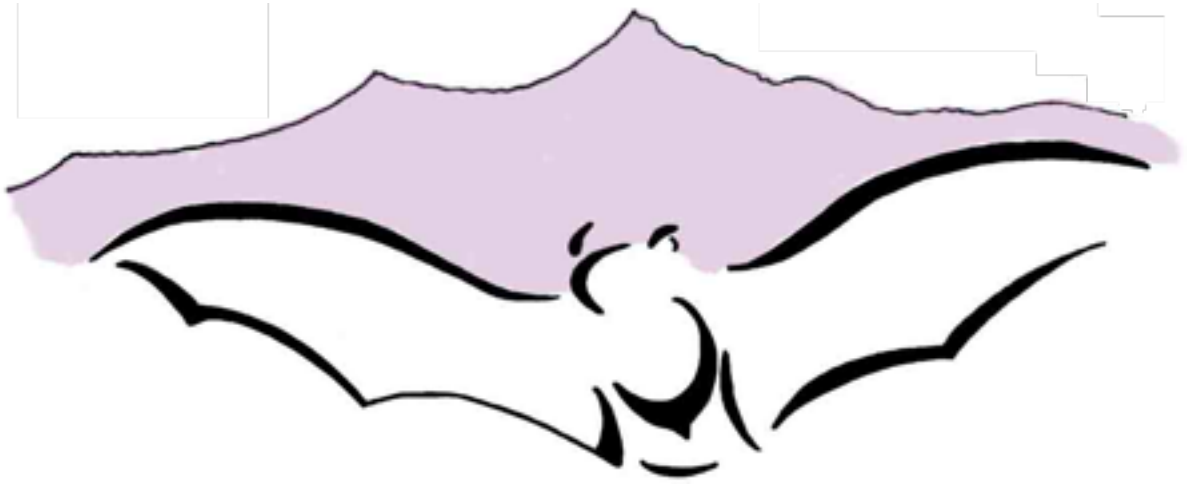
3rd International

Infectious Diseases of Bats Symposium



July 24-27, 2022
Lory Student Center Ballrooms C and D
Colorado State University
Fort Collins, CO, USA

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Program

Venue: **Lory Student Center, Ballrooms C and D**, Colorado State University

Sunday, July 24

5:30 PM Registration, **Ballroom D**

5:30 PM to 7:30 PM Reception - *Wine, beer, non-alcoholic drinks and hors d'oeuvres*, **Ballroom D**

Monday, July 25

7:30 AM **Registration, Lory Student Center**

8:30 AM **Tony Schountz**. Colorado State University. **Welcoming remarks**

8:45 AM **Session I - Coronaviruses I (Joel Rovnak, moderator)**

8:45 AM **Susanna KP Lau**, University of Hong Kong. *Isolation of a MERS-related coronavirus from lesser bamboo bats that uses DPP4 receptor and infects human-DPP4-transgenic mice* (prerecorded presentation)

9:00 AM **Kevin J Olival**, EcoHealth Alliance, USA. *Bat coronaviruses surveillance in Western Asia (2017-2022)*

9:15 AM **Anne Balkema-Buschmann**, Friedrich-Loeffler-Institut, Germany. *Intranasal versus oral SARS-CoV-2 inoculation of Rousettus aegyptiacus fruit bats induce distinct differences in the course of infection*

9:30 AM **Ariful Islam**, EcoHealth Alliance, USA. *Epidemiology and genetic diversity of novel coronaviruses in Bats, Bangladesh*

9:45 AM **Vera C Mols**, Erasmus University Medical Center, Netherlands. *Intestinal tropism of a* Betacoronavirus *(*Merbecovirus*) in its natural Nathusius' pipistrelle (*Pipistrellus nathusii*) host*

10:00 AM **Break**

10:30 AM ***KEYNOTE ADDRESS***: **Peter Daszak**, EcoHealth Alliance, USA. *Bat-origin coronaviruses provide a unique platform for a strategy to predict and prevent disease emergence*

11:00 AM **Session II - Immunology I (Paul Cryan, moderator)**

11:00 AM **Hannah K Frank**, Tulane University, USA. *Strong selective signatures of viruses on bat innate and adaptive immunity*

11:15 AM **Amanda Vicente-Santos**, Emory University, USA. *Differential eco-immunological response to human disturbance in cave-dwelling bats*

11:30 AM **Julia R Port**, Rocky Mountain Laboratories, NIAID, USA. *Suitability of* Artibeus jamaicensis *bats for modeling SARS-CoV-2 reservoir infections*

11:45 AM **Daniel Becker**, University of Oklahoma, USA. *Leveraging serum proteomics to characterize bat immune phenotypes and response to viral infection*

12:00 PM **Lunch, Ram's Horn Dining Hall, Academic Village**

1:30 PM **Session III - Ecology (Kathryn Stoner, moderator)**

1:30 PM **Robert Martin Kityo**, Makerere University, Uganda. *Bat Ecology in the pathogen mix in the Mt Elgon foothill landscape* (prerecorded presentation)

1:45 PM **Raina Plowright**, Cornell University, USA. *Dynamics of bat pathogens: drivers of spillover risk*

2:15 PM **DeeAnn Reeder**, Bucknell University, USA. *Viral evidence from African bats: the good, the bad, and the ugly*

2:30 PM **Lisa Worledge**, Bat Conservation Trust, UK. *Bat Disease Surveillance in the UK: engaging conservation volunteers & bat workers*

2:45 PM Break

3:15 PM **Rebekah Kading**, Colorado State University, USA. *Common ground: the foundation of interdisciplinary research on bat disease emergence*

3:30 PM **Tigga Kingston**, Texas Tech University, USA. *Bat Research in the Time of Covid-19 – Risks and Recommendations from the IUCN Bat Specialist Group*

3:45 PM **Cara E Brook**, University of Chicago, USA. *Reservoir host immunology and life history shape virulence evolution in zoonotic viruses*

4:00 PM **Joanna Shisler**, National Science Foundation, USA. *Funding opportunities from the National Science Foundation*

Tuesday, July 26

8:00 AM Registration, Lory Student Center

8:00 AM Session IV - Filoviruses (Rebekah Kading, moderator)

8:00 AM **Xing-Lou Yang**, Kunming Institute of Zoology, Chinese Academy of Sciences. *Ecological study of cave nectar bats reveals low risk of direct transmission of bat viruses to humans* (prerecorded presentation)

8:15 AM **Jonathan Epstein**, EcoHealth Alliance. *Ebola virus (Zaire ebolavirus) sequence and antibody detection in bats in Liberia, 2016-2018*

8:30 AM **Gábor Kemenesi**, University of Pécs, Hungary. *Isolation of Lloviu virus from Schreiber's bat: the hunt for Lloviu virus in Europe*

8:45 AM **Lisa Powers**, Bucknell University, USA. *Response of a North American bat species to immunization with Ebola-like virus particles*

9:00 AM **Emily Cornelius Ruhs**, University of Chicago, USA. *Seasonal patterns in the serology of henipa- and filoviruses in Madagascar fruit bats*

9:15 AM **Brian R. Amman**, Centers for Disease Control and Prevention, USA. *Assessment of Zoonotic Disease Risk Associated with Nightly Foraging and Dispersal Activity by Egyptian Rousette Bats (*Rousettus aegyptiacus*) in Southwest Uganda*

9:30 AM **Silke Riesle-Sbarbaro**, Robert Koch Institute, Germany. *Selective and high replication of Zaire ebolavirus following experimental inoculation of the Angolan free-tailed bat (*Mops condylurus*) with filoviruses*

9:45 AM Break

10:15 AM Session V - Paramyxoviruses (Anne Balkema-Buschmann, moderator)

10:15 AM **Alison J Peel**, Griffith University, Australia. *Using multiplexed assays to investigate serological cross-reactivity and identify the presence of novel viruses: a case study of henipaviruses and pararubulaviruses in Australian flying foxes*

10:30 AM **Moushimi Amaya**, Uniformed Services University, USA. *A recombinant chimeric Cedar virus based surrogate neutralization assay for pathogenic henipaviruses*

10:45 AM **Claude Kwe Yinda**, Rocky Mountain Laboratories, NIAID, USA. *Genetic variability of Hendra virus in Australian pteropodid bats over time and space*

11:00 AM **Chanakha K Navaratnarajah**, Mayo Clinic, Rochester, MN USA. *The role of the fusion glycoprotein in henipavirus particle assembly*

11:15 AM **Wolfgang Preiser**, University of Stellenbosch, South Africa. *Paramyxoviruses in insectivorous bats in South Africa*

11:30 AM Lunch, Ram's Horn Dining Hall, Academic Village

1:00 PM This Week in Virology with Vincent Racaniello and Brianne Barker (Ballroom C)

2:00 PM Poster Session (Ballroom D)

3:30 PM Session VI - Coronaviruses II (Amy Gilbert, moderator)

3:30 PM **Vincent Munste**r, Rocky Mountain Laboratories, NIAID, USA. *Preparedness for bat-borne coronaviruses, Lessons from the pandemic*

4:00 PM **Ken Field**, Bucknell University, USA. *Transcriptomic Responses to Coronavirus Infections in African and North American bats*

4:15 PM **Tyler N Starr**, University of Utah, USA. *ACE2 binding is an ancestral and evolvable trait of SARS-related coronaviruses*

4:30 PM **Caitlin Kollander**, University of Alaska Anchorage, USA. *Alaskan* Myotis lucifugus *Virome Reveals Bat Alphacoronavirus Prevalence and Likely Secondary Acquisition of Diet and Habitat-related Viruses*

4:45 PM **Cecilia A. Sánchez, EcoHealth Alliance**, USA. *A strategy to assess spillover risk of bat SARS-related coronaviruses in Southeast Asia*

5:00 PM **Michael Letko, Washington State University**, USA. *An ACE2-dependent Sarbecovirus in Russian bats is resistant to SARS-CoV-2 vaccines*

Wednesday, July 27

8:30 AM Session VII - Immunology II (Arinjay Banerjee, moderator)

8:30 AM **Peng Zhou**, Wuhan Institute of Virology, China. *On the evolution of bat type I interferons* (prerecorded presentation)

8:45 AM **William J Liu**, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, China. *The characteristics of bat MHC class I and CD8 reveal unique anti-viral immunity* (prerecorded presentation)

9:00 AM **Angela R. Mingarelli**, McGill University, Canada. *Interrogating anti-viral innate immune responses of Chinese* Hipposideros *bats*

9:15 AM **Karen Mossman**, McMaster University, Canada. *Differential IRF3-independent innate immune signaling in human and bat cells*

9:30 AM **Jonathan Guito**, Centers for Disease Control and Prevention, USA. *Immunoprotective disease tolerance in Marburg virus-infected reservoir host bats*

9:45 AM **Tony Schountz**, Colorado State University, USA. *Profiling virus-specific helper T cells from Jamaican fruit bats*

10:00 AM Break

10:30 AM Session VIII - Other Infectious Agents of Bats (Ashley Malmlov, moderator)

10:30 AM **Stefania Leopardi**, University of Pecs, Hungary. *Co-circulation of West Caucasian bat Virus, Lleida bat virus and Lloviu virus in* Miniopterus schreibersii *in Italy and Hungary* (prerecorded presentation)

10:45 AM **Eugenia Corrales-Aguilar**, Universidad de Costa Rica. *Serological Positivity against Selected Flaviviruses and Alphaviruses in Free-Ranging Bats and Birds and in Domestic and Peri-domestic Mammals Evidence Exposure to Arboviruses Seldom Reported Locally in Humans*

11:00 AM **Davide Lelli**, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy. *Bat-borne Issyk-Kul virus in Italy: isolation and genome characterization*

11:15 AM **Richard Yanagihara**, University of Hawaii at Manoa, USA. *Molecular Phylogeny of Bat-borne Gammaherpesviruses in Vietnam*

11:30 AM **Paul Cryan**, U.S. Geological Survey, USA. *Globalizing accessible field observation tools for bats*

11:45 AM Lunch, Ram's Horn Dining Hall, Academic Village

1:15 PM Session IX - Other Infectious Agents of Bats (continued) (Anna Fagre, moderator)

1:15 PM **Amy J Schuh**, Centers for Disease Control and Prevention, USA. *Kasokero Virus-Inoculated Egyptian Rousette Bats Shed Virus through Multiple Routes*

1:30 PM **Peter Reuther**, University Medical Center Freiburg, Germany. *Molecular characterization of the interactions between Bat-borne Influenza viruses and host MHC-II molecules*

1:45 PM **Kevin Ciminski**, University Medical Center Freiburg, Germany. *Characterization of the bat-derived influenza A viruses H17N10 and H18N11*

2:00 PM Session X - Lyssaviruses (Anna Fagre, moderator)

2:00 PM **Wanda Markotter**, University of Pretoria, South Africa. *Bats and rabies; An African perspective and implications for lyssavirus taxonomy*

2:15 PM **Celeste Huaman**, Uniformed Services University, USA. *Control of established, CNS-resident lyssavirus infection by a $CD4^+$ T cell dependent immune response stimulated by single-dose monoclonal antibody therapy*

2:30 PM **Amy Gilbert**, National Wildlife Research Center, USDA, USA. *Rabies virus spillover and host shifts from bats into meso-carnivores*

2:45 PM **Open Discussion**

3:30 PM Adjourn

Posters

1. **Akshamal M. Gamage**, Wharton Chan, Feng Zhu, Randy Jee Hiang Foo, Lin-Fa Wang. Single-cell transcriptome analysis of the in-vivo response to viral infection in the cave nectar bat *Eonycteris spelaea.* Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

2. **Catherine E. Arnold**[1,2], Gregory K. Rice[1,3,] Priscila Ikeda[4,5], Clifton L. Dalgard[6,7], Regina Z. Cer[1], J. Stephen Dumler[8], Marcos Rogério André[4], Kimberly Bishop-Lilly[1]. Virome analysis of both bats and ectoparasites of bats captured in Campo Grande, Brazil. [1] Genomics and Bioinformatics Department, Biological Defense Research Directorate, Naval Medical Research Center-Frederick, Fort Detrick, MD, USA. [2] Defense Threat Reduction Agency, Fort Belvoir, VA, USA. [3] Leidos, Reston, VA, USA. [4] Laboratório de Imunoparasitologia, Departamento de Patologia, Reprodução e Saúde Única, Universidade Estadual "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [5] Veterinary Department, Universidade Estadual do Centro-Oeste UNICENTRO, Guarapuava, Paraná, Brazil. [6] Department of Anatomy, Physiology & Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA. [7] The American Genome Center, Uniformed Services University of the Health Sciences, Bethesda, MD, USA. [8] Department of Pathology, Uniformed Services University, Bethesda, Maryland, USA.

3. **Bradly E. Burke**[1], Savannah M. Rocha[2], Ronald B. Tjalkins[2], Wenjun Ma[3], Tony Schountz[1]. Antibody Validation for immunophenotyping T and B cells of the Jamaican fruit bat**.** [1]Department of Microbiology, Immunology, and Pathology,Colorado State University; [2]Department of Environmental and Radiological Health Sciences, Colorado State University; [3]Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO

4. **Nicole Castaneda**[1], Jorgensen, Marcus A.[1], Starbuck, Clarissa A.[2], O'Keefe, Joy M. [2], & Hews, Diana K.[1] Does wing damage from white-nose syndrome predict other health measures? [1]Department of Biology, Indiana State University, Terre Haute, IN 47809; [2]College of Agricultural, Consumer & Environmental Sciences, University of Illinois Urbana-Champaign, Urbana 61801.

5. **Kevin Castle**[1], Miles Eckley[2], Shijun Zhan[2], Pedro Boscan[3], Tony Schountz[2]. Injectable, reversible anesthesia in captive Jamaican fruit bats (*Artibeus jamaicensis*). [1]Wildlife Veterinary Consulting, LLC; Livermore, CO, USA. [2]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA. 3Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA.

6. **Daniel Crowley**[1], Caylee Falvo[1], Aga Apple[2], Raina Plowright[1]. Measuring Affinity Maturation and Somatic Hypermutation in Jamaican Fruit Bats. [1]Department of Public and Ecosystem Health, Cornell University, Ithaca, NY.; [2]Department of Microbiology and Immunology, Montana State University, Bozeman, MT.

7. **Michelle Culbertson**[1], Clay Carey[2], Zoë Hilbert[1], Nels Elde[1] Evolutionary conflicts between bats and diarrheal pathogens. Department of Human Genetics, University of Utah, Salt Lake City, United States[1]; Department of Biology, University of Utah, Salt Lake City, United States[2]

8. **Caylee Falvo**[1,] Dan Crowley[1], Raina Plowright[1], Aga Apple[2]. Effect of diet on viral shedding in experimental infection of Jamaican fruit bats. [1]Department of Public and Ecosystem Health, Cornell University, Ithaca, NY; [2]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT.

9. **Gábor Endre Tóth**[1,2], Tamás Görföl[1,2], Ágota Ábrahám[1,2], Zsófia Lanszki[1,2], Gábor Kemenesi[1,2]. Demonstration of the feasibility of on-site laboratory tools to study bat viruses. [1] National Laboratory of Virology (Hungary), [2] University of Pécs.

10. **Sophia Horigan**[1], Gwenddolen Kettenburg[1], Hafaliana Christian Ranaivoson[2,3], Angelo Andrianiana[2], Santino Andry[4,] Anecia Gentles[5], Amy Kistler[6], Ny Anjara Fifi Ravelomanantsoa[2], Cara Brook[1]. Detection of whole genome astrovirus sequence in Madagascar fruit bats. [1]Department of Ecology and Evolution, University of Chicago, Chicago, USA; [2]Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar; [3]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar; [4]Department of Entomology, University of Antananarivo, Antananarivo, Madagascar; [5]Odum School of Ecology, University of Georgia, Athens, USA; [6]Chan Zuckerberg Biohub, San Francisco, USA

11. **Gwenddolen Kettenburg**[1], Hafaliana Christian Ranaivoson[2,3], Angelo Andrianiaina[2], Santino Andry[4], Anecia Gentles[5], Amy Kistler[6], Sharline Madera[6], Ny Anjara Fifi Ravelomanantsoa[2], Cara Brook[1] Insights from whole genome sequences of Madagascar bat viruses. Department of Ecology and Evolution, University of Chicago, Chicago, USA[1]; Department of Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar[2]; Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar[3]; Department Entomology, University of Antananarivo,

Antananarivo, Madagascar[4]; Odum School of Ecology, University of Georgia, Athens, USA[5]; Chan Zuckerberg Biohub, San Francisco, USA[6].

12. **Ashley Malmlov**[1] and Anna Fagre[1,2]. Batabase: a publicly-available relational database of species-specific physiological bat data. Bat Health Foundation, Fort Collins, USA[1]; Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, USA[2].

13. **Marana Tso**[1], Spencer L. Sterling[1,2], Hafaliana Christian Ranaivoson[3,4], Gwenddolen Kettenburg[5], Angelo Andrianiaina[3], Santino Andry[3], Fifi Ravelomanantsoa[3], Jean-Michel Héraud[4,6], Eric D. Laing[1], Cara Brook[5]. Serologic evidence of marburgviruses in Madagascan rousettes. [1]Department of Microbiology and Immunology, Uniformed Services University of Health Sciences, Bethesda, USA. [2]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Rockledge, MD, USA. [3]Department of Zoology and Animal Biodiversity at the University of Antananarivo, Madagascar. [4]Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar. [5]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA. [6]Virology Department, Institut Pasteur de Dakar, Dakar, Senegal.

14. J. Low de Vries and **Wanda Markotter**. Wing tattoos: A cost-effective and permanent method for marking bats. Centre for Viral Zoonoses, Department of Medical Virology, School of Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa.

15. **Wanda Markotter**[1], de Vries JL[1], Dietrich M[2], Epstein JH[1,3], Geldenhuys M[1], Mortlock M1, Pawęska JT[1,4], Weyer J[1,4]. Maintenance and excretion dynamics of paramyxo- and coronaviruses within South African frugivorous bat populations. [1] Centre for Viral Zoonoses, Department of Medical Virology, University of Pretoria, Pretoria, South Africa. [2]UMR Processus Infectieux en Milieu Insulaire Tropical, Sainte-Clotilde, Reunion, [3]EcoHealth Alliance, New York, United States of America, [4]Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases of the National Health Laboratory Services, Johannesburg, South Africa.

16. **Mitra Gultom**[1], Laura Laloli[1,2], Lukas Probst[1,2], Manon Wider[1], Andres Moreira-Soto[3], Eugenia Corrales-Aguilar[3] and Ronald Dijkman[1]. Investigating the innate immune response of bat respiratory epithelium during influenza virus infection. [1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland. [2]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland. [3]Virology, Research Center for Tropical Diseases (CIET), Faculty of Microbiology, University of Costa Rica, Costa Rica.

17. **Betty Nalikka**[1], Robert M. Kityo[1], Benard Matovu[1], Lillian Nalukenge[1], Jack-Michael Mutebi[1], Aggrey Siya[1], Natalie Wickenkamp[2], Kalani Williams[2], Emma Harris[2], Anna C. Fagre[2], Teddy Nakayiki[3], Charity Nassuna[3], Leonara Becky[3], John Kayiwa[3], Kevin Castle[4], Tanya Dewey[5], Julius Lutwama[3], and Rebekah C. Kading[2]. Preliminary observations on ranging patterns of two rhinolophid bats in the Mt Elgon area.[1]Department of Zoology, Entomology, and Fisheries Sciences, Makerere University, Kampala, Uganda; [2] Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA; [3]Uganda Virus Research Institute, Entebbe, Uganda; [4]Wildlife Veterinary Consulting, Fort Collins, CO, USA; [5]Department of Biology, Colorado State University, Fort Collins, CO, USA.

18. **Andrea D Ordonez**, Conor J Kelly, Alex Hirano, Giulia Irene Maria Pasquesi, Edward B Chuong. Investigating the evolution of bat immune transcriptomes by short and long-read RNA-seq. BioFrontiers Institute, University of Colorado Boulder, Boulder, CO USA.

19. **Mariëtte Pretorius**[1,2], Wanda Markotter[1] and Mark Keith[2]. Assessing the extent of land-use change around important bat-inhabited caves. Centre for Viral Zoonoses, Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, South Africa[1]; Mammal Research Institute, Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa[2]

20. **Randy Foo**[1], Ying Ying Hey[1], Justin Ng Han Jia[1], Yok Teng Chionh[1], Wan Ni Chia[1], Pui San Kong[1], Benjamin P. Y-H. Lee[2], Adrian Eng Zheng Kang[1], Sophie Alison Borthwick[1], Dolyce Low Hong Wen[1], Ian Hewitt Mendenhall[1], and Lin-fa Wang[1]. Establishment of a captive cave nectar bat (*Eonycteris spelaea*) breeding colony in Singapore. [1]Programme in Emerging Infectious Disease, Duke-Nus Medical School Singapore, Singapore. [2]Wildlife Management Division, National Parks Board (NParks), Singapore

21. **Aggrey Siya**[1], Richardson Mafigiri[2], Richard Migisha[3] and Rebekah C. Kading[4] Bat-borne viruses from an anthrozoological lens and community-based surveillance capacities in Mount Elgon, Uganda. [1]Department of Environmental Management, Makerere University, P.O. Box 7062, Kampala, Uganda; [2]Infectious Diseases Institute, Global Health Department, Makerere University, Kampala, Uganda; [3]Department of Physiology, Mbarara University of Science and Technology, Mbarara, Uganda; [4]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA.

22. **Spencer L. Sterling**[1,2], Phireak Hip[3], Piseth Ly[3], Pidor Ouch[3], Menghou Mao[3], Dolyce H.W. Low[4,5], Christopher C. Broder[1], Jeffrey C. Hertz[3], Ian H. Mendenhall[5], and Eric D. Laing[1]. Serologic evidence of human exposure to known and unknown henipaviruses, Cambodia. Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD, USA[1];Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockledge, MD, USA[2];Navy Medical Research Unit No. 2, Singapore, SGP and [4]Detachment Phnom Penh, Phnom Penh, KHM[3];NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, SGP[4];Programme in Emerging Infectious Disease, Duke-NUS Medical School, Singapore, SGP[5]

23. **Taylor Pursell**[1, 2], James Ellison[3], Scott Boyd[2], Hannah Frank[4]. Characterization of *Eptesicus fuscus* splenic immune cell landscape following rabies infection. Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, USA$_1$; Department of Pathology, Stanford University School of Medicine, Stanford, USA[2]; Division of High-Consequence Pathogens and Pathology, Center for Disease Control, Atlanta, USA[3]; Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, USA[4].

24. **Touseef Ahmed**[1], Aitezaz Ahsan[2], Sajida Noureen[3], Muhammad Farooq Tahir[4], Hamid Irshad[2,] Arshad Javid[5], Mamoona Irshad[5], Mudassar Hussain[5], Tigga Kingston[1]. The role of Indian flying foxes (*Pteropus medius*) in propagating antimicrobial resistance in the environment. [1]Department of Biological Sciences, Texas Tech University, Lubbock, Texas, USA; [2]Animal Health Research Laboratories, National Agricultural Research Centre (NARC), Islamabad; [3]University of Haripur, Khyber Pakhtunkhwa, Pakistan; [4]Health Security Partners, Washington DC, USA; [5]University of Veterinary and Animal Sciences Lahore, Pakistan.

25. **Touseef Ahmed**[1], Aitezaz Ahsan[2], Wajahat Ali[3], Adeel Kazam[4], Ahmad Bilal[4], Muhammad Nauman Faisal[4], Abdul Ali[2], Muhammad Ramathan Shahzad[2], Muhammad Farooq Tahir[5], Tigga Kingston[1]. Isolation of antimicrobial resistant *Salmonella* from Indian flying foxes (*Pteropus medius)* in Pakistan. [1]Department of Biological Sciences, Texas Tech University, Lubbock, Texas, USA; [2]Animal Health Research Laboratories, National Agricultural Research Centre (NARC), Islamabad; [3]University of Haripur, Khyber Pakhtunkhwa, Pakistan; [4]University of The Punjab, Lahore, Pakistan; Health Security Partners, USA

26. **Sarah van Tol**[1], Ricardo Rajsbaum[1,2], Alexander N. Freiberg[2,3,4,5]. *Pteropus vampyrus* TRIM40 is an interferon stimulated gene that antagonizes RIG-I-like receptors. Microbiology and Immunology, University of Texas Medical Branch in Galveston, Galveston, TX. USA[1]; Institute for Human Infections and Immunity, University of Texas Medical Branch in Galveston, Galveston, TX. USA[2]; Pathology, University of Texas Medical Branch in Galveston, Galveston, TX. USA [3]; Galveston National Laboratory, University of Texas Medical Branch in Galveston, Galveston, TX. USA [4]; Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch in Galveston, Galveston, TX. USA[5].

27. **Wenjun Ma**[1,2], Liping Wang[1,2], Zhenyu Shen[1,4], Nirmalendu Deb Nath[3], Yonghai Li[3], Timothy Walsh[3], William Mitchell[1,4], Jinhwa Lee[3], Susan Moore[3,4], Shuping Zhang[1,4]. Isolation and identification of mammalian orthoreoviruses from bats in the United States. Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA[1]; Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO, USA[2]; Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA[3]; Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA[4].

28. **Natalie Wickenkamp**[1,] Kalani Williams[1], Emma Harris[1], Anna C. Fagre[1], Jack-Michael Mutebi[2], Benard Matovu[2], Lillian Nalukenge[2], Betty Nalikka[2], Aggrey Siya[2], Teddy Nakayiki[3], Charity Nassuna[3], Leonara Nabatanzi[3], John Kayiwa[3], Kevin Castle[4], Tanya Dewey[5], Julius Lutwama[3], Robert M. Kityo[2], and Rebekah C. Kading[1] Ecology and biosurveillance methods for bats and their viral pathogens in Uganda. Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA[1]; Department of Zoology, Entomology, and Fisheries Sciences, Makerere University, Kampala, Uganda[2]; Uganda Virus Research Institute, Entebbe, Uganda[3]; Wildlife Veterinary Consulting, Fort Collins, CO, USA[4]; Department of Biology, Colorado State University, Fort Collins, CO, USA[5].

29. **Scott T. Yohe**[1], Francesca Yalong[2], Rintaro Kato[2], Mateo D. Saenz[2], Miranda Margulis-Ohnuma[1,] Timothy D. Smith[3], Liliana M. Dávalos[4], Angelique Corthals[2], Laurel R. Yohe[5]. Comparative nasal anatomy and goblet cell distribution reveal possible adaptation to viruses in bats. Department of Earth and Planetary Sciences, Yale University, New Haven, USA[1]; Department of Sciences, John Jay College of Criminal Justice, New York, USA[2]; Department of Physical Therapy, Slippery Rock University, Slippery Rock, USA[3]; Department of Ecology and Evolution, Stony Brook University, Stony Brook, USA[4]; Department of Bioinformatics and Genomics, University of North Carolina Charlotte, Charlotte, USA[5].

30. **Samantha Zepeda**[1], Tyler Starr[2,3,4], Allison Greaney[2,4], Lexi Walls[1,3], Young Park[1], John Bowen[1], Davide Corti[5], Jesse Bloom[2,3,4], and David Veesler[1,3]. Molecular Basis of *Rhinolophus* ACE2 Receptor Association with Cambodian bat sarbecovirus RshTT200 Spike Glycoprotein. [1]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA, [2]Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [3]Howard Hughes Medical Institute, Seattle, WA, USA, [4]Department of Genome Sciences, University of Washington, Seattle, WA, USA, [5]Vir Biotechnology, San Francisco, CA 94158, USA.

31. **Emma Harris**[1], Charity Nassuna[2], Leonara Becky[2], Natalie Wickenkamp[1], Betty Nalikka[3], Kalani Williams[1], Benard Matovu[3], Aggrey Siya[3], Kevin Castle[4], Tanya Dewey[5], Lillian Nalukenge[3], Teddy Nakayiki[2], Jack-Michel Mutebi[3], Anna Fagre[6], John Kayiwa[2], Julius Lutwama[2], Robert M. Kityo[3], and Rebekah C. Kading[1]. Viral Surveillance in *Rhinolphus* and *Hipposideros* bats from Eastern Uganda. Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO[1]; Uganda Virus Research Institute, Entebbe, Uganda;[3]Department of Zoology, Entomology and Fisheries Sciences, Makerere University, Kampala, Uganda[2]; Wildlife Veterinary Consulting LLC, Fort Collins, CO[4];Department of Biology, Colorado State University, Fort Collins, CO[5];Division of Vector-borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO[6].

32. **Phillida A. Charley**, Kira W. Douglas, and Tony Schountz. Innate immune responses of Jamaican fruit bat cells infected with Middle Eastern respiratory syndrome coronavirus. Department of Microbiology, Immunology, and Pathology; Colorado State University, Fort Collins CO, USA.

33. **Juliette Lewis**[1], John Nicholas Allen[1], Joseph Prescott2, Eric Laing[3], and Tony Schountz[1]. Jamaican fruit bats as a potential model of Cedar virus infection. [1]Department of Microbiology, Immunology, and Pathology, Colorado State University; [2]Robert Koch Institute, Berlin, Germany; [3]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD, USA.

34. **Clara Reasoner**[1], Bradly Burke[1], Miles Eckley[1], Wenjun Ma[2], and Tony Schountz[1]. Generation of Dendritic Cells and Macrophages from the Jamaican Fruit Bat (*Artibeus jamaicensis*). [1]Department of Microbiology, Immunology, and Pathology, Colorado State University. [2]Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.

35. **Shijun Zhan**, Maggie Priore and Tony Schountz. Sosuga virus infection of Jamaican fruit bat primary kidney epithelial cells. Department of Microbiology, Immunology, and Pathology, Colorado State University.

36. **Kaitlynn Williams**, Bradly Burke and Tony Schountz. The synthesis of a self-regenerative monolayer of Jamaican fruit bat gastrointestinal epithelial cells. Department of Microbiology, Immunology, and Pathology, Colorado State University.

Presentation Abstracts

1. Isolation of a MERS-related coronavirus from lesser bamboo bats that uses DPP4 receptor and infects human-DPP4-transgenic mice

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Introduction. After the outbreaks of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2003 and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, another SARS-related-CoV (SARSr-CoV), SARS-CoV-2, has recently emerged to cause the even more disastrous COVID-19 pandemic. While a number of human coronaviruses are believed to be originated from ancestral viruses in bats, none of these bat coronaviruses including those bat merbecoviruses have been successfully isolated *in vitro*, their potential for emergence and causing direct bat-to-human transmission remains unclear. **Methods**. A 6-year surveillance study of merbecoviruses in different bat species from Hong Kong and mainland China was conducted and virus isolation was attempted using different cell lines. **Results**. Tylonycteris-bat-CoV-HKU4 (Ty-BatCoV HKU4) from lesser bamboo bats (*Tylonycteris pachypus*) was successfully isolated using human colorectal adenocarcinoma (CaCo-2) cells for the first time. It replicated efficiently in Caco-2 and hepatocarcinoma (Huh-7) cells with cytopathic effects. Ty-BatCoV HKU4 replication was inhibited by interferons-α/β, suggesting their potential as antiviral candidates. Exogenous expression of human-dipeptidyl-peptidase-4 (hDPP4) and dromedary camel-DPP4 (dcDPP4) rendered non-permissive cell lines to become susceptible for Ty-BatCoV HKU4 infection, suggesting the use of hDPP4 and dcDPP4 by Ty-BatCoV HKU4 as the receptors for cell entry. Flow cytometry, co-immunoprecipitation and surface plasmon resonance assays showed that Ty-BatCoV HKU4-receptor-binding-domain (RBD) can bind hDPP4, dcDPP4, and *Tylonycteris pachypus*-DPP4 (TpDPP4). Ty-BatCoV HKU4 can infect hDPP4-transgenic mice by intranasal inoculation with self-limiting disease. Lung and brain pathologies, associated with suppression of antiviral cytokines and activation of proinflammatory cytokines and chemokines, were observed in infected mice. **Conclusion**. The results suggest that MERS-related bat CoVs may overcome species barrier by utilizing DPP4 and potentially emerge in humans by direct bat-to-human transmission.

2. Bat coronaviruses surveillance in Western Asia (2017-2022)

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Introduction. Western Asia, a region of 20 countries extending from Turkey in the west, Georgia in the north, Yemen in the south, and Afghanistan and Pakistan in the east, represents a major gap in global bat research and bat virus surveillance. Western Asia and its bat fauna are positioned at the biogeogeographic cross-roads of Europe, Asia, and Africa. Current understanding of coronavirus (CoV) diversity and the risk of CoV emergence in this region is almost non-existent, highlighted by the emergence of MERS-CoV in 2012. **Methods**. In 2017, we established the Western Asia Bat Research Network (WAB-Net) -- a regional One Health network with the aim of integrating bat ecological and virological research – the first of its kind in the region and a model for other global networks. Bat researchers, veterinarians, virologists, public health experts, and policy makers were brought together via annual meetings and in-person field and lab trainings. We conducted active field surveillance for bat CoVs in 7 countries, and conducted molecular screening using conserved CoVs primers for the RdRp gene in two regional labs in Amman, Jordan and Tbilisi, Georgia. **Results**. We non-lethally sampled 4,278 bats (of 37 species in 9 families) from 50 sites in 7 countries (Armenia, Azerbaijan, Georgia, Jordan, Oman, Pakistan, and Turkey) from 2018-2022. As of April 2022, 60% of bats have been screened for CoVs. Overall CoV prevalence was 15% across all bat individuals (fecal samples), but prevalence varied by species, site, and age class. Some well-sampled species had even higher prevalence, e.g. *Rhinolophus ferrumequinum* exhibited the highest prevalence at 33%. We discovered a large clade of novel sarbecoviruses found at a relatively high prevalence in *Rhinolophus* spp., as well as a diversity of other alpha and beta-CoV lineages (at least 12 unique clades). Viral phylogenetic patterns largely followed host associations, but evidence of cross-species transmission between diverse bat families was also observed. **Conclusions**. Our collaborative WAB-Net has strengthened, and continues to build, the capacity for bat virus and zoonotic disease research in a region with limited ongoing surveillance. We are working with our in-

country partners to share this information across sectors, develop spillover risk models, and pilot community outreach tools with the goal of reducing the threat of future bat-CoV emergence, while simultaneously promoting bat ecological research and conservation.

3. Intranasal versus oral SARS-CoV-2 inoculation of *Rousettus aegyptiacus* fruit bats induce distinct differences in the course of infection

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Introduction. Rhinolophus bats of the suborder *Yinpterochiroptera* have been postulated as reservoir hosts since closely related coronaviruses have been isolated from this species. Our pilot experiment had confirmed the general susceptibility of *Rousettus aegyptiacus* fruit bats to SARS-CoV-2. Since in vitro analyses revealed a preferential virus propagation in gut epithelial cells over respiratory epithelial cells, we were interested in the pathogenesis and agent distribution upon intranasal and oral challenge. **Methods**. In two independent experiments, we challenged groups of 6 bats each by intranasal or orotracheal inoculation of $1*10^5$ $TCID_{50}$ SARS-CoV-2. Oral and anal swabs were collected daily and nasal wash samples were collected on days 2, 4, 6 and 8. Three animals per inoculation route were sacrificed on days 2, 4 and 14, and the respiratory and digestive tract were analyzed. 50% of the animals carried transponders to monitor their body temperature, respiratory rate and locomotive activity. **Results**. Upon intranasal challenge, animals shed viral RNA for at least 8 days and replication competent virus in titers up to $1*10^4$ $TCID_{50}$ until day 4. In contrast, we only detected minute amounts of viral RNA or replication competent virus in any of the swab samples collected from animals challenged orally. Virus replication was detected in the nasal conchae of intranasally infected animals up to $1*10^6$ copy numbers / ml, while other samples of the respiratory and digestive tract contained only minute amounts of viral RNA or replicating virus. All samples from orally infected bats were negative or very weakly positive. However, these animals showed a clear drop in the respiratory rate and locomotion activity upon infection. Moreover, expression of immune factors was more upregulated in orally infected than in intranasally infected animals. **Conclusions**. Our data show a clear preference of the intranasal route concerning virus accumulation in the upper respiratory tract and virus shedding. However, oral inoculation seems to have a more severe effect on the organism as a whole, causing loss in body weight, and a reduced respiratory rate and locomotion activity within the first 6 days post inoculation. Further histological, virological and immunological analysis will help elucidate this enigma.

4. Epidemiology and genetic diversity of novel coronaviruses in Bats, Bangladesh

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Introduction. Bats are considered the natural progenitors for mammalian coronaviruses. Both severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) CoV have been associated with bat hosts and cause human morbidity and mortality. The WHO includes SARS and related coronaviruses among its top threats to global health. Here we report on coronaviruses (CoV) detected in bats in Bangladesh in the pre-pandemic period of COVID-19. **Methods.** We collected oral and rectal samples and blood from fruit (n=1056) and insectivorous bats (n=493) twice a year (winter and summer) from eight districts in Bangladesh from 2016 to 2019. We tested swab samples using consensus PCR targeting the RdRp gene to screen a total of 3098 samples from 18 different species of the bat. Bat species were confirmed with both morphometric measurements and genetic barcoding. **Results.** Overall, 4.6% (n=69; 95% CI: 3.6-5.8) bats were positive for CoV, including 1.7% were fruit bats (n=17) and 10.7% (n=52) were insectivorous bats. *Tylonycteris pachypus* (20.7%) and *Megaderma lyra* (15.8%) were most frequently positive for CoV. The presence of CoVs was associated with the season, body conditions, bat type, feeding habit, landscape, and interfaces ($p<0.001$). Insectivorous bats were 4.6 (95% CI: 2.7-7.8) times more likely to harbor CoVs than fruit bats. We identified 10 CoV strains from 96 samples of eight bat species. Of these, five were alpha (α)-CoVs, and five were beta (β)-CoVs. Four α-CoVs and three β-CoVs were novel in nature; others were closely related to bat coronaviruses from southeast Asia and Africa, suggesting that co-circulation of coronaviruses is common in multiple bat species with overlapping geographical distributions. Phylogenetic analysis revealed that novel β-CoVs, detected in *Tylonycteris pachypus*, are genetically related to a known human pathogen- MERS CoVs and therefore warrant further investigation. **Conclusions.** This study demonstrates that diverse coronaviruses, including MERS-related β-CoVs, are present in various bat species in Bangladesh. We recommend One Health surveillance at human-animal interfaces to detect novel coronaviruses before emerging to humans and to prevent future epidemics and pandemics by Disease X.

5. Intestinal tropism of a *Betacoronavirus* (*Merbecovirus*) in its natural Nathusius' pipistrelle (*Pipistrellus nathusii*) host

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Introduction. Coronavirus surveillance in bats has been driven by the emergence of several bat coronavirus-related disease outbreaks in humans and domestic animals. Since then, we have learned that coronaviruses can be detected in virtually every bat species investigated. Fecal samples were the most common sample types tested in previous studies. However, detection of viral RNA in feces does not demonstrate that the intestinal tract is the primary replication site. Not knowing where bat coronaviruses replicate hampers our understanding of how they interact with their natural hosts. We aimed to investigate the interaction of a *Betacoronavirus* (subgenus *Merbecovirus*), PN-βCoV, with its natural host, the Nathusius' pipistrelle (*Pipistrellus nathusii)*. **Methods**. Samples of the intestinal tract of Nathusius' pipistrelle bats, found dead or euthanized due to poor prognosis, were tested for PN-βCoV RNA presence by RT-qPCR. From PN-βCoV RNA-positive bats, a standard set of additional tissues was further tested for virus RNA using RT-qPCR and virus antigen using MERS nucleoprotein MAb to identify the origin of the detected viral RNA. PN-βCoV-specific in situ hybridization was used to confirm the virus antigen test. If virus antigen was detected, we investigated colocalization with lesions by microscopic examination of hematoxylin-and-eosin-stained sections. **Results**. We show that 25 of 89 (28%) bats tested positive for PN-βCoV RNA by RT-qPCR. Varying per bat, PN-βCoV RNA was detected in intestinal tissue (36%), rectal swab (16%), or both (48**%**), as well as 12 of 21 fecal samples (57%). Low levels of viral RNA were detected in nose wash (20%), lung (8%), pharyngeal swab (4%), liver (16%), kidney (16%), brain (16%) and spleen (12%). Viral RNA loads in intestinal samples were higher compared to other samples, on average and in each individual bat. PN-βCoV antigen and RNA were detected in intestinal epithelium and lamina propria fibroblasts, in at least one bat (analysis ongoing). Lesions were not detected. **Conclusions**. Our results show that a *Betacoronavirus* replicates in intestinal tissue of its natural host, the Nathusius' pipistrelle. These results help us to understand how bat coronaviruses interact with their natural hosts and could be used to further investigate transmission, perhaps via the fecal-oral route.

6. Bat-origin coronaviruses provide a unique platform for a strategy to predict and prevent disease emergence

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Since the emergence of the COVID-19 pandemic, there has been an unprecedented focus on how we can be better prepared for pandemics, and respond to them more rapidly. However, most of the policies and funding requests for pandemic preparedness are based on control measures post-emergence, rather than on strategies to predict and prevent emergence. With the majority of emerging disease events, and all known pandemics, caused by zoonotic microbes, analyzing viral diversity in wildlife, and trends, risk and drivers of zoonotic viral emergence has huge potential as a pandemic prevention strategy. In this talk, I will lay out some of the critical challenges to predicting pandemics, some of the research that has already begun to answer them, and some of the key questions that remain.

While predicting the exact time and place of the origin of future EIDs is unrealistic, I will review approaches to addressing the following questions, and show how these can provide value in enhancing surveillance and helping prevent disease emergence at different scales:

1. Where will the next emerging virus originate?
2. What are the key causes of disease emergence?
3. Which reservoir species will the next EID likely emerge from?
4. How many unknown viruses do these species harbor, and how many can infect us?
5. How can we prevent emergence, and how much will it cost?

Finally, I will use our work on bat-origin coronaviruses to demonstrate how disease ecology, lab virology, and social science approaches can provide a unique platform for a pandemic prediction and prevention strategy. While our understanding of the diversity and risk of coronaviruses in wildlife is still rudimentary, work on bat-CoVs has already demonstrated value in enhancing surveillance, and developing therapeutics and vaccines of direct public health relevance. The emergence of a global pandemic caused by a virus with its likely ancestral origin in bats should be a wake-up call to double-down on this approach, rather than close it down as some have argued.

7. Strong selective signatures of viruses on bat innate and adaptive immunity

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Introduction. Bats are unique among mammals in their ability to fly and to host and shed numerous highly lethal viruses seemingly asymptomatically, leading to great interest in the adaptations that allow bats to survive these infections. However, the field of bat immunology is still relatively young, and few of the over 1,400, ecologically-diverse species of bats have been studied in depth. **Methods**. We investigated both the innate and adaptive immune system, as well as host proteins targeted by pathogens, of multiple bat species to understand the basis of their unique relationship with pathogens. **Results.** We present genomic data from over 90 species of bats showing that pattern recognition receptors, specifically Toll-like receptors and RIG-I-like receptors, in bats evolved early to recognize viral pathogens. Comparative genomic analysis demonstrates that bats are under exceptional pressure to adapt to coronaviruses in their ACE2 and DPP4 genes compared to other mammals. Additionally, data from seven bat species show that bats express all three canonical superfamilies of Ig heavy chain V genes, with species-specific diversification and similar CDR3 lengths to those observed in other mammals. **Conclusions**. Our data suggest that pathogen pressure has shaped bat immunity and evolution differently from other mammals, shaping bats' unique relationship with pathogens.

8. Differential eco-immunological response to human disturbance in cave-dwelling bats

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Introduction. By providing critical roosting sites, caves have the potential to bolster bat densities in degraded forests where populations would otherwise be expected to decline over time. However, habitat degradation may reduce individual bat health (i.e., reduced foraging quality, increased travel costs). Thus, apparently healthy cave-roosting bat populations in modified landscapes may suffer from chronic stress, which may make them more susceptible to pathogens, thereby enhancing the spread of diseases. While some bat species may acclimatize to repeated stressors and even thrive in human-modified habitats, other species may experience higher stress levels that subsequently impair immune response. Due to wide taxonomic variation in morphology, foraging behavior, and habitat use, bats are good models to assess the physiological effects of human disturbance on wildlife. **Methods**. We conducted our study in 16 caves in Costa Rica along a gradient of human disturbance ranging from undisturbed caves in protected areas, to caves subject to high levels of disturbance resulting from previous mining and surrounded by agriculture, livestock, and other human-dominated landscape. Surveys were repeated in dry and wet season. **Results**. We collected samples from 1,230 adult individuals, representing 12 species from four families with diverse ecological niches. We evaluated markers of physiological stress (neutrophil-to-lymphocyte ratio), downstream health measures, such as body condition and white blood cell counts, and innate (lysozyme) and adaptive (IgG) immune markers. We contrast these results with infection prevalence of four common and divergent pathogens: *Bartonella* (242/1230), *Leptospira* (61/192), *Trypanosoma* (393/1230) and microfilaria (82/1230). **Conclusions**. Our results suggest differential responses among bat species to pathogen prevalence and immune markers. Factors that may explain this variation, such as species identity, ecological traits, season, sex, and human perturbation, will be discussed. This work provides insight into the complex eco-immunology of wild bats in changing environments.

9. Suitability of *Artibeus jamaicensis* bats for modeling SARS-CoV-2 reservoir infections

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Introduction. SARS-CoV-2 emerged in late 2019, highlighting the need to increase our understanding of virus-reservoir interactions and transmission dynamics. The putative reservoir are bats. Jamaican fruit bats, *Artibeus jamaicensis* (Ajs), have been successfully used to model MERS-CoV infection. Here, we investigate the suitability of this bat species to model infections of other betacoronaviruses like SARS-CoV-2. **Methods**. We first set out to characterize the immune-landscape of the species in more detail for uninfected adult bats to establish a robust baseline. **Results**. We hypothesized that a SARS-CoV-2 infection would be focused on the

respiratory or intestinal tract in the reservoir species. We determined sex-specific differences in immune gene expression patterns across the respiratory tract and intestinal system and the assigned lymphatic organs. Additionally, we described the relative frequency of innate immune cells, T cells, B cells and NK cells during steady state conditions by flow cytometry in circulating PBMCs and in the respiratory and intestinal tract by immunohistochemistry (IHC). We also, for the first time, characterized the intestinal microbiome of this species in captivity. We assessed the compatibility between SARS-CoV-2 and host *in vitro*. Interestingly, variants of concern (VoC) Alpha and Delta, demonstrated increased entry over the ancestral lineage A in a pseudotype system, while Omicron did not. We confirmed the entry and growth kinetics in BHK cells, as well as primary and immortalized Aj cell lines, transfected with Aj ACE2 and TMPRSII. Both ACE2 and TMPRSII were present in both the respiratory and intestinal tract, as demonstrated by IHC, strongly suggesting the suitability of this bat species to model *in vivo* infection. Hence, we plan to inoculate Aj bats with SARS-CoV-2 VoCs and measure the viral titers and shedding, as well as pathology, host immune response and the microbiome. We additionally will assess the capability of transmission by measuring environmental contamination and successful exposure of naïve co-housed sentinels. **Conclusions**. This work highlights the suitability of Jamaican fruit bats to model coronavirus infections. It lays the foundation for additional analyses of the host immune response, but also demonstrates the need to generate additional tools to conduct more detailed immunological work.

10. Leveraging serum proteomics to characterize bat immune phenotypes and response to viral infection

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Introduction. Bats are natural hosts for many viruses, including coronaviruses (CoVs). However, the ability to characterize immune mechanisms of viral tolerance in wild bats is limited by small sample volumes and few species-specific reagents. Proteomics holds promise for illuminating immune factors involved in bat responses to infection, because it can accommodate small sera volumes and can thus be applied to large and small species and in longitudinal studies. As the serum proteome includes proteins secreted from not only blood cells but also proximal organs, it provides a broader characterization of immune proteins. **Methods**. We used 2 µL of serum, LC-MS/MS, and data-independent acquisition to characterize serum proteomes of 36 wild vampire bats (*Desmodus rotundus*) from Belize in 2015 and 2019. We compared abundance of immunological proteins in bat and human sera and identified a core serum proteome of vampire bats. We compared effects of heat inactivation, a common treatment for shipping bat sera, on proteome composition. We used multivariate tests, classifier algorithms, and enrichment analyses to ask how proteomes differed by CoV infection. **Results**. We identified nearly 600 proteins in vampire bat serum covering five orders of magnitude. Bat serum shared most proteins with humans, but many innate immune proteins, proteasomes, and redox-related proteins had greater abundance in bats. Over 90% of bat serum proteins were shared among years, and abundance of most proteins changed less than 17% with heat inactivation. By analyzing paired oral and rectal swabs from our 2019 bats, we identified novel α-CoVs (related to human CoVs NL63 and 229E) in 21% of bats. Infected and uninfected bats did not differ in proteome composition, and we observed weak evidence for differential protein abundance. Classifier analyses identified 32 protein biomarkers of infection, and enrichment analyses suggested CoV-infected bats had upregulated cellular immune response and downregulation of complement, immune effector processes, and humoral immunity. **Conclusions**. Our findings highlight the untapped potential of serum proteomics to characterize wild bat immune phenotypes and pathways involved in viral infection. Applying a proteomic approach across bat species and to distinct life history stages could improve our understanding of pathogenesis and mechanisms of zoonotic virus tolerance.

11. Dynamics of bat pathogens: drivers of spillover risk

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Bats are hosts of human pathogens that have pandemic potential, including coronaviruses, henipaviruses, and filoviruses. Bats are also key pollinators, seed dispersers, and insect consumers and may be sensitive to environmental change. Over the past four years, we have been sampling bats on multiple continents to understand the factors that drive pathogen shedding and spillover. At the same time, we have been collecting and analyzing long-term data on land use change, bat ecology, bat behavior, and henipavirus spillover events. We find that bat virus shedding is more likely during stressful conditions and from populations that have been displaced to novel habitats. Spillover may be more likely when bats are shedding more infectious virus. Our work indicates that ecological stressors may trigger the cascade of events that leads to spillover of bat pathogens to humans. We consider how to proactively prevent spillover by addressing the upstream factors that drive the transmission of pathogens from animals to humans.

12. Reservoir host immunology and life history shape virulence evolution in zoonotic viruses

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Introduction. Future pandemic risk management requires better understanding of the mechanisms that determine the virulence of emerging zoonotic viruses. Bats host viruses that cause higher case fatality rates upon spillover to humans than those derived from any other mammal, suggesting that reservoir host immunological and life history traits may be important drivers of cross-species virulence. **Methods**. We first analyzed trends in human case fatality rates, transmission capacities, and total death burdens across a comprehensive dataset of mammalian and avian zoonotic viruses. Then, using a nested population-level and within-host modeling approach, we generated virulence predictions for viral zoonoses derived from diverse mammalian reservoirs, successfully recapturing corresponding virus-induced human mortality rates from our meta-analysis. **Results**. Our work first demonstrates that bat-borne zoonotic viruses cause higher case fatality rates in human hosts than do viruses derived from any other mammal or bird host. Next, our within-host model offers a mechanistic explanation for the observed virulence of these bat-borne zoonoses—and, more generally, demonstrates how key differences in reservoir host longevity, tolerance, and population density impact the evolution of viral traits that generate severe disease following spillover to humans. Critically, we demonstrate that bat-borne zoonoses do not cause the highest death burdens in the human population, an outcome which appears to result more from intrinsic viral traits than from reservoir host relationships. **Conclusions**. We provide a theoretical framework that offers a series of testable questions and hypotheses designed to stimulate future work comparing cross-species virulence evolution in zoonotic viruses derived from diverse mammalian hosts, including bats.

13. Viral evidence from African bats: the good, the bad, and the ugly

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Introduction. African bats are frequently described in the literature as probable zoonotic virus hosts. However, because study design and detection methods vary widely, comparison and interpretation of data are challenging. **Methods**. We comprehensively reviewed all virus-related African bat research published through 2020, extracting data on bat species identity and numbers sampled, virus identity, sampling methodology and sample type, study locality, and ecological parameters. **Results**. In total, 1283 unique papers were identified. Of these, 160 contained new virus findings from free-ranging bats and were included in our analysis. Of the 328 bat species in 12 families currently recognized in Africa and neighboring islands, viral data were recorded from 160 bat species from ten families (all but Cistugidae and Myzopodidae); noting that 8 studies did not taxonomically identify bats, that thousands of samples were collected from bats only identified to genus or family, that species misidentification appeared frequent, and that negative data were often not reported. At a minimum, 86,353 bats were sampled across these studies, 45% of them lethally. Pteropodid fruit bats and several insectivorous bats (*Mops condylurus*, *M. pumilus, Coleura afra,* and *Macronycteris gigas*) were sampled more often than others, with cavernicolous habitats most frequently targeted. The vast majority of studies (79%) included no ecological data about the captured species or the study site. We report the findings from Filo-, Paramyxo-, Corono-, and Rhabdoviruses (and 14 other families) by bat species and locality, as well as type of viral evidence (serology, NAAT). Upon reviewing the data, we propose a framework that evaluates the strength of presumed virus-host relationships by evidence type and quality, which facilitates the identification of zoonotic risk knowledge gaps. **Conclusions.** This review, for the first time, systematically presents all viral findings from African bats and highlights critical data that are not being collected and that will enhance our ability to simultaneously characterize viral spillover risks and conservation. Future surveillance studies should be expanded to include: sampling of non-bats, standardized collection of ecological and other metadata, more rigorous viral detection methods and reliable host species identification, characterization of viral properties that elucidate spillover potential, and social science perspectives.

14. Bat Disease Surveillance in the UK: engaging conservation volunteers & bat workers

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Surveillance for bat related pathogens (of consequence for human and/or bat health) can be facilitated in a cost-effective manner by collaborative action that engages with conservation organizations, volunteers, and bat workers. Through consideration of different approaches for viral (rabies and SARS-CoV-2), fungal (*Pseudogymnoascus destructans*) and other pathogens (internal parasites) we'll explore how conservation, research, governmental, human health, and veterinary organizations work together, in both active and

passive surveillance, to better understand bats and diseases in the UK. We will highlight the central role that bat conservationists and volunteers can play, how they are engaged in disease work, their contribution, and the importance of their support in educating the public and promoting best practice. Bat conservation is inherently linked to the public's perception of bats. We all have a role to play in making sure that there is a clear understanding of the actual public health risks associated with diseases of bats.

15. Common ground: the foundation of interdisciplinary research on bat disease emergence

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Introduction Human perturbation of natural systems is accelerating the emergence of infectious diseases, mandating integration of disease and ecological research. **Methods**. We conducted a bibliometric analysis of co-author relationships to investigate cross-disciplinary collaboration between ecological- and infectious disease-oriented bat researchers. Publication metadata were extracted from the Web of Science database on over 5,600 journal articles published between 1950 and 2019. Ongoing analyses are extending this evaluation through mid-2022 to assess how the co-authorship network may have changed following the emergence of SARS-CoV-2. **Results**. This analysis identified a separation of bat ecologists and infectious disease researchers with few cross-disciplinary relationships. Of 5,645 papers, true interdisciplinary collaborations occurred primarily in research focused on White Nose Syndrome (WNS). **Conclusions**. This finding is important because it illustrates how research with outcomes favoring both bat conservation and disease mitigation promotes domain integration and network connectivity. Just as WNS provides common ground for convergent research, understanding and mitigating other emerging zoonoses with One Health implications, like SARS-CoV-2, involve common challenges that are best met through cross-disciplinary engagement. We advocate for increased engagement between ecology and infectious researchers to address such common causes, and suggest that efforts focus on leveraging existing activities, building interdisciplinary projects, and networking individuals and networks to integrate domains and coordinate resources. We provide specific opportunities for pursuing these strategies through the Bat One Health Research Network.

16. Bat Research in the Time of Covid-19 – Risks and Recommendations from the IUCN Bat Specialist Group

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In May 2020, the IUCN SSC Bat Specialist Group (BSG) assembled an interdisciplinary working group (WG) to assess the risk of spillover of SARS-CoV-2 from humans to bats (also referred to as spillback and reverse zoonosis) and to identify mitigation strategies for those working at the human-bat interface. The WG found the risk to be small but credible, with highly significant negative consequences for bat conservation, and focused on mitigation in four stakeholder groups: bat researchers, bat rehabilitators and rescuers, cavers, and guano collectors. Guidelines responsive to the global constituency of the BSG were developed and disseminated, with recent updates reflecting the availability of vaccines and our improved understanding of SARS-CoV-2 transmission. Current efforts focus more broadly on best field hygiene practices for working safely with bats and minimizing the risk of bi-directional disease transmission. An equally great and realized risk to bat conservation of COVID-19 has been the proliferation of misinformation and misperceptions about bats and viruses. The WG identified four types of miscommunication about disease research among both professional and non-professional audiences that have consequences for research agendas, public policy and bat conservation. Here we illustrate each type and make recommendations for best practice. The WG was renamed the BSG One Health WG in July 2021 with a vision of "a world with healthy ecosystems in which society has a fair and science-based understanding of impacts that people and bats have on each other's health".

17. Bat Ecology in the pathogen mix in the Mt Elgon foothill landscape

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The Foothills of the Mt Elgon Area is a heavily settled agricultural area in which we have recorded a community assemblage of 20 species of bats. Of these, 8 species have confirmed cave roosts that we continue to monitor for a number of years now. We have observed that:

- 6 of the species continue to be steadfast with the caves and locations in the cave where they roost
- One species has been lost from the caves we monitor
- The populations in the caves have mostly shown a decline although we are documenting almost continuous reproductive activity
- One species seems to move around to other roost sites.
- We have documented 6 caves with guano but no bats
- Members of the local community continue to interact with the caves having bats with evidence of bat capture

Because bats can host pathogenic organisms such as viruses and fungi, it is our interest to demonstrate the role of bat ecology in the potential for spillover. Using acoustic detection of bats' activity, we attempt to demonstrate to what extent the cave dwelling bats spread into human settlement areas as well as understanding overall bat fauna activity of all the general bat fauna in our study area.

18. Ecological study of cave nectar bats reveals low risk of direct transmission of bat viruses to humans

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Introduction. Bats are reservoirs of various viruses. The widely distributed cave nectar bat (*Eonycteris spelaea*) is known to carry both filoviruses and coronaviruses. However, the potential transmission of theses bat viruses to humans is not fully understood. **Methods**. In this study, we tracked 16 *E. spelaea* bats in Mengla County, Yunnan Province, China, using miniaturized GPS devices to investigate their movements and potential contact with humans. Furthermore, to determine the prevalence of coronavirus and filovirus infections, we screened for the nucleic acids of the Měnglà virus (MLAV) and two coronaviruses (GCCDC1 and HKU9) in anal swab samples taken from bats and for antibodies against these viruses in human serum samples. **Results**. None of the serum samples were found to contain antibodies against the bat viruses. The GPS tracking results showed that the bats did not fly during the daytime and rarely flew to residential areas. The foraging range of individual bats also varied, with a mean cumulative nightly flight distance of 25.50 km and flight speed of up to 57.4 km/h. **Conclusions**. Taken together, these results suggest that the risk of direct transmission of HKU9-CoV, GCCDC1-CoV, and MLAV from *E. spelaea* bats to humans is very low under natural conditions.

19. Ebola virus (*Zaire ebolavirus*) sequence and antibody detection in bats in Liberia, 2016-2018

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Introduction. From 2013-16, West Africa experienced the largest outbreak of Ebola virus disease in history, with more than 28,000 cases and 11,000 deaths. The magnitude of the epidemic was attributed to the high connectivity among people in rural and urban areas throughout Guinea, Sierra Leone and Liberia. However, the initial outbreak likely started from a single spillover event of Ebola virus (*Zaire ebolavirus*) from an unidentified wild animal in Guéckédou, Guinea. Various bat species have been associated with filoviruses in Africa and Asia, suggesting that bats may be natural reservoirs for these viruses. Yet, the ecology of Ebola virus, including the bat species associated with it in West Africa, remains poorly understood. **Methods**. In 2016, in response to the West Africa Ebola virus disease epidemic, the USAID funded PREDICT project initiated a 3-year surveillance effort to identify natural reservoirs for filoviruses in West Africa. In Liberia, we collected blood, throat swabs, urine and fecal samples from bats. We screened the swabs & fecal samples for filovirus RNA using a combination of a broadly reactive pan-filovirus consensus PCR assay and a specific Ebola Zaire real time assay. We screened sera using a multiplexed pan-filovirus serology assay designed to detect IgG antibodies that bind to recombinant

native-like envelope glycoproteins from each known filovirus. **Results**. A total of 9465 samples were tested by PCR and 563 serum samples by serology. Illumina was used to perform unbiased whole genome sequencing on PCR positive samples. We detected Ebola virus RNA (with ~20% of the full genomic sequence obtained) in a sample from one Nimba long-fingered bat (*Miniopterus nimbae*) roosting in a mine shaft in northern Liberia. Anti-Ebola virus IgG antibodies were detected in one serum sample from *M. nimbae*. Interestingly, the highest Ebola virus seroprevalence and reactivity with the Ebola virus glycoprotein was observed in sera collected from Noack's round-leaf bats (*Hipposideros cf. ruber*). Additionally, seropositive *Hipposideros* bats were detected at two different locations more than 150km apart. **Conclusions**. The finding of viral RNA and antibodies in two bat species known to commonly co-roost, suggests that Ebola virus circulates in bats in West Africa and one, or both, of these species may be involved in viral maintenance. Longitudinal surveillance in these and other ecologically associated bat species in the region will shed further light on their role as hosts for Ebola virus or related filoviruses.

20. Isolation of Lloviu virus from Schreiber's bat: the hunt for Lloviu virus in Europe

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Introduction. Only Marburg and Ravn viruses were isolated directly from bats so far and the role of *Rousettus aegyptiacus* as a reservoir is now clear. Lloviu virus (LLOV) is the only member of the *Filoviridae* family which was ever found in European bats. Since the initial appearance of the virus in 2002 Spain, the seroprevalence and RNA positivity were reported from Schreiber's bats (*Miniopterus schreibersii*) in Spain and Hungary. We established a monitoring system of Schreiber's bats in Hungary in 2013 and after the detection of viral RNA in 2016, we started an active surveillance. Here we present the experiences and results of this surveillance spanning from 2016 to 2020. **Methods**. Bats were collected directly at the colony's resting sites, during daytime for the collection of blood, feces, urine and ectoparasites. We operated a mobile laboratory unit on-site and screened the blood samples of these bats with LLOV-specific real-time RT-PCR. Neutralizing antibodies against LLOV were measured with pseudotyped virus neutralization assay and the viral genomic sequences were determined with amplicon-based sequencing technology, using the Nanopore sequencing platform. Positive animals were re-sampled at the collection site, these materials were used for *in vitro* isolation experiments at the Biosafety Level 4 laboratory, University of Pécs. **Results**. Bat blood samples and ectoparasites of positive animals were positive for LLOV RNA. The LLOV seropositivity among live animals were 12,16 %. During the study period we occasionally found dead animals, exclusively after, or at a late stage of hibernation period. One-third (33%) of these animals were LLOV RNA positive, which was 1,14% in live animals. After multiple attempts we successfully isolated the virus on bat, monkey, and human cell lines. **Conclusions**. Schreiber's bat has a wide geographic distribution in Europe and the Mediterranean region. By the successful infection of human cell lines, we demonstrated the zoonotic potential of the virus. Our results emphasize the importance of bats in the evolution of filoviruses and nominates Schreiber's bats as possible reservoirs for this virus. The identification of the virus in a different ecosystem compared to the tropics, raises several questions about the ecology, evolution and zoonotic risk of filoviruses.

21. Response of a North American bat species to immunization with Ebola-like virus particles

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Introduction. As part of a broadly comparative research initiative examining the bat immune response to Ebola antigens, we conducted a pilot study of the response to stimulation with Ebola virus-like particles (eVLPs, containing GP, NP, and VP40 proteins) and the adjuvant poly(I:C) (a dsRNA analog) in big brown bats (*Eptesicus fuscus*). **Methods.** We immunized bats with eVLPs and poly(I:C) at two dosages: 0.075ug/g ("low dose", typical of most eVLP studies, n=6) and 0.375ug/g ("high dose", n=5). All bats were boosted with eVLP alone 21 days after the first inoculation. As non-natural hosts without previous filovirus exposure, we predicted a modest response to primary inoculation and a robust response to the booster shot, with greater responses at the higher dose. Body temperature was continuously measured, beginning 7 days before immunization with Star-Oddi data loggers affixed to the skin with medical-grade adhesive. We measured body mass and collected blood samples at days 1, 7, 14, 21, 22, 35, and 56 after primary immunization. We developed an ELISA with Ebola glycoprotein (GP)-coated plates to assay anti-GP titers in plasma samples. **Results.** Body mass varied over time in these captive bats in ways that were not relatable to the study design. ELISA results were as expected with samples from a parallel study done in mice but, unexpectedly, 9 of the 11 bats had positive titers on day 0, before inoculation. There were no consistent trends in anti-GP antibodies over time in low-dose inoculated bats. However, 4 of the 5 high dose bats displayed a robust response to the booster. In concordance with this finding, bats at the high dose displayed a greater febrile response to the booster dose than low dose bats, evident as an elevated temperature at the trough of the thermal circadian rhythm for two days post booster inoculation In other words, bats delayed the normal drop into torpor on these days by 4-5 hours. **Discussion.** The results of this pilot study have informed the design of our ongoing eVLP studies in Ugandan bats. They suggest that bats may require exposure to greater levels of Ebola proteins than other mammals in order to generate a response. The unexpected finding of positive anti-GP

antibody titers in baseline samples and of a complete lack of response to eVLPs in some bats, especially at the high dose, should be explored further.

22. Seasonal patterns in the serology of henipa- and filoviruses in Madagascar fruit bats

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Introduction. The current SARS-CoV2 pandemic, as well as several past epidemics of zoonotic origin, highlight the role of bats as reservoir hosts for many viruses that are highly virulent and often lethal to humans and domestic animals. Bat species are confirmed reservoir hosts for henipa- and filoviruses; however, these viruses rarely cause clinical disease in their hosts. These viruses transmit between bats and across species through contact with virus shed in bat excreta, such as urine, feces, and saliva. Intriguingly, the viral load shed within excreta fluctuates dramatically over the course of a year but is often elevated during periods of reproductive or nutritional stress. In fact, many of these bat-borne viruses have a spatiotemporal signature, such that spillover occurs preferentially when transitioning between seasons. **Methods.** Using longitudinally collected data, we determine serological status for over 1250 individuals from three different species of fruit bats (*Eidolon dupreanum, Pteropus rufus, Rousettus madagascariensis*) endemic to Madagascar. Serum samples were run on in a multiplex serology assay to measure the presences of antibodies against six henipaviruses and eight filoviruses. For the full dataset, we then fit separate generalized additive models (GAMs) to the data to determine seasonality of serostatus by species and virus. **Results.** We found clear evidence of specific henipa- and filovirus seropositivity in samples from all three species of fruit bats. As expected, African henipa- and filoviruses showed seasonal trends, with seroprevalence increasing shortly after the dry season and around the time of parturition. **Conclusions.** Using a unique dataset, we describe the serological profile of three species of Malagasy fruit bats to a number of zoonotic and potentially-zoonotic henipa- and filoviruses. These results aim to advance understanding of the seasonal signatures viral shedding and can thus shed light on when public health efforts or animal-disease interventions are most likely to be most effective. Further research should examine drivers of the seasonality of these viruses, including how age influences the patterns observed here.

23. Assessment of zoonotic disease risk associated with nightly foraging and dispersal activity by Egyptian rousette bats (*Rousettus aegyptiacus*) in Southwest Uganda

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Introduction. Marburg virus (MARV), the causative agent of Marburg virus disease, emerges sporadically in sub-Saharan Africa and is often fatal in humans. The natural reservoir for this zoonotic virus is the Egyptian rousette bat (ERB; *Rousettus aegyptiacus*), that when infected, sheds virus in highest amounts in oral secretions and urine. These bats forage nightly throughout the year for ripened fruit, including those types often preferred by humans. During feeding, they continually discard partially eaten fruit to the ground that could then be consumed by other MARV-susceptible animals or humans. Previous experimental MARV inoculations of fruit showed that infectious MARV can persist on bananas and mangos for up to 6 hours. The objectives of this study were to use global positioning system and passive integrated transponder technology to track nightly movement and longer-term dispersal of ERBs and assess the risk of MARV and other zoonotic pathogen spillover to humans in Uganda. **Methods**. Bats were captured, sampled (oral and rectal swabs, venous blood), and fitted with small, lightweight GPS trackers (<7g) or PIT tags before being released. The GPS trackers were programmed to begin logging data points at 7:00 pm each night and continue to log points every 5 minutes until 5 am the next morning. Data collected by the loggers was downloaded wirelessly to base stations deployed at the mouth of the cave. PIT tags were deployed for future mark recapture efforts and satellite cave dispersal surveillance. **Results**. Data collected from 45 of 50 GPS trackers showed that the bats flew predominately to one of two sites, both of which are near human occupied structures. Some bats also frequented areas of larger scale agricultural activity. Samples tested indicate that MARV, Sosuga and Kasokero viruses are actively circulating in bats roosting in Python Cave in southwest Uganda. **Conclusions**. The GPS data show that ERBs routinely forage in fruiting trees near human occupied structures. Coupled with the findings that MARV can persist on certain fruits for up to 6 hours, these visitations to fruiting trees by bats potentially infected with MARV and other zoonotic viruses increase the risk of virus spillover in those communities.

24. Selective and high replication of Zaire ebolavirus following experimental inoculation of the Angolan free-tailed bat (*Mops condylurus*) with filoviruses.

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Introduction. Outbreaks of Ebola hemorrhagic fever still burden the Central African region, yet the natural reservoir host for ebolavirus remains unknown. Ebola and Marburg -virus are genera within the Filoviridae, with Zaire ebolavirus (EBOV) as the type species and the most frequently transmitted in humans, among five others so far discovered:including, Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV), Reston ebolavirus (RESTV) and Bombali ebolavirus (BOMV). Evidence from multidisciplinary research, including experimental infection and transmission of Marburg marburgvirus (MARV) in the Egyptian rousette fruit bat, has cemented its role as a reservoir host for MARV. Similar reservoir competence stemming from experimental infections studies, however, have not been demonstrated for any ebolavirus using any bat species. **Methods**. Due to the information scarcity on ebolavirus ecology, we developed a comparative filovirus infection model using the insectivorous Angolan free-tailed (AFB, *Mops condylurus*); a reservoir host of the recently discovered BOMV and as shown by previous studies, a possible reservoir host of EBOV. We inoculated cohorts of AFBs with EBOV, TAFV, RESTV or MARV to assess filovirus tissue tropism, pathology, host immune responses and infection kinetics at 5 and 10 days post-infection (dpi). **Results**. Efficient and disseminated replication of EBOV was detected in all inoculated bats in absence of clinical signs of disease. Microscopically, even though EBOV antigen was abundant in tissues, very mild pathology was associated with virus replication. Furthermore, we isolated infectious EBOV from tissues, oral and rectal swabs and fecal samples, evidencing a plausible route of horizontal transmission. In contrast, AFBs did not support similar replication of MARV, TAFV or RESTV, nor a possible route of their transmission between conspecifics. Nonetheless, most bats seroconverted by 10 dpi. **Conclusions**. AFBs could play a role as a reservoir host of EBOV due to the unique selective and high reservoir-competence shown by our results, as well as previous experimental and epidemiological findings, including harbouring BOMV. Further, results from this and other future experimental infections will allow for targeted field studies of AFBs populations. Overall, identifying reservoir host species for ebolavirus is an important step to better-understand spillover events and human outbreaks.

25. Using multiplexed assays to investigate serological cross-reactivity and identify the presence of novel viruses: a case study of henipaviruses and pararubulaviruses in Australian flying foxes

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Introduction. Viral serological assays generally aim to detect antibodies specific to the target virus of interest. However, infected individuals also generate antibodies against viral antigens that are conserved across a range of closely related viruses, which can make results from serological assays challenging to interpret. This is a particular challenge in wildlife studies, where the composition of the broader viral community, and therefore expectations about the presence of potentially cross-reactive viruses in host populations, are generally poorly defined. Yet, this cross-reactivity also presents opportunities. In isolated or poorly studied populations, initial serosurveys targeting cross-reactive antibodies may be used to suggest the presence or absence of broad viral genera. We also argue that multiplexing both specific and cross-reactive serological assays is a valuable approach for assessing history of exposure to multiple viruses of interest, as well as identifying the presence of related viruses yet to be characterized. **Methods.** We analyze multiviral serological datasets from Australian *Pteropus* spp. flying foxes to look for evidence of cross-reactivity amongst henipavirus glycoproteins (G), pararubulavirus hemagglutinin neuraminidase (HN) proteins and pararubulavirus nucleoproteins (N) in a multiplex microsphere assay. **Results.** Compared with the strongly cross-reactive relationship observed between Hendra virus G and Nipah virus G proteins, we found no evidence for cross-reactivity between Hendra virus G and Cedar virus G. Four distinct clusters of henipavirus serostatus were discerned (positive for either Hendra virus or Cedar virus, or both, or neither). Pararubulavirus N assays (Menangle virus and Tioman virus) were highly correlated with each other and demonstrated strong cross-reactivity with HN assays for pararubulaviruses and related paramyxoviruses (Menangle virus, Grove virus, Yeppoon virus). Patterns of cross reactivity suggested the presence of additional unknown paramyxoviruses circulating within Australian flying foxes. **Conclusions.** While results from serological assays can be challenging to interpret in the context of multiviral communities, multiplexing diverse viral antigens within

serological assays can provide opportunities to distinguish cross-reactivity from co-seropositivity and identify the presence of unknown viruses.

26. A recombinant chimeric Cedar virus based surrogate neutralization assay for pathogenic henipaviruses

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Introduction. Bats are known reservoirs for a variety of emerging zoonotic viruses, particularly the highly pathogenic henipaviruses, Nipah virus (NiV) and Hendra virus (HeV). NiV and HeV possess a broad species tropism, and can induce a fatal respiratory and/or neurological disease in a wide range of mammals including humans. Henipaviruses are enveloped RNA viruses with two membrane anchored glycoproteins on the virion surface, the attachment (G) glycoprotein and the fusion (F) glycoprotein that facilitate virus entry. The G and F glycoproteins are the major antigenic targets for neutralizing antibodies and the main focus of vaccine development strategies. The conventional plaque reduction neutralization test (PRNT) using authentic NiV and HeV in the BSL-4 setting is the current gold standard to test and evaluate antisera and monoclonal antibodies (mAbs) for virus neutralization, which can be more laborious, time-consuming, and costly. **Methods.** Here, we developed a surrogate henipavirus platform based on the nonpathogenic Cedar virus (CedV) suitable for use at BSL-2 containment. By replacing the CedV F and G glycoprotein genes in a recombinant CedV (rCedV) antigenome with that from NiV-Bangladesh (NiV-B) or HeV, non-reporter gene containing versions of each chimera (rCedV-NiV-B and rCedV-HeV) were generated. Two reporter gene versions for each chimera were also prepared that express either a green fluorescent protein (GFP) (rCedV-NiV-B-GFP and rCedV-HeV-GFP) or a luciferase protein (Luc) (rCedV-NiV-B-Luc and rCedV-HeV-Luc). **Results.** Successful rescue of all chimeras resulted in replication-competent viruses that induced an interferon-β response similarly to rCedV. The rCedV-NiV and rCedV-HeV chimeras mirrored the B-class ephrin receptor tropisms of NiV-B and HeV. We tested a panel of well-characterized neutralizing mAbs specific against HeV/NiV F or G glycoproteins and revealed very high correlations between the GFP expressing chimeras and authentic NiV-B and HeV by PRNT. A rapid high-throughput fluorescence reduction neutralization test (FRNT) was established, which also produced very high correlations between the PRNT and FRNT. We also evaluated this rapid assay for applicability in testing immunized animal sera samples. **Conclusion.** These results demonstrate the suitability of the rCedV chimera platform as a rapid and cost-effective surrogate for conducting pathogenic henipaviruses neutralization assays that can be carried out in the BSL-2 setting.

27. Genetic variability of Hendra virus in Australian pteropodid bats over time and space

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Introduction. The genus *Henipavirus* includes zoonotic and highly pathogenic viruses Hendra virus (HeV) and Nipah virus (NiV) which can cause respiratory distress and fatal encephalitis with case fatality rates ranging from 40-100%. Since its discovery in 1994, equine spillover of HeV continue despite the availability of an effective vaccine since 2012. Temporal and spatial risk of HeV spillover is not fully described. **Methods**. To describe the ecology of HeV in bats, we sampled five bat roosts monthly over approximately 3 years and screened 9853 samples for Hendra virus. Using next generation sequencing techniques, we sequenced full genomes of positive samples with higher viral loads. **Results**. We identified intra- and inter-annual variation in high-prevalence pulses of viral shedding from bat roosts. We detected viral RNA year-round, but high viral loads only occurred in winter. Viral shedding from bats suggests that spillover is not directly linked with overall HeV prevalence in the bat populations but more with the magnitude of virus shed from these bat populations. Upon full genome analyses, we identified the circulation of HeV closely related to those causing fatal outbreaks in humans and horses; and novel lineages, suggesting that a larger area of Australia is at risk for HeV spillover. During our investigations, a novel genetically distinct HeV was discovered by next-generation sequencing in a horse that died after acute illness. The genetic variation in the novel HeV resulted in primer dropout and was subsequently missed in all the HeV surveillance programs. Upon reanalysis of 4,539 samples, we detected the novel HeV variant in the black flying fox and the grey-headed flying fox urine obtained from a wide geographical distribution. **Conclusions**. Bats, which fly long distances and often aggregate in mixed-species roosts, may maintain a more diverse population of HeV variants than was previously known. The detection of the novel HeV variant in black flying fox and grey-headed flying fox populations suggests that the distribution of HeV risk to horses and their carers should be expanded to include the distribution of grey-headed flying fox. Lastly, our data support the need for surveillance of emerging and highly pathogenic viruses in reservoir host populations to enable the development of a more comprehensive pre-emptive strategy that targets spillover into human population.

28. The role of the fusion glycoprotein in henipavirus particle assembly

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Introduction. Hendra (HeV) and Nipah (NiV) are zoonotic henipaviruses that frequently jump from bat reservoirs into humans or domestic animals. Their BSL-4 designation has limited most studies to transient expression systems of viral components and virus-like particles leaving important aspects of henipavirus particle assembly ill-defined. We are studying particle assembly using Cedar virus (CedV), a non-pathogenic henipavirus that can be grown under BSL-2 containment. The attachment (G), fusion (F) and matrix (M) proteins mediate particle assembly. Here, we focus on how CedV F-trafficking governs F-G interactions and glycoprotein incorporation into virus particles. HeV and NiV F-proteins are initially transported to the cell surface as inactive precursors, F_0. Endocytosis allows for F_0 cleavage by endosomal cathepsin proteases. The fusion-active F-protein is transported to the surface via $Rab11^+$-recycling endosomes. **Methods**. Sequence alignment of the F-glycoproteins reveal a shared Tyr-based endocytic motif in the cytoplasmic tails (CT) that conforms to the consensus YxxΦ motif. The CedV F-CT is longer due to a 12-residue insertion which includes a di-tyrosine motif. To assess the importance of endocytosis for CedV F-processing and particle assembly we mutated residues in the F-CT and assessed F-processing, fusion function, and particle assembly. **Results**. We first mutated the Tyr in the YxxΦ motif to Ala (Y524A) and monitored a 2-fold reduction in F-processing for CedV, while for NiV we documented a 10-fold decrease. To attain a similar level of F-processing reduction as observed for NiV-F, we had to mutate four Tyr residues in the CedV F-CT, indicating that multiple Tyr-based motifs direct CedV F-protein endocytosis. While wildtype F-protein co-localized with G in intracellular vesicles, the mutant F-protein failed to co-localize with G, as determined by super-resolution microscopy. To study the impact of F-trafficking on particle assembly, we engineered a recombinant CedV encoding the Y524A substitution in the F-CT. The mutant virus exhibits aberrant particle assembly: it incorporated 5-times more F-protein and 5-times less G-protein in comparison to the wildtype virus. **Conclusions**. Reduced F endocytosis may disrupt intracellular F-G interactions, leading to lower G incorporation into virus particles. Endocytosis of the F-protein, in addition to regulating F-processing, may also regulate particle assembly.

29. Paramyxoviruses in insectivorous bats in South Africa

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Introduction. Paramyxoviruses are a highly abundant viral group with public and veterinary health relevance. Over the past decades an increasing number of novel paramyxoviruses, especially from the *Orthoparamyxovirinae* and *Rubulavirinae* subfamilies, have emerged from small mammal reservoir hosts. Most related research in South Africa has focused on paramyxoviruses in fruit bats, with little to no data available so far on insectivorous bats and other small mammals such as rodents, shrews and sengis. **Methods**. Faecal specimens from 359 bats belonging to 16 species in five families were collected at 36 field sites representing different biomes. Samples were screened using two broadly reactive, genus-subgroup specific semi-nested screening PCR assays, RMH and AR, for the detection of respiro-, morbilli- and henipaviruses and of avula- and rubulaviruses, respectively. PCR-positive samples underwent sequencing. **Results**. A total of 23/359 (6.4%) insectivorous bats were PCR-positive, representing nine different species in four families, of which eight species were previously not implicated as hosts for paramyxoviruses. Cape horseshoe bats (*Rhinolophus capensis*) harbour multiple paramyxovirus variants / strains. Viral sequences displayed multiple nonsynonymous mutations resulting in amino acid changes and although the zoonotic potential of these presumptive viruses was not investigated in this study, this raises concerns as to whether such viruses may be able to cross the species barrier and infect humans and / or livestock. **Conclusions**. Similar to previous studies, the majority of sequences identified in a particular species and / or genus displayed a relatively high likeness to one another, suggesting that bat paramyxoviruses are species- or genus-specific, likely due to the coevolution within their respective hosts. Given the abundance and diversity of novel potential paramyxoviruses discovered in this study and the multitude of previously unimplicated species as potential reservoir hosts, this study reaffirms the need for ongoing surveillance efforts in Southern Africa. It is important to not only identify novel paramyxoviruses but also characterize them, especially those closely related to human-pathogenic members within the family. Further investigation into host-pathogen dynamics at the wildlife – domestic animal – human interface is warranted to establish the potential ability of these viruses to cross the species barrier and cause zoonotic infections.

30. Preparedness for bat-borne coronaviruses, lessons from the pandemic

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In the past two decades, three coronaviruses have emerged and caused widespread outbreaks in humans. The origin of these zoonotic viruses have been traced back to bats as natural reservoir. Since the SARS epidemic in 2002–2003, the appreciation of bats as key

hosts of zoonotic coronaviruses has advanced rapidly. However, despite decades of research into bats and the pathogens they carry, the fields of bat virus ecology and molecular biology are still nascent, with many questions largely unexplored. Here we discuss the origin and evolution of SARS-CoV and MERS-CoV. Highlighting the ecological distribution, genetic diversity, transmission dynamics, pathogenicity and development of countermeasures.

31. Transcriptomic responses to coronavirus infections in African and North American bats

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Introduction. Bats are the likely ancestral hosts of nearly all coronavirus lineages but, like most reservoir hosts, do not appear to experience significant illness. Using archived gastrointestinal (GI) tissue samples from two different species of bats, one from North America and one from Africa, we have characterized the presence of coronaviruses using viromic and PCR-based approaches. To determine how these hosts do (or do not) respond to infection we are examining the whole-transcriptome changes in host gene expression that accompany coronavirus infection in the GI tract. **Methods**. RNA was isolated from the GI tracts of 26 North American bats (*Myotis lucifugus*) and 150 African fruit bats (*Epomophorus labiatus*) and RNASeq was performed to a read depth of 40-80 million read pairs per sample. Coronaviruses were detected either by using Kraken2 or STAR to map reads to viral transcripts or by PCR using nested degenerate primers for coronaviruses. **Results**. The levels of alpha-coronavirus BtCoV-CDPHE15 detected in the *M. lucifugus* RNASeq reads correlated with their white-nose syndrome status, as expected, and was significantly higher in juveniles than adults. However, no coronaviruses were detected in the RNASeq reads from 150 *E. labiatus* GI samples using this viromic approach. Using consensus PCR, we have identified up to 14 of these same African fruit bats as positive for coronaviruses in RNA isolated from GI tract, blood, or fecal swab. **Conclusions**. We have found that consensus PCR is more sensitive to detect coronaviruses than deep sequencing RNA-Seq of the GI tract in bats. We will next identify the coronaviruses present in the African fruit bat samples and then look for changes in host gene expression that correlate with infection.

32. ACE2 binding is an ancestral and evolvable trait of SARS-related coronaviruses

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Introduction. Two different sarbecoviruses have caused major human outbreaks in the last two decades. Both of these sarbecoviruses, SARS-CoV-1 and SARS-CoV-2, engage ACE2 via the spike receptor-binding domain (RBD). However, binding to ACE2 orthologs from humans, bats, and other species has been observed only sporadically among the broader diversity of bat sarbecoviruses. **Methods**. Here, we use phylogenetic reconstruction and high-throughput biochemical binding assays to trace the evolutionary history of ACE2 binding across a diverse range of sarbecoviruses and ACE2 orthologs. **Results**. We find that ACE2 binding is an ancestral trait of sarbecovirus RBDs that has subsequently been lost in some clades. Furthermore, we demonstrate for the first time that bat sarbecoviruses from outside Asia can bind ACE2. Moreover, ACE2 binding is highly evolvable: for many sarbecovirus RBDs there are single amino-acid mutations that enable binding to new ACE2 orthologs, including the acquisition of human ACE2 binding in non-Asian sarbecoviruses. However, the effects of individual mutations can differ markedly between viruses, as illustrated by the N501Y mutation which enhances ACE2 binding affinity within SARS-CoV-2 variants of concern but severely dampens it for SARS-CoV-1 and other sarbecoviruses. **Conclusions**. Our results point to the deep ancestral origin and evolutionary plasticity of ACE2 binding, broadening consideration of the range of sarbecoviruses with spillover potential. Ongoing and future work seeks to understand the molecular evolutionary and ecological factors that drive latent acquisition of human receptor binding as sarbecoviruses circulate in bat reservoirs.

33. Alaskan *Myotis lucifugus* virome reveals bat alphacoronavirus prevalence and likely secondary acquisition of diet and habitat-related viruses

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Introduction. *Myotis lucifugus* (little brown bat) is one of the most widespread bat species in North America, yet little is known about its virome. Little brown bats are the only bat species in Southcentral Alaska and hence provide a unique opportunity to understand how ecology influences the bat virome and the likelihood of viral spillover events. **Methods**. Fecal samples and swabs were collected from multiple bat populations around Southcentral Alaska and screened for bat alphacoronaviruses. Several samples were sequenced on an

Illumina HiSeq platform and analyzed for viral diversity. **Results**. Each sample contained a large diversity of bat alphacoronaviruses and insect and plant viruses. Alphacoronaviruses were detected in most bat populations sampled. Although no data suggests Alaskan little brown bats harbor viruses capable of infecting humans, the results suggest these bats acquire many of these viruses through diet and through intraspecies interactions. **Conclusions**. In order to understand the full zoonotic potential of bats, both within and outside of Alaska, we are also studying the ecological interactions and natural history of the host. The results of this study show how transdisciplinary research is necessary to understand when, where, and how bat-borne infections diseases may emerge.

34. A strategy to assess spillover risk of bat SARS-related coronaviruses in Southeast Asia

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Introduction. Emerging diseases caused by coronaviruses of likely bat origin (e.g. SARS, MERS, SADS and COVID-19) have disrupted global health and economies for two decades. Evidence suggests that some bat SARS-related coronaviruses (SARSr-CoVs) could infect people directly, and that their spillover is more frequent than previously recognized. Each zoonotic spillover of a novel virus represents an opportunity for evolutionary adaptation and further spread; therefore, quantifying the extent of this spillover may help target prevention programs. **Methods.** We derived biologically realistic range distributions for known bat SARSr-CoV hosts in Southeast Asia and quantified their overlap with human populations. We then performed a probabilistic risk assessment that incorporated this spatial overlap data with literature-informed estimates of human-bat contact, viral seroprevalence among humans with bat contact, and human SARS antibody duration. **Results.** The highest richness of SARSr-CoV bat host species was observed in southern China, eastern Myanmar, and northern Lao PDR. We estimated that a median of ~66,000 people are infected with SARSr-CoVs annually in Southeast Asia. Sensitivity analyses indicated that two parameters—the probability of human contact with a bat, and the probability of antibody detection given contact with a bat—primarily contributed to variance in the estimate of spillover. **Conclusions.** These data on the geography and scale of spillover can be used to target surveillance and prevention programs for potential future bat-CoV emergence.

35. An ACE2-dependent Sarbecovirus in Russian bats is resistant to SARS-CoV-2 vaccines

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Introduction. Spillover of sarbecoviruses from animals to humans has resulted in outbreaks of severe acute respiratory syndrome SARS-CoVs and the ongoing COVID-19 pandemic. Efforts to identify the origins of SARS-CoV-1 and -2 has resulted in the discovery of numerous animal sarbecoviruses – the majority of which are only distantly related to known human pathogens and do not infect human cells. The receptor binding domain (RBD) on sarbecoviruses engages receptor molecules on the host cell and mediates cell invasion. **Methods**. Here, we tested the receptor tropism and serological cross reactivity for RBDs from two sarbecoviruses found in Russian horseshoe bats. **Results**. While these two viruses are in a viral lineage distinct from SARS-CoV-1 and -2, one virus, Khosta 2, was capable of using human ACE2 to facilitate cell entry. Viral pseudotypes with a recombinant, SARS-CoV-2 spike encoding for the Khosta 2 RBD were resistant to both SARS-CoV-2 monoclonal antibodies and serum from individuals vaccinated for SARS-CoV-2. **Conclusions**. Our findings further demonstrate that sarbecoviruses circulating in wildlife outside of Asia also pose a threat to global health and ongoing vaccine campaigns against SARS-CoV-2.

36. On the evolution of bat type I interferons

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Introduction. Bats are the natural reservoir hosts of viruses, some of which may cross-species spillover to humans and cause diseases. The mechanism of how bats coexist with viruses is still largely unknown. Others and we demonstrated that the constitutively expressed IFNs in *P.alecto* and dampened STING-IFN or NLRP3-inflammation responses may render bats the ability to quickly inhibit viral replication or tolerance viral diseases. However, this evolutionary pattern of bat type I IFNs was challenged following the sequencing of Egyptian Rousette bat genomes, which showed expanded IFN locus with no basal expression. This species difference hindered our understanding of a pan-bat character of type I IFNs, which play vital role against viruses. **Methods**. We dig into the 10 high quality bat genomes published online, and compared the type I IFN locus with that from human and other representative mammals. We also compared the individual IFN expression profiles, antiviral functionality and anti-proliferation activity in a representative *Rhinolophus* bat. **Results**. Our data showed a pan-bat contraction nature of this locus, including the Rousettus

aegyptiacus that was reported expansion in previous study that based on poorly assembled genome. Moreover, the bat type I IFNs locus appear to largely lost the second half, from KLHL9 to IFNB, compared to all other mammals. Of note, bat prefers IFNW to IFNA genes, and the IFNA was even absent in certain bat species, which was not observed in other mammals except armadillo. To understand why bats choose IFNW but not IFNA, we did the following analysis: (1) a phylogeny analysis indicates that some of the IFNWs are actually more ancient IFNAW; (2) based on *Rhinolophus* bat cell, we found IFNW showed a higher basal expression level than IFNA and IFNAW; (3) the IFNW showed better anti-viral, weaker anti-proliferation activity and be more thermo stable than IFNA protein. **Conclusions**. These analyses demonstrated the unique nature of bat type I IFNs, which was probably shaped by the flying ability. The nature of a constitutive expression undermined the evolutionary pressure for IFN gene expansion, resulting in ancient but effective type I IFN locus. The reservation of IFNAW genes, which were considered as ancient IFNs during evolution, is another evidence of a relatively ancient IFN locus in bats. The IFNW preference was also rare in other mammals. As shown in our data, the higher anti-viral activity and better thermo stability (that could tolerant the elevated body temperature during flight) should be the driving forces. Collectively, we revealed unique evolutionary patterns in bat type I IFNs that contributed to a unique ability for bats to co-exist with viruses.

37. The characteristics of bat MHC class I and CD8 reveal unique anti-viral immunity

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Introduction. The capacity of bats to carry highly pathogenic zoonotic viruses without producing symptoms of viral infection has prompted researchers to consider possible immunological differences between bats and other mammals, and especially the features of adaptive immunity in bats remain largely unknown. Although the critical cellular and molecular components in the adaptive immune system are relatively conserved in bats, a large number of unique amino acid substitutions or insertions occur in bat immune genes. The T-cell receptor, assisted by CD8 or CD4 receptors, recognizes peptide antigen in the context of MHC molecules, which is the basis of the activation of adaptive immunity against the virus. **Methods**. Our study has screened and identified a series of bat MHC I Ptal-N*01:01-binding peptides derived from bat-related viruses, i.e. Hendra virus, Ebola virus, MERS-CoV and influenza H17N10 virus. **Results**. The structure of Ptal-N*01:01 shows unusual peptide presentation characteristics, with a 3AA insertion at the N-terminus of the α1 helix. The hydrogen bond network herein allows the first amino acid Asp of the peptide anchored in the A pocket of the bat MHC class I. The F pocket as traditionally main anchoring site shows a preference for Pro (PLoS Biol, 2019). Interestingly, this structural feature in the A pocket of bat MHC I can be found in marsupials' MHC I as well (J Immunol, 2021). We also determined the structure of bat CD8aa, the overall structure and key positions are quite conserved although the sequence conservation is low compared to other mammals. Meanwhile, the high affinity between bat MHC I and CD8aa molecules has also been verified, which may enhance the T-cell immune effect mediated by MHC I (Unpublished). We also explored the differences in peptide recognition upon binding of bat MHC I Ptal-N*01:01 to bat β_2m and human β_2m, and found that the conformation of peptide presentation by bat MHC I is not affected by β_2m (J Visual Exp, 2020). **Conclusions**. Our findings provide a structural basis for the understanding of bat adaptive immunity and help to explore differences in antiviral responses between bats and other mammals.

38. Interrogating anti-viral innate immune responses of Chinese hipposideros bats

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Introduction. SARS-CoV-2 is only the latest from a string of zoonotic RNA viruses whose origin has been traced back to bats, yet the immunological reasons for the unique association of bats with RNA viruses in particular remain a tantalizing mystery. Studies of antiviral immune responses of bats to the viruses they harbour have largely focused on *Pteropus* and *Rousettus* flying foxes, but little is known about immune responses of Old World Rhinolophoidea species that harbour coronaviruses at prevalences of up to 30% and are likely origins of SARS and SARS-CoV-2. **Methods.** In 2017 we collected blood samples from a total of 20 *Hipposideros pratti* bats at 3 sites located in the Guangdong province in southwestern China and performed stimulations with lipopolysaccharide (LPS) at various doses followed by RNA-sequencing at 2, 6, and 24 hours post stimulation. A comparison dataset was generated using human blood samples from 6 healthy donors. **Results.** Our analyses of the RNAseq data showed that following stimulation bat primary circulating blood cells have substantially delayed responses to LPS with very few differentially expressed genes (DEGs) detected at all time points examined post stimulation. Moreover, the magnitude of the response in bats was significantly subdued compared to human cells. In addition, the specific gene expression changes elicited were qualitatively distinct between bats and humans. While DEGs in human cells were enriched for gene ontology terms such as defense response to virus, lymphocyte activation, response to LPS, and regulation of cytokine production, DEGs in bats were enriched for metabolic processes, and regulation of mRNA processing. Interestingly, some DEGs representing key response genes to LPS were shared between the species, including the upregulation of *IL6, IFNg, NOD2,* and *TRAF2.* **Conclusions.** Thus, together our data suggest that there are key differences between bat and human anti-viral innate responses and provides clues how bats support the establishment of a disease-free host-pathogen equilibria.

39. Differential IRF3-independent innate immune signaling in human and bat cells

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Introduction. Interferon regulatory factor 3 (IRF3) is one of the most well-characterized transcription factors involved in the regulation of innate immune responses. Many studies have used IRF3 knockout systems (mice or cells) to analyze the functions of IRF3 in immunity and other biological systems. Understanding mechanisms of IRF3 signaling in taxa that act as viral reservoirs, such as Chiroptera (bats), can identify natural mechanisms of viral suppression in non-human species. **Methods.** In this study, we used RNA-seq to compare human and bat transcriptomic responses to viral infection and uncovered potential commonalities and differences in antiviral signaling between distantly related mammalian lineages. We simulated viral infection by exposing human and bat (*Eptesicus fuscus*) cells in vitro to polyinosinic:polycytidylic acid (poly(I:C)), and investigated subsequent interferon and cytokine signaling responses. Further, we interrogated IRF3-independent immune responses by comparing gene expression in vitro in wildtype and IRF3-deficient human and bat cells. **Results.** Loss of IRF3 dramatically altered global gene expression in human and bat cells following mock and poly(I:C) treatment. Human and bat cells with IRF3 responded similarly to poly(I:C) treatments, while IRF3-deleted bat cells exhibited a unique, IRF3-independent transcriptomic response to poly(I:C) stimulus. Details on differential signaling in the absence of IRF3 will be discussed in this presentation, along with implications for SARS-CoV-2 replication. **Conclusion.** Despite similarities in innate signaling pathways and components in bats and humans, bats have evolved unique responses that enable them to be carriers of diverse viruses, including pandemic viruses, without causing disease. Understanding both similarities and differences inform strategies to prevent humans to pandemic virus replication and spread.

40. Immunoprotective disease tolerance in Marburg virus-infected reservoir host bats

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Introduction. Marburg virus (MARV) is among the deadliest pathogens in humans and nonhuman primates (NHPs). Contributors to severe MARV disease include immune response suppression and inflammatory gene dysregulation ("cytokine storm"), leading to systemic damage and often death. The only known MARV reservoir host is the Egyptian rousette bat (ERB, *Rousettus aegyptiacus*). MARV-infected ERBs support virus replication and oral shedding without the immunopathology typically seen in infected humans and NHPs. Growing evidence supports that unique ERB antiviral responses likely underlie control of MARV disease. Recent studies by our lab and others led us to hypothesize that a major mechanism facilitating this control is disease tolerance, whereby a finely-tined immune response limits virus replication while avoiding the inflammatory dysregulation underlying severe MARV disease. **Methods**. Using custom species-specific antibodies and an immune gene probe array (NanoString), we temporally characterized transcriptional host responses of MARV-infected ERBs. Our *in vivo* targeted transcriptomics analysis included monocytes/macrophages (critical early immune response mediators and primary MARV targets in humans and NHPs) and tissues (particularly skin at the site of inoculation) known to support MARV replication in ERBs. **Results**. We found tightly-regulated induction of canonical antiviral genes (e.g., *IRF7*, *ISG15* and *OAS3*) typical of viral infection in mammals, but strikingly, almost no significant changes in expression of traditional markers of adaptive immunity or inflammation, including cytokines and chemokines like *CCL8*, *FAS* and *IL6*, classically implicated in filoviral pathogenesis in humans and NHPs. **Conclusions**. Our study offers the first *in vivo* functional evidence for disease tolerance in a filovirus reservoir. The remarkable absence of differentially-expressed inflammatory genes thought to cause severe human and NHP immunopathology holds major implications regarding the differences in pathogenesis observed in natural reservoir versus spillover hosts and the physiological avenues by which reservoirs and viruses co-evolved a tolerant host environment.

41. Profiling virus-specific helper T cells from Jamaican fruit bats

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Introduction. Bats serve as reservoir hosts for a number of important viruses that cause significant morbidity and mortality in humans. Little to no pathology is observed in these virus/bat infections. To date, no studies have examined bat T cell responses to infection, principally because of a lack of methodologies and colonized bats for such studies. We sought to cultivate helper T cells from infected bats to determine their profiles upon in vitro antigen recall challenge. We also examined T cell responses from Jamaican fruit bats transduced with human ACE2 in the lungs and subsequently challenged with SARS-CoV-2. **Methods**. For helper T cell culturing, bats were infected with H18N11 influenza A virus. Nine days later, spleen cells and mesenteric lymph node cells were collected for in vitro antigenic stimulation with H18N11 nucleocapsid. Concurrently, bone marrow derived dendritic cells were generated with recombinant Jamaican fruit bat GM-CSF to serve as antigen presenting cells (APC). Restimulation of T cells occurred at 2 week intervals with fresh APC, antigen and T cells. In a second set of experiments, Jamaican fruit bat lungs were transduced with human ACE2 using replication-defective adenovirus, then challenged with SARS-CoV-2 5 days later. Examination of T cells was performed by stimulating splenocytes with SARS-CoV-2 nucleocapsid peptide library and assessment of CD154 expression by flow cytometry after 6 hours. **Results**. Only short-term cultures could be generated despite the use of autologous, MHC-matched bone marrow-derived antigen presenting cells and addition of exogenous Jamaican fruit bat interleukin-2. Gene expression profiling showed prominent IL-10 expression and moderate IL-4 expression, but other cytokines were expressed at lower levels. SARS-CoV-2 antigen was readily detected in the lungs of human ACE2-transduced bats up to 7 days, and flow cytometric analysis of splenocytes showed increased levels of CD154 expression, suggesting $CD4^+$ helper T cell activation. **Conclusions**. We have demonstrated that bat helper T cells participate in immune responses to viral infections. A significant obstacle is the inability to perform long-term T cell culturing; however, development of new assays that rely on single cell analysis of T cells are within reach and will provide substantially more detailed analysis of how bat T cells respond to viral infections.

42. Globalizing accessible field observation tools for bats

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Introduction. Global climate change will result in more frequent and widespread instances of zoonotic pathogens spilling into human populations (and vice versa). Bats might play an increasingly dark role in future spillover events unless we develop efficient ways to keep tabs on their many populations. Recent calls to “pair viral surveillance and discovery efforts with biodiversity surveys tracking species’ range shifts, especially in tropical regions that harbor the most zoonoses and are experiencing rapid warming” highlight the importance of efficiently tracking the multi-scale movements of bats in the entwined contexts of public health and wildlife conservation. **Methods**. The openBatMonitoring Project involves leveraging and integrating trends in open-source computing and sensing hardware, computer-vision and deep-learning methods, and new battery/solar storage tech to develop open-source bat observation systems that more people can afford and learn to build and program themselves. System design has benefitted from lessons learned during long-term studies of wild bats with white-nose syndrome and in other remote field settings and ecological contexts. **Results**. Devices currently being developed and tested include weatherproof, off-the-grid infrared time-lapsing, video, and acoustic detection systems that cost less than about $200US, are entirely open source, and can run bat detection and classification algorithms in real time. **Conclusions**. openBatMonitoring aims to create accessible bat observation technologies that people all over the world can use to address issues critical to conservation and public health.

43. Serological positivity against selected flaviviruses and alphaviruses in free-ranging bats and birds and in domestic and peri-domestic mammals evidence exposure to arboviruses seldom reported locally in humans

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Introduction. Many arboviruses have virus transmission cycles that involve wildlife mammals as amplifiers and mosquitoes as vectors. For some of them, such as West Nile virus (WNV), Saint Louis encephalitis virus (SLEV) and some alphaviruses, most mammals, including humans, are dead-end-hosts that may be asymptomatic or develop more severe symptoms. Costa Rica harbors several

flaviviruses, though while Dengue and Zika are hyperendemic, previous research indicates limited and restricted circulation of WNV, SLEV and alphaviruses in horses, sloths, and monkeys. SLEV, WNV or alphaviruses seroprevalence and high transmission areas have not yet been identified. We aimed to i) determine the extent of putative circulation for these viruses by sampling peri-domestic and domestic animals, humans, and mosquitoes and ii) study the role of wildlife in a putative arbovirus sylvatic cycle by sampling free-ranging bats and birds. **Methods**. Sampling took place in rural households located in two DENV and ZIKV hyperendemic regions during the rainy and dry seasons of 2017-2018. We conducted PRNT assays for serology and RT-PCR for virus detection. **Results**. In both regions no current infection in the sampled mammals or mosquitoes with any of the sought arboviruses was detected. Nevertheless, we obtained past serological evidence; but more interestingly we recorded seroconversion events for some of these arboviruses that are seldom reported locally in humans. In bats, positivity in 34.95% for DENV-1, 16.26% for DENV-2, 5.69% for DENV-3, 4.87% for DENV-4, 2.43% for WNV, 4.87% for SLEV, 0,81% for YFV, 7.31% for EEEV, and 0.81% for VEEV was found. Antibodies against ZIKV were not detected. In birds, PRNT results were positive against WNV in 0.80%, SLEV in 5.64%, EEEV in 8.4%, and VEEV in 5.63%. An additional retrospective PRNT analysis was performed using bat samples from three additional DENV endemic sites resulting in a 3.27% prevalence for WNV and 1.63% for SLEV. Interestingly, one sample from these bats resulted unequivocally WNV positive confirmed by serum titration. **Conclusions**. These results suggest that free-ranging bats and birds are exposed to not currently reported hyperendemic-human infecting Flavivirus and Alphavirus; however, their role as reservoirs or hosts is still undetermined. Also, the seroconversion events recorded support the silent but recent circulation of SLEV and WNV in these two regions. This study provides clear-cut evidence for WNV, SLEV and alphaviruses presence and thus, they should be considered by health authorities for future prevention campaigns and arboviruses differential diagnostics.

44. Bat-borne Issyk-Kul virus in Italy: isolation and genome characterization

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Introduction. *Issyk-Kul virus* (ISKV), *Nairoviridae* family, was firstly isolated in 1970 from a *Nyctalus noctula* bat trapped near Lake Issyk-Kul, Kyrgyzstan. ISKV was subsequently detected in nearby countries in Central Asia (Tajikistan and Kazakhstan) from organs of several bat species and bat ticks and it has been described to cause sporadic outbreaks of febrile illness in humans with headache, myalgia, and nausea. It has been assumed that bats and ticks are both reservoirs of ISKV with transmission to humans being associated with tick bites and exposure to bat feces and urine. Recently, ISKV expanded its geographical range to Europe following the detection of portions of its genome in *Eptesicus nilssonii* in Germany. Herein we report the isolation and the whole-genome characterization of an ISKV strain detected in a *Hypsugo savii* bat collected within the survey implemented in Italy on emerging viruses of bats. **Methods**. A fresh carcass of an adult female of *Hypsugo savii* spontaneously dead in a wildlife rehabilitation center in Northern Italy was fully necropsied. Identification of bat species was achieved by morphological and molecular methods. Tissue samples from different organs (lung, hearth, intestine) were subjected to viral isolation on cell culture. Virus identification was performed using negative staining electron microscopy (nsEM) and NGS sequencing. Molecular and phylogenetic analyses were also performed. **Results**. Anamnesis reported sensory depression, inappetence and weight loss. The death occurred eleven days after the admission to the rehabilitation center and no traumatic lesion, nor pathological lesions indicative of infectious diseases were observed at necropsy. CPE was observed on MARC 145 cells inoculated with a pool of organs and nsME performed on cells supernatants revealed the presence of spherical enveloped virions of 80-100nm morphologically referable to Nairovirus. The complete genome sequence revealed the 3 typical Nairovirus genome segments L (11,978 nt), M (4,907nt) and S (1,457nt) encoding protein corresponding to the polymerase protein, polyglycoprotein and nucleoprotein respectively. Blast analysis showed the highest nucleotide identity with ISKV strain LEZ 86-787 for L gene (95.34%), strain LEIV-315K for M gene (81.34%) and strain ISKV PbGER for S gene (97.51%). Phylogenetic trees based on full-length of L, M and S genome sequences confirmed that the new isolate belong to the ISKV clade within the Keterrah group, genus Orthonairovirus. **Conclusions**. A crucial point to asses viral zoonotic risks that may emerge from bats consist in determining first the excreting non-infectious nucleic acids and then the eventual shedding of infectious virus particles. The successful of ISKV isolation from a synanthropic bat species such as *Hypsugo savii* suggests possible implications for Public Health and the need for further investigation aimed to better define the viral ecology and diffusion in bats population and its zoonotic potential.

45. Molecular phylogeny of bat-borne gammaherpesviruses in Vietnam

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Background: Bats (order Chiroptera) serve as reservoir hosts of multiple zoonotic pathogens, including Ebola virus, Nipah virus, rabies virus and SARS coronaviruses. Recently, genetically distinct hantaviruses have also been detected in multiple species of bats in the suborders Yangochiroptera and Yinterochiroptera, but no virus isolates are available. **Methods:** In an attempt to isolate hantaviruses from bats, kidney tissues were collected from 28 bat species in Vietnam to establish cell lines. **Results:** Cytopathic effect was observed in primary kidney cell cultures from two acuminate horseshoe bats (*Rhinolophus acuminatus*) and one Côn Đảo horseshoe bat (*Rhinolophus chaseni*). Analysis by next-generation sequencing showed gammaherpesviruses (GHV). To ascertain the prevalence of the newfound *Rhinolophus* gammaherpesvirus (RGHV, 139,222-bp), RNA*later*™-preserved lung tissues from 151 bats (representing 30 species), collected in Vietnam, during 2002–2019, were screened by PCR using newly designed oligonucleotide primer sets. RGHV sequences were confirmed in 14 of 26 *R. acuminatus*, three of seven *R. chaseni*, and five of 13 *R. microglobosus*. Phylogenetic analysis indicated that the newfound RGHV shared a common ancestry with GHV from *Rhinolophus blythi* in China and was distantly related to bat gammaherpesvirus 8 (BGHV8), previously detected in an interscapular tumor from a cave bat (*Myotis velifer incautus*). **Conclusions:** Future studies are warranted to determine the genetic diversity and geographic distribution of RGHV, as well as other bat-borne GHV.

46. Kasokero virus-inoculated Egyptian rousette bats shed virus through multiple routes

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Introduction. The human-pathogenic Kasokero virus (KASV; family *Nairoviridae*, genus *Orthonairovirus*) has been isolated from serum specimens from Egyptian rousette bats (ERBs; *Rousettus aegyptiacus*) captured at Kasokero Cave, Uganda and from unengorged ticks collected from the rock crevices of ERB colonies at Lanner Gorge Cave, South Africa and Python Cave, Uganda. Although evidence suggests that KASV is maintained in an enzootic transmission cycle between (*Ornithodoros (Reticulinasus) faini*) ticks and ERBs with potential for incidental virus spillover to humans through the bite of an infected tick, the vertebrate reservoir status of ERBs for KASV has never been experimentally evaluated. Further, the potential for bat-to-bat and bat-to-human transmission of KASV is unknown. **Methods**. Groups of ERBs were intradermally inoculated with 4.0 $\log_{10}TCID_{50}$/mL of KASV and either serially-sampled through 18 days post infection (DPI) to assess viremia, oral, rectal, and urinary shedding, or serially-sacrificed at regular intervals post-infection to assess virus-tissue tropism. **Results**. Throughout the study, all bats appeared clinically healthy, and maintained normal body weights and temperatures. KASV viremia (mean peak load: 5.6 $\log_{10}TCID_{50}$eq/mL; mean duration: 7.7 days) and oral (mean peak load: 5.0 $\log_{10}TCID_{50}$eq/mL; mean duration: 8.6 days), rectal (mean peak load: 3.2 $\log_{10}TCID_{50}$eq/mL; mean duration: 4.9 days), and urinary (mean peak load: 4.3 $\log_{10}TCID_{50}$eq/mL; mean duration: 3.9 days [opportunistic collection]) shedding was detected in all KASV-inoculated bats. All KASV-inoculated bats assigned to the serial-sampling group seroconverted by 14 DPI. KASV loads that were greater than the virus inoculum dose and greater than the viremia at the time of euthanasia were detected in the skin at the inoculation site (load range: 4.1─5.0 $\log_{10}TCID_{50}$eq/g, time: 3, 6, and 9 DPI), spleen (load range: 4.2─5.7 $\log_{10}TCID_{50}$eq/g, time: 3 and 9 DPI), and inguinal lymph node (load: 4.1 $\log_{10}TCID_{50}$eq/g, time: 20 DPI). **Conclusions**. The magnitude and duration of viremia and the high viral loads in the skin at the inoculation site indicate the potential for KASV transmission between infectious ERBs and feeding *O. (R.) faini* ticks. Viral shedding data highlight the potential for KASV spillover to humans through direct or indirect contact with infectious oral secretions or urine.

47. Molecular characterization of the interactions between bat-borne influenza viruses and host MHC-II molecules.

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Introduction. Zoonotic transmission of viruses from bat reservoirs to humans poses a constant threat to global health and therefore a better understanding of the biology of bat viruses is of utmost importance. Recently, two novel Influenza virus strains (H17N10 and H18N11) were discovered in New World bats. In contrast to classical Influenza viruses these novel, bat-borne viruses use MHC-II molecules as receptor to enter the host cell. Interestingly, MHC-II molecules from various vertebrate species can be used for cell entry, including human HLA-DR, possibly allowing spill over to other species. This indicates that conserved domains/amino acids of MHC-II molecules mediate cell entry. However, the low affinity between MHC-II and H17/H18 precludes simple biochemical interaction studies. **Methods and Results**. Performing *in silico* guided mutagenesis coupled to an infection-based entry assay, we could narrow down the interface between H17/H18 and HLA-DR to single, highly conserved amino acid positions. Employing fluorescently-labeled and photoswitchable HLA-DR molecules, we provide evidence that attachment of Bat influenza viruses induces clustering of MHC-II molecules on the surface of the host cell followed by internalization in a clathrin-dependent manner. Consistently, clustering was absent with MHC-II variants lacking these highly conserved amino acids. **Conclusions**. Finally, the use of highly conserved amino acids of

MHC-II molecules by H17/H18 might explain the observation that several quite diverse bat species can be infected by these novel bat-borne influenza viruses.

48. Characterization of the bat-derived influenza A viruses H17N10 and H18N11.

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Introduction. Influenza A viruses (IAVs) originating from aquatic waterbirds circulate among a wide variety of different hosts. They can cross species barriers and establish new virus lineages in avian and mammalian species. As a consequence, IAVs are exposed to recurrent selective pressures, leading to a range of virus variants that are able to cope with the new host environment. Bats were not considered to be part of the IAV ecology until recently, when two phylogenetically distinct IAV lineages, designated H17N10 and H18N11, were identified in the New World bats *Sturnira lilium* and *Artibeus* spp, respectively. Although bat H17N10 and H18N11 overall resemble classical IAVs of avian origin, their major surface glycoprotein hemagglutinin (HA) fails to utilize sialic acid receptors for cell entry. **Results**. We show that bat IAVs can instead enter cells by using major histocompatibility complex class II (MHC-II) molecules from various species, including different bats, chickens, pigs and humans. Moreover, following experimental infection, H18N11 was able to replicate in mice, ferrets as well as Seba's Short-Tailed Bats (*Carollia perspicillata*) and Jamaican fruit bats (*Artibeus jamaicensis*); however, horizontal transmission was only observed between Jamaican fruit bats, suggesting they are a putative natural reservoir. Histopathological examination of infected animals revealed that viral antigen and genomic RNA was predominantly present in the pharyngeal and palatine tonsils and the follicle-associated epithelium of the jejunal Peyer's patches. **Conclusions**. We are currently addressing the nature of H18N11-infected cells in these organs and determining the innate immune response induced upon infection by the means of single cell RNA sequencing.

49. Bats and rabies; An African perspective and implications for lyssavirus taxonomy

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Background: Rabies is caused by bullet-shaped and negative-sense RNA viruses in the lyssavirus genus with bats the principal reservoir host for 14 of the 17 officially recognised species. Rabies virus, prototype virus species, is the only lyssavirus that is well established in terrestrial carnivores (worldwide) and bats (but only in the Americas). The other bat lyssaviruses (rabies-related lyssaviruses) occur only outside the Americas. They have a distinct geographical distribution and association with specific bat species, with limited cross-species transmission to other animals and humans, resulting in dead-end infections. The diversity of lyssaviruses described in recent years has increased due to increased surveillance. **Methods:** Since 2004, we have been doing passive surveillance for lyssavirus infections in bats in South Africa. A real-time pan-lyssavirus reverse transcriptase PCR tests are performed targeting a partial conserved nucleoprotein gene of the viral genome extracted from bat brain samples. Sample submission was from bat rehabilitators, bat biologists, national museums, the general public, national rabies reference laboratories and research programmes and represented over 45 bat species including frugivorous and insectivorous bats. Sequencing additional genome regions further characterised positive samples to assess the taxonomic position**. Results**: We identified new Duvenhage and Lagos bat virus infections in South African bat species associated with new reservoir hosts. In addition, did we also identify two new lyssavirus species; Matlo bat lyssavirus and an Aravan-like lyssavirus. These viruses are related to known lyssaviruses previously only identified in Eurasia, expanding the diversity. **Conclusions:** Molecular detections of lyssavirus RNA in bats are still rare and sporadic due to a low incidence and inadequate surveillance. Serological evidence indicates a more widespread and even higher diversity, suggesting that the incidence of known lyssaviruses is underestimated, and several new lyssavirus species are yet to be discovered. However, cases are still rare, with less than 1% of bats infected and transmitting the virus via infected saliva. The increased diversity of lyssaviruses reported challenge the current guidelines for taxonomic characterisation of new lyssavirus species and should be reconsidered.

50. Control of established, CNS-resident lyssavirus infection by a CD4$^+$ T cell dependent immune response stimulated by single-dose monoclonal antibody therapy

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Introduction. Lyssaviruses cause uniformly fatal disease once the infection progresses to the central nervous system (CNS). Current lyssavirus immunotherapies (post exposure prophylaxis) have not resulted in protection from mortality when delivered after onset of clinical rabies. In this study, using anti-lyssavirus human monoclonal antibody (mAb) F11, we evaluated the efficacy of immunotherapy on established lyssavirus infections in mice. **Methods.** We used luminescence-based longitudinal imaging to trace recombinant luciferase expressing Australian bat lyssavirus (ABLV) in a mouse model of lethal disease. We treated ABLV infected mice with F11 at days 5 and 7 post infection. To investigate the mechanism of F11 efficacy, we treated mice with an F11 mutant (F11-N297G), which has defective FcRy binding. **Results.** We found that a single dose of F11 reverses disease signs and protects animals from lethality, even when administered after initiation of virus replication in the CNS. Investigation of the mechanisms of F11 efficacy revealed that virus neutralization is insufficient to protect animals from mortality. Control of infection requires an intact adaptive immune response, particularly CD4$^+$ T cells. In vivo analysis of F11-N297G showed a transiently increased viral load and increased morbidity when compared to F11 treated mice, suggesting involvement of Fc dependent immune mechanisms. Despite long-term survival and absence of disease signs in F11-treated animals, we found that lyssavirus infection persists chronically, concomitant with elevated expression of cellular immune response genes. Notably, treatment with F11 correlates with a large reduction in CD3+/CD4-negative T cells in the brains of infected animals that are controlling infection. **Conclusion.** These findings demonstrate that single-dose mAb therapy can stimulate a durable T cell-dependent response that is highly effective against an established CNS infection by a lethal neurotropic virus.

51. Co-circulation of West Caucasian bat virus, Lleida bat virus and Lloviu virus in *Miniopterus schreibersii* in Italy and Hungary

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Introduction. The recent pandemic of COVID-19 prompted innovative research for the management and the ready response to emerging viruses, but, up to now we are far from unraveling the triggers that allow or even push viruses to jump from the wild reservoir to humans directly or through intermediate hosts. Although recognized hotspots of emerging pathogens have been identified in tropical areas, there is increasing evidence that pathogens with zoonotic potential also circulate in European wildlife. In particular, the almost 50 species of bats found in Europe are known to carry a wide variety of viruses whose potential for emergence has not been fully addressed. Among these, lyssaviruses are of particular concern because of their lethality for humans, with particular concern posed for divergent species whose infection cannot be managed with current vaccines or antibodies, such as west Caucasian bat virus (WCBV) and Lleida bat virus (LLEBV). In addition, recent studies showed that Lloviu virus (LLOV), belonging to the family *Filoviridae*, can infect human cells, suggesting its zoonotic potential. Interestingly, these three priority pathogens have been all described from the common bent-winged bat (*Miniopterus schreibersii*) a migratory bat with one of the greatest geographical range among mammals. The spillover is a stochastic event that requires several determinants manifesting altogether, among which the distribution of viruses across the geographical range of reservoir species is a sine qua non condition. The aim of this study was to detect the circulation of lyssaviruses and filoviruses across the European range of the bent-winged bat, with preliminary data obtained from four roosts Italy and Hungary. **Methods**. Bats were trapped within roosts during the day for the conservative collection of blood and salivary swabs. Carcasses found within each site were collected and fully necropsied. Sera were screened for the presence of neutralizing antibodies against WCBV and LLEBV using a modified RFFIT; salivary swabs, blood clots and organs were screened for the presence of lyssaviruses and LLOV using standard molecular approaches. Viruses detected within the study were fully sequenced using Nanopore technique. **Results**. Serological analyses confirmed the circulation of WCBV in all study sites in Italy and Hungary, with highest prevalence detected in 2020 when a single colony from Italy was tested. This transient colony was peculiar for its urban position and was, indeed, the source of a spillover event that lead to the death of a cat and the exposure of six persons. All colonies screened in Italy and Hungary in 2021 and archive samples from 2018 and 2019 showed lower percentage of positivity. Preliminary testing using LLEIDA showed co-circulation of the two lyssaviruses of phylogroup III but no individuals reacting against both viruses. No salivary samples of carcasses were positive from any time point or locations, confirming low prevalence and shedding within populations. Bats were found LLOV RNA positive at one site in Hungary. Here the virus is circulating continuously, and recently an infectious isolate was established from an infected bat, suggesting the role of this species as a reservoir. In addition, for the first time Lloviu presence was confirmed in Italy, where a single blood clot was found positive in September 2020. Phylogenetic analyses showed that the two viruses from Hungary and Italy share a

high genomic similarity, and are more genetically related compared to the original Isolate found in Spain in 2002. **Conclusions**. The bent winged bat is a migratory bat species widespread in Southern Europe, with high mixing between populations supported by genetic data. Our preliminary data confirmed that all priority viruses associated with the reservoir species are circulating in roosts up to 900 km apart in Italy and Hungary, suggesting they might be spread across its wide distribution range, spanning from Portugal to Russia.

52. Rabies virus spillover and host shifts from bats into meso-carnivores

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Introduction. There is global interest in understanding emerging and re-emerging zoonoses transmitted from wildlife to humans and animals. In North America, rabies virus (RABV) perpetuates as distinctive variants in reservoir populations of wild meso-carnivores and bats. Spillover occurs commonly, usually as discrete, dead-end transmission events, when RABV variants are transmitted to non-reservoirs or when reservoirs are infected with atypical variants. Together with public health surveillance addressing human exposures to RABV, the enhanced rabies surveillance (ERS) of wildlife using a field-friendly direct, rapid, immunohistochemical test has proved essential for detection of CST in the USA. **Methods**. Unusual CST events of a bat RABV to wild meso-carnivores were detected in Flagstaff, Arizona during 2001 (19 striped skunks), 2004-05 (6 striped skunks, 2 gray foxes), and 2009 (23 gray foxes, 6 striped skunks, and a ringtail). During 2021, CST involving the same bat RABV variant re-occurred (16 striped skunks to date). **Results**. Rabies management actions in response to the 2021 cases have focused on public education, animal quarantine, domestic animal and wildlife vaccination, as well as ERS. **Conclusions**. Defining practical diagnostic tools and surveillance strategies targeting at-risk wildlife remains fundamental for timely detection of CST. As such, the 'Flagstaff phenomenon' may serve as a basis for understanding infectious disease origins and adaptations, and guide studies designed for the detection, prevention, and control of zoonoses associated with wildlife.

Poster Abstracts

1. Single-cell transcriptome analysis of the in-vivo response to viral infection in the cave nectar bat *Eonycteris spelaea*

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Introduction. Bats are reservoir hosts of many zoonotic viruses with pandemic potential in humans. Understanding the *in-vivo* mechanisms responsible for viral disease tolerance in bats is therefore of significant importance. **Methods**. Here, we utilized single-cell transcriptome sequencing (scRNA-seq) to provide detailed comparative analyses of the immune repertoire and the transcriptional responses in the bat lungs upon *in-vivo* infection with a double-stranded RNA virus, *Pteropine orthoreovirus* PRV3M. **Results**. In uninfected bats, neutrophils were observed to have basally high *IDO1* expression, uniquely amongst mammals currently profiled by scRNA-seq. NK/T cells were the most abundant immune cell type in lung tissue, and included three distinct $CD8^+$ effector T cell populations delineated by the differential expression of *KLRB1*, *GFRA2* and *DPP4*. We identified NK/T clusters which up-regulated genes involved in T-cell activation and effector function early after viral infection. Alveolar macrophages and classical monocytes were key drivers of antiviral interferon signaling. Infection also resulted in the expansion of a $CSF1R^+$ population expressing collagen-like genes, which became the predominant myeloid cell type after infection. **Conclusions**. In conclusion, this work uncovers novel features relevant to viral disease tolerance in bats, lays a foundation for future *in vivo* and *in vitro* experimental investigations, and serves as a key resource for comparative immunology studies across bats and other mammals.

2. Virome analysis of both bats and ectoparasites of bats captured in Campo Grande, Brazil

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Introduction: Bats are a source of zoonotic spillover for many human viral pathogens including filoviruses and coronaviruses. In order to thwart and better prepare for future outbreaks of disease, monitoring and identification of known and novel viruses in bat species is critical. **Methods:** Ten different species of bats were captured from two different sites within Campo Grande, Brazil. Ectoparasites as well as oral and fecal swabs were collected from the captured bat species. DNA was isolated from ectoparasites and DNA and RNA were isolated from oral and fecal swabs and processed for metagenomic sequencing. Resulting next-generation sequencing reads were processed through two separate bioinformatics pipelines to investigate the virome of these samples. An in-house pipeline, MetaDetector, cleans reads, filters out host contaminants, and performs *de novo* assembly followed by blastx analysis of assembled contigs and reads for taxonomy classification. A secondary analysis tool called VirusSeeker 2.0., an extended version of publicly available VirusSeeker, was also utilized with an updated viral database, to perform a viral-centric analysis similar to MetaDetector. **Results:** DNA sequencing of ectoparasites includes a large number of hits to various cycloviruses, betaretroviruses, and circoviruses. DNA and RNA-sequencing of oral and fecal swabs showed a large number of hits to betaretroviruses. Of special interest is an oral swab from a white-lined broad-nosed bat, *Platyrrhinus lineatus,* in which both MetaDetector and VirusSeeker 2.0 detected reads and assembled contigs that aligned with the family *Coronaviridae.* Further analysis of these contigs showed similarity to other bat betacoronaviruses. **Conclusions:** Sequencing of the virome of ectoparasites of bats as well as bats hosts themselves allows for identification of known and novel viruses. This data provides a glimpse of viral species circulating in the bat population in Central-West Brazil.

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3. Antibody Validation for Immunophenotyping T and B Cells of the Jamaican Fruit Bat

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Introduction: Bats are reservoir hosts for viruses that are of concern to humans and livestock, including, henipaviruses, filoviruses, coronaviruses, and lyssaviruses. Other pathogens are of concern directly to bats. Foundational immunology based on humans and rodents only allows us to assume bat immune systems and responses are similar, even though clear differences exist between well understood species and bats. Jamaican fruit bats (*Artibeus jamaicensis*) represent an underdeveloped animal which gives rise the inherent scarcity of bat specific reagents, such as antibodies. Limited availability of antibodies make the use of high throughput flow cytometry, cell sorting, and data from fluorescent microscopy limited. **Methods.** In this study, commercially available antibody clones against CD3e and CD19 were tested for cross-reactivity with Jamaican fruit bat immune cells. The potential for cross-reactivity was identified by pre-screening protein homology *in silico*, then screened by flow cytometry and florescent microscopy, and validated by cell sorting and RT-qPCR. Additionally, we generated hybridomas against two Jamaican fruit bat peptide epitopes, CD3g and CD4. This was done by immunizing BALB/c mice with Jamaican fruit bat KLH-CD3g peptide, and KLH-CD4 peptide, fusion of splenocytes to Sp2/10 Ag14 cells, hybridoma selection and cloning, and screening of CD3g/CD4 reactive clones by flow cytometry. **Results.** CD3e (clone Hit3a), CD19 (Clone 1D3), and CD3g (Clone X-E2) demonstrate reactivity for flow cytometry, and immunofluorescent microscopy. Jamaican fruit bat splenocyte CD3e+, CD3g+, CD3e+ CD3g+, and CD19+ populations were successfully enriched by cell sorting. Gene enrichment for the respective proteins of interest, CD3e, CD3g, CD19, was also observed in the RNA isolated from these cell sorted populations by RT-qPCR. **Conclusion.** Characterization of bat immune responses using flow cytometry, cell sorting, and fluorescent microscopy will provide valuable insight into the Jamaican fruit bat immune system. These reagents will provide important tools for Jamaican fruit bat T and B cells, and likely other bat species, respond to infections, and therefore the selection pressures placed on bat-borne viruses driving spill-over events in human and livestock populations. Furthermore, insights into the bat immune systems will provided aid to bolster current and future conservation efforts.

4. Does wing damage from white-nose syndrome predict other health measures?

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Introduction. White nose syndrome can be fatal to bats, but longer-term effects on health are not as well-understood. Wing damage index has been used as a surveillance method to monitor the health of bats, but it is unclear how damage from a past fungal infection might affect or predict future health. **Methods**. We mist netted for Indiana bat (*Myotis sodalis*), big brown bat (*Eptesicus fuscus*), evening bat (*Nycticeius humeralis*), and eastern red bat (*Lasiurus borealis*) across multiple sites. Working on four bat species in northeastern Missouri (USA) from late May-August (2021) we measured wing damage using an index (0-2) and also several health measures. These measures included ectoparasite load (the majority of which were mites), fecal egg counts (nematodes, etc.), neutrophil to lymphocyte ratio (a widely-used indicator of infection), and hair cortisol (a longer-term indication hair follicle exposure to cortisol). **Results**. Combining all species, we found some significant relationships between wing damage index and other health measures. For example, bats with higher wing damage had lower ectoparasite loads (Anova $F_{2,214} = 4.68$, $p = 0.01$). **Conclusion**. Several mechanisms might contribute to this pattern. High wing damage might 1) impede mite attachment or mite feeding success, 2) alter bat behavior resulting in reduced mite transmission, or 3) it reflects upregulated immune system components due to the WNS infection. Relationships with the other health measures will also be presented.

5. Injectable, reversible anesthesia in captive Jamaican fruit bats (*Artibeus jamaicensis*)

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Introduction. This study was conducted to determine safe, effective injectable protocols for microbats, using Jamaican fruit bats (*Artibeus jamaicensis*). Inhalant anesthesia is commonly used for minimally invasive procedures (blood and other sampling for infectious disease studies). Injectable agents offer several advantages over inhalants. Injectable drugs are more portable and may be more available in some areas/countries. Injectable drugs are reliable under extreme ambient temperatures. Injectable equipment and supplies are generally less expensive than inhalants. An advantage of injectable anesthetic agents for disease transmission studies is that they may minimize unwanted side effects on non-target species. Arthropods succumb to the effects of inhalants provided via induction chamber and so studies must often rely on mask induction and maintenance of anesthesia, which exposes workers unnecessarily. **Methods.** Bats were weighed, and doses of drugs drawn up accordingly. Drugs (Alfaxalone/Dexmedetomidine, "A/D" and Ketamine/Dexmedetomidine, "K/D") were injected subcutaneously between the scapulae. Measurements and observations included: heart rate, respiratory rate, oxygen saturation, temperature, time to anesthesia, response to noxious stimulus, time to arousal after reversal agent was given, and time to flight. **Results**. Time to anesthesia was similar for both groups (mean +/- SD: A/D 3.3 +/- 2.7 min; K/D 5.3 +/- 3.1 min). The A/D group had a slower time to arousal after given the reversal (9.5 min vs 6.1 min), however time to full flight was shorter in the A/D group (28 min vs 46 min). Bats anesthetized with isoflurane were fully flighted after 16 min. All bats recovered from the anesthetic events and were flighted within 90 minutes of reversing the dexmedetomidine. Both combinations provided adequate anesthesia for minimally invasive procedures for the duration of the event. The A/D combination was more reliable; about half of the bats given the K/D combination did not become anesthetized. **Conclusions**. The combinations of injectable drugs

used in this study are safe and effective and offer an alternative to gas anesthesia. Rapid recovery and post-anesthesia flight are important components of bat anesthesia, especially in field settings, where return to natural behavior and activity is critical.

6. Measuring affinity maturation and somatic hypermutation in Jamaican fruit bats

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Introduction. While indirect evidence suggests bats undergo limited somatic hypermutation (SHM) and affinity maturation, this hypothesis has not been directly tested. **Methods**. We immunized and repeatedly boosted *Artibeus jamaicensis* bats with a T-dependent antigen (*KLH in IFA, SC, N = 8*) or a T-independent antigen (*NP-Ficoll in sterile saline, IP, N = 7*). The titer and affinity of serum IgG antibodies were measured weekly for 43 days after the initial immunization. Affinity was measured by assessing the antigen:antibody bond strength using a chaotropic agent, ammonium thiocyanate. Bats were sacrificed on day 43 and RNA from spleen single cell suspensions was harvested. We used 5' Rapid Amplification of cDNA Ends (5' RACE) to amplify B cell receptor genes. BCR RNA transcripts were sequenced on a Nanopore platform. **Results**. Post boosting, we were not able to detect any change in the affinity of serum anti-KLH antibodies from 5/7 bats. We could measure increased affinity in the serum anti KLH antibodies from 2/7 bats. By sequencing the IgG BCR mRNA transcripts, we found the CDR2 regions had higher frequencies of non-synonymous mutations than the framework regions. **Conclusions**. Artibeus bats have genetic (BCR sequencing results) and physical evidence (antigen binding strength) of SHM and affinity maturation. However, we could not detect affinity maturation in most bats when immunized and boosted with a T-dependent antigen. We have yet to determine what factors caused 2/7 bats to undergo affinity maturation during our experiment. While we tend to think of stickier antibodies as better, this may not always be the case. In humans and mice, memory B cells (MBC) secrete less sticky antibodies than long lived plasma cells (LLPCs). MBCs are thought to be better at responding to escape mutants than LLPCs. There may be an evolutionary advantage for bats to focus primarily on "MBC-like" antibodies.

7. Evolutionary conflicts between bats and diarrheal pathogens

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Introduction. 179 million people in the US suffer from infectious diarrhea every year, and deaths have increased four times since 1980. A major cause of infectious diarrhea in the US is enterotoxigenic *E. coli,* which produces heat-stable enterotoxins (ST) to overactivate host guanylyl-cyclase C (GC-C), a major regulator of fluid balance in the gut. STs cause diarrhea in the host, benefiting the bacteria by facilitating spread to new hosts. We previously identified the ST–GC-C interface as an ongoing evolutionary conflict between hosts and pathogens, where GC-C was undergoing positive selection in primates and especially bats. Bats also present compensatory evolution in the host ligand for GC-C, uroguanylin, maintaining its ability to activate GC-C despite the evolution of GC-C in response to the bacterial evolutionary pressure. **Methods**. We identified GC-C and uroguanylin sequences using gene models constructed with MAKER and identified coevolving residues with spider monkey. **Results**. We identified signals of co-evolution between residues in uroguanylin and GC-C and are analyzing the functional consequences of those residues in a GC-C model of infection. **Conclusions**. These findings demonstrate a rare example of co-evolution between a host ligand and receptor driven by selection from a pathogen outside the immune system and will expand our understanding of how diarrheal pathogens specialize to their host to cause disease.

8. Effect of diet on viral shedding in experimental infection of Jamaican fruit bats

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Introduction. Periods when bats shed viruses into the environment are often coincident with periods of nutritional stress. However, how periods of nutritional stress influence the immune system and its control of viral shedding is poorly understood. We hypothesize that periods of nutritional stress weaken bats' immune systems and make viral shedding more likely. To test this hypothesis, we provided Jamaican fruit bats (JFBs, *Artibeus jamaicensis*) with three unique diets for 21 days and then infected them with H18N11 influenza virus and monitored viral shedding while maintaining the bats on their diets. **Methods**. Jamaican fruit bats (JFBs, *Artibeus jamaicensis*) were given three different diets (fruit alone, fruit supplemented with fat, or fruit supplemented with protein) for three weeks before infection with H18N11 influenza A virus, a virus naturally hosted by *Artibeus* bats. We collected daily rectal swabs to measure viral RNA and cytokine expression for 20 days post infection. **Results**. JFBs in the high fat group had lower levels of H18N11 RNA (higher Ct values) and stopped shedding detectable viral RNA sooner than the other two groups. Bats provided fruit only had the highest levels of detectable viral RNA and shed viral RNA for the longest duration. JFBs in the high fat group had the lowest level of TNF expression in rectal swabs on day 3, while the fruit with protein supplement group had the highest levels of TNF expression. **Conclusions**. Diet is a major driver of H18N11 infection dynamics in JFBs. By manipulating bats' diets, we were able to have dramatic effects on the duration

and intensity of viral RNA detection in rectal swabs. However, the mechanism by which diet is impacting shedding remains unclear without more investigation. We found statistically significant differences in TNF expression in the three diet groups, but these patterns do not explain the viral shedding data. Nonetheless, if we expand this research beyond JFBs and find similar patterns, it may have important implications for how we manage spillover from bats.

9. Demonstration of the feasibility of on-site laboratory tools to study bat viruses

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Introduction. The importance of bats in the area of emerging infectious diseases is unquestionable as they harbour a notable diversity of viruses, some of them with known or suspected zoonotic potential. With technological advancements during the last decades now multiple tools are available such as rapid enzymes for molecular analysis, real-time PCR equipment without moving parts or portable next generation sequencing platforms. The field application of these tools on-site holds the potential to extend our knowledge and priorities on-site during sampling events and facilitate the collection of high-quality samples for additional experiments (such as virus isolation). Nipah virus (NiV) and a neglected member of the *Paramyxoviridae* family, the Lloviu virus (LLOV) are relevant emerging viruses with known or in the case of LLOV, suspected zoonotic potential. Performing these experiments on-site allows to implement multiple interventions through rapid data generation and most importantly opens the possibility for high-quality, targeted sampling of positive animals. **Methods.** We implemented field sampling with the support of field laboratory equipment in the case of Lloviu virus and *Miniopterus schreibersii* bats (Hungary) and Nipah virus in *Pteropus* bats (Bangladesh. We used the blood samples of *Miniopterus* bats, and the colony sampled urine of *Pteropus* bats and performed qRT-PCR on-the-spot to identify positive animals or colonies. We supplemented this activity with re-sampling and Nanopore-based complete viral genomic sequencing capacities. Novel amplicon-based Nanopore sequencing methods were established for LLOV and NiV. **Results** We were able to detect LLOV and NiV RNA in the field within 1-3 hours of samples taken. Passive surveillance was suitable to determine the NiV affected colonies, opening the possibility for interventions (such as banning of palm-sap consumption in the area). The LLOV on site surveillance and resampling of live bats resulted successful in vitro isolation as we were able to re-sample positive animals. **Conclusions.** Our protocol is a suitable tool which can support multipurpose (detection, isolation, serological, genomic) research studies. Field laboratory practices and on-site surveillance techniques could advance in the understanding of the natural circulation of bat viruses and fasten research efforts due to sequence and ecological data. In addition, this concept has multiple possibilities for intervention activities to reduce spillover risks.

10. Detection of whole genome astrovirus sequence in Madagascar fruit bats

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Introduction. The COVID-19 spillover and resulting global pandemic highlight the need for a comprehensive surveillance of viruses maintained in bat populations. This is particularly true of fruit bats in Madagascar, where widespread hunting and consumption by humans increases the risk of zoonotic spillover. Astroviruses (family *Astroviridae)* cause acute gastroenteritis, and exhibit a history of frequent cross-species transmission events, yet remain understudied in Malagasy fruit bats. **Methods.** We carried out Metagenomic Next Generation Sequencing on fecal, urine, and throat samples from three species of endemic Malagasy fruit bats (*Pteropus rufus, Eidolon dupreanum,* and *Rousettus madagascariensis*) collected as part of a long-term longitudinal study. We utilized BLAST to search for putative astrovirus sequences, and performed phylogenetic analysis on resulting hits. **Results.** We detected both whole and partial astrovirus genome sequences from fecal samples of *E. dupreanum* and *R. madagascariensi.* In addition, we found surprising evolutionary relationships between these sequences and previously described astroviruses from the African continent. **Conclusions**. The detection of astroviruses indicates that the bats commonly consumed in Madagascar may be reservoirs of a large diversity of astroviruses and thus pose substantial spillover risk. The comparison of genetic relatedness between these sequences and sequences from the African continent shows both surprising similarity and divergence, suggesting the evolutionary history of these viruses in Madagascar is more complex than previously understood.

11. Insights from whole genome sequences of Madagascar bat viruses

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Introduction. Recent advances in bat virus research and the COVID-19 pandemic have led to more pronounced efforts to undertake comprehensive studies encompassing both the ecology and molecular biology of novel pathogens. Our research focus incorporates longitudinal sampling efforts and molecular techniques to take a One Health approach to bat virus discovery. **Methods**. Metagenomic Next Generation Sequencing (mNGS) was carried out on fecal, urine, and throat samples collected from endemic Malagasy fruit bats (*Pteropus rufus*, *Eidolon dupreanum*, and *Rousettus madagascariensis*). From these, coronavirus and henipavirus hits were identified in Chan Zuckerberg ID (CZID) that showed significant nucleotide or protein BLAST alignment to coronaviruses or henipaviruses present in NCBI databases. Phylogenies of full sequences and proteins of interest were constructed in concert with bootscan recombination analyses. **Results**. We describe three novel *Nobecovirus* sequences (two from *R. madagascariensis* and one from *P. rufus*) and one novel henipavirus (named Angavokely virus) from *Eidolon dupreanum.* **Conclusions**. These sequences define unique clades that are ancestral to all currently described Nobecoviruses and bat henipaviruses, lending to interesting ecological questions about the timing of the divergence of Madagascar's endemic fauna and their related viruses. We are using these genomic insights to develop more accurate ecological models of bat virus dynamics and population-level persistence. In turn, these models will inform public health efforts to develop more focused zoonotic disease surveillance and even, possibly, wildlife vaccines. We anticipate continued discovery of more divergent bat viruses from the isolated Madagascar ecosystem, which we will continue to study holistically, using the tools of dynamical modeling, antibody and PCR assays, and virus isolation.

12. Batabase: a publicly-available relational database of species-specific physiological bat data

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As technology advances, more tools are created to capture, store, analyze, and report data. Currently, the estimated global value of electronic medical health records (EHR) and associated works is $23.94 billion dollars, and predicted to grow to $101 billion dollars by 2031. Companion and food animal medicine are increasingly using EHR systems as well as other databases to track animal disease and gauge public health risk. While in the early stages of leveraging database technology, wildlife and exotic veterinary medicine also use these data platforms. However, the field of wildlife medicine differs greatly from human and companion/food animal medicine because for most wildlife species, reference intervals for physiological parameters have not been defined. Further, most wildlife databases are structured around morbidity and mortality data, resulting in insufficient data for healthy wildlife. To address this, Bat Health Foundation (BHF) is developing Batabase, a publicly-available relational database serving as a comprehensive data repository for physiological parameters. Batabase will be populated by scrubbing literature, requesting contributions from researchers and collaborators within our network (ensuring proper attribution), and generating data via BHF's own research efforts. We predict that structuring bat-related data in a relational schema will facilitate queries of 3 over-arching types: 1) queries that aid scientists in their research efforts by providing baseline parameters/reference intervals for comparison to their own data to rapidly identify deviations, 2) queries that allow for testing of hypotheses restricted to data within Batabase, and 3) queries that guide research efforts by identifying target populations for which knowledge gaps exist. Here we demonstrate the utility of a centralized hub of physiologic bat data in identifying correlations and discrepancies across taxa and geographic regions—an important step in ensuring the scientific community's ability to promptly identify threatened or unhealthy populations.

13. Serologic evidence of marburgviruses in Madagascan rousettes

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Introduction. Marburgviruses are the causative agents of Marburg virus disease (MVD), a viral hemorrhagic fever disease with a high case fatality in humans and non-human primates. Marburg virus (MARV) and Ravn virus (RAVV) are distinct lineages within the Marburg marburgvirus species, both isolated from Egyptian fruit bats (Rousettus aegyptiacus). Outbreaks of MVD in Kenya, Uganda, Angola, Democratic Republic of Congo, and South Africa are associated with mining or cave-tourism where cave bats like the Egyptian fruit bat roost. These bats geographic distribution span East Africa, the southern tip of Africa, and West Africa. Recently, MARV was isolated from bats in Sierra Leone and a fatal case of MVD occurred in Guinea, despite no regional history of MARV outbreaks, highlighting gaps in our current knowledge of the geographic distribution of marburgviruses and at-risk areas for spillover. Madagascar contains a single species of rousette bat, Madagascan rousette (Rousettus madagascariensis). Here, we conducted serology-based biosurveillance to assess marburgviruses circulation in Madagascan rousettes. **Methods**. Serum samples were collected from 579 Madagascan rousettes and tested by a multiplex microsphere-based immunoassay for immunoglobulin (Ig) G reactivity against soluble envelope glycoprotein (GP) ectodomain trimers of RAVV, Ebola, Bundibugyo, Bombali, Sudan, Reston, Mengla, and Lloviu virus. Antigen-antibody complexes were detected via Luminex xMAP-based technologies, and IgG levels were reported as a median fluorescence intensity. We applied a three-sigma-rule (99.7%) probability distribution of naïve Egyptian fruit bat sera and latent cluster

analysis of field-collected Madagascan rousettes sera to identify IgG positivity threshold cutoffs. **Results**. We detected RAVV IgG-binding antibodies in 15.9% (92/579) serum samples above both threshold cutoffs. Another 69 samples had anti-RAVV IgG levels that were between our two thresholds, thus statistically indeterminate as negative or positive. **Conclusions**. This serological profile of Madagascan rousette bats provides the first evidence to our knowledge that marburgviruses are maintained and circulate in Madagascar. More broadly, the serological footprints of RAVV in Rousettus species native to Madagascar support the current understanding of rousette bats as reservoirs for marburgviruses. Future research is necessary to determine possible evidence of Marburgvirus chatter between bats and other wildlife or domestic animals, and spillover risk to humans.

14. Wing tattoos: A cost-effective and permanent method for marking bats

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Introduction. While the monitoring of animals over extended periods may provide valuable information about their ecology and behaviour, it has also become important for virological studies in wildlife. Longitudinal monitoring of viral pathogens in wildlife can provide insight into their maintenance within populations and information on the host-virus dynamics. However, it requires animals to be marked to allow resampling upon recapture, which have posed a problem for some taxa. Marking methods have been proposed and successfully used for numerous species, and yet a reliable, inexpensive method has not been found for bats with methods used thus far causing injury, and, in some cases, lead to death. Given the association of bats with zoonotic viruses, mark-recapture is becoming increasingly important as many questions regarding viral maintenance remains unanswered. **Methods**. Our research proposes wing tattoos as an alternative method to mark bats, as this has been extensively used to mark small mammals in both laboratory and field conditions. Initially tattoo equipment from the Animal Identification and Marking System (AIMS™) was used, but we adapted this and bought commercially available tattoo equipment, for human use, to set up our own tattoo system. **Results**. In a captive population of *Rousettus aegyptiacus*, wing tattoos were shown to have no negative effect on the animals, with no deaths and no infections, and remained legible over 927 days. We captured and tattooed 7 725 bats from 12 species across four years in the wild at three locations in South Africa. Of these animals a total of 435 were recaptured with one individual captured 2 465 days after the initial tattoo, indicating the longevity of this method. **Conclusions**. Wing tattoos offer a non-invasive, cost-effective and permanent method to mark bats and monitor populations over time and space.

15. Maintenance and Excretion Dynamics of paramyxo- and coronaviruses within South African Frugivorous Bat Populations

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Introduction: Potentially zoonotic viruses include members of the paramyxo- and coronavirus families. However, detection in bat species does not constitute a spillover risk, and several factors influence this. We examine how interdisciplinary longitudinal biosurveillance is essential to understanding infection dynamics in a maternal colony of the Egyptian Rousette bat (*Rousettus aegyptiacus*) in South Africa. **Methods**: Non-destructive sampling of bat urine and fecal started in 2012. Excretory samples from monthly collections were tested for viral RNA using reverse transcriptase PCR targeting a partial conserved viral genome region. Positive samples were characterised by DNA sequencing and subsequent Bayesian phylogenetics. Data on the bat population size, composition, age class, sex and reproductive condition were collected. Tattooing and pit tags were used to mark and identify individuals and environmental data were captured. **Results**: There were three different coronavirus genetic lineages with non-identical temporal excretion dynamics. The study identified near-constant shedding from the colony throughout the year, with significant excretion peaks during specific times (up to 58% positivity) that coincided with increased infections of young bats following the waning of maternal antibodies. Among adults, higher detection frequency among reproductively active female bats was correlated to lower body conditions, suggesting a metabolic cost or higher infection susceptibility in pregnant and lactating females. Phylogenetic analysis of paramyxoviruses indicated 18 putative *Henipavirus* and genus-related viral species. The winter and spring periods were at increased risk for virus spillover and transmission. Additionally, annual peaks in viral excretion are again likely driven by subadults and might be linked to the waning of maternal immunity and recolonisation of the roost. Analysis of recaptured bats suggests that viral clearance may occur within one month. **Conclusions**: Given that longitudinal excretion studies of bat-borne pathogens are limited, and these findings provide insight into this bat-virus relationship which can be extrapolated to other countries across the geographical distribution of the Egyptian rousette bat. These findings assist in our understanding of viral persistence and the identification of higher-risk periods associated with viral circulation in a colony that may be useful in developing risk reduction strategies for potential zoonotic transmission.

16. Investigating the innate immune response of bat respiratory epithelium during influenza virus infection

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Introduction. Bats are the second most speciose group of mammals after rodents and are an important reservoir host for emerging and re-emerging viruses that pose a serious risk to both human and animal health. Despite this importance, our current understanding of virus – host interactions in bats, and in particular our knowledge of the interferon (IFN) system in bats, remains rudimentary. While much progress has been made in recent years, most of the underlying viral and host determinants affecting the successful establishment and immune control of viral infections in bats remain elusive. Herein, a major hurdle is the lack of an appropriate unified biologically relevant model system and molecular tools to study virus – host interactions in bats that can be directly compared to analogous models of other mammals. **Methods**. To overcome this limitation, we have established a primary well-differentiated bat airway epithelial cell (AEC) culture model from a neotropical bat *Sturnira lilium* that is analogous to the human AEC culture model. **Results**. Following the establishment, we show that these bat AEC cultures are responsive to exogenous recombinant Type III interferon and poly-I:C, but not to Type I IFN. More importantly, using an Orthomyxovirus with a broad host-tropism, influenza D virus, we observed distinct viral replication kinetics at ambient temperatures observed during torpor (32°C), normal (37°C), or flight (39°C), as well as a temperature-dependent sensitivity to exogenous interferon pretreatment. **Conclusions**. Combined these preliminary results demonstrate that the bat AEC model is a viable surrogate model to investigate fundamental virus-host interactions during respiratory virus infection in bat respiratory epithelium. That will increase our understanding on what makes bats 'special' as reservoir hosts for emerging viruses.

17. Preliminary observations on ranging patterns of two rhinolophid bats in the Mt. Elgon area.

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Introduction. While bats are known reservoirs of zoonotic pathogens, knowledge gaps still remain on their interactions with the environment. It is mainly through such interactions that spillover events to humans could take place and as such, it is necessary to have a concrete understanding of a number of aspects of their ecology, such as ranging patterns in order to predict and curb the occurrence of potential spillover events. **Methods**. We present preliminary observations on ranging patterns for two *Rhinolophid* bats in the Mt Elgon foothills area of Eastern Uganda. The bats were tagged with GPS units which were set to periodically record coordinates in the areas they foraged through. The tagged bats were recaptured and the GPS data downloaded. We then physically visited these locations to assess the nature of the habitats and determine what could have attracted the bats to them. **Results**. Looking through the human eye, the locations visited by the bats do not seem to have critical landscape features to draw bats to them, however, there were gardens, tree stands, and a river flowing through some locations that could hold the secret to their ranging. The fact that such small-sized cave-dwelling bat species could fly a distance of up to 16 km in one night shows the potential such species could have in the dispersal of viral pathogens. **Conclusions**. These observations are still very preliminary and we need more time to come up with more concrete conclusions.

18. Investigating the evolution of bat immune transcriptomes by short and long-read RNA-seq

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Introduction: Bats are a major reservoir of zoonotic viruses, including Rabies viruses, Dengue viruses, Ebolaviruses, and Coronaviruses. For this reason, there is a growing interest in characterizing bat-specific adaptations that affect innate immunity and inflammation. Recent studies have identified that key immune genes, such as interferon (IFN) and interferon stimulated genes (ISGs), have unique expression patterns and differential functions in bats. Nonetheless, bat-specific immune adaptations remain largely uncharacterized and we have a poor understanding of the genetic mechanisms behind these changes. Therefore, in this project we propose to study the immune transcriptomes of two bat species, the little brown bat (*Myotis lucifugus)* and the Jamaican fruit-eating bat (*Artibeus jamaicensis)*, in response to interferon stimulus. We are particularly interested in the potential role of transposable elements, which are a source of genetic novelty that shapes immune evolution in other mammals. **Methods:** *Myotis* primary fibroblast and *Artibeus* primary kidney cell lines were treated with recombinant universal type I IFN. Cells were collected at different time points of treatment and messenger RNA (mRNA) was extracted for short-read (Illumina) and long-read (Nanopore MinION) sequencing. Following transcriptome assembly, bat-specific isoforms of immune genes were identified and differential gene expression between

treated and controlled samples was carried out for all transcripts at the different time points. **Results:** We have found extensive evidence of bat-specific isoforms of immune genes, including NLRC5 and IFITM2 in *Myotis*. Interestingly, we found that transposable elements are a source of new exons and regulatory regions for some of these bat-specific transcripts, which points to a potential genetic mechanism behind these changes. **Conclusions:** Evolution at the transcriptomic level, comprised of novel gene isoforms and unique genes expression patterns, has contributed to the distinctive immune response in bats. Additionally, we found that one of the genetic mechanisms that potentially explains these changes are transposable elements capable of re-wiring gene expression or creating new gene isoforms through exonization.

19. Assessing the extent of land-use change around important bat-inhabited caves

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Introduction. Bats are important zoonotic transmission vectors, and the destruction of natural habitats is bringing them into closer contact with humans than before. Caves and cave-dwelling bats are under-represented in conservation plans. In South Africa, at least two cavernicolous species are of interest as potential zoonotic hosts: the Natal long-fingered bat *Miniopterus natalensis* and the Egyptian fruit bat *Rousettus aegyptiacus*. Both bats are numerous and widespread throughout the country; land-use changes and urban expansions are a rising concern for both conservation and increased bat-human contact, yet little information is available about the anthropogenic pressures these species face around important roost sites. **Methods**. Our study addressed this shortfall by determining the extent of land-cover change around 47 roosts between 2014 and 2018 using existing land cover datasets. We determined the land-cover composition around important roosts (including maternity, hibernacula and co-roosts), distances to urban settlements and assessed the current protection levels of roost localities. **Results**. We detected an overall 4% decrease in natural woody vegetation (trees) within 5km buffer zones of all roosts, with a 10% decrease detected at co-roosts alone. Agricultural land cover increased the most near roosts, followed by plantations and urban land-cover. Overall, roosts were located an average 4.15 ± 0.91 km from urban settlements in 2018, the distances decreasing as urban areas expand. Concerningly, the majority (72%) of roosts were located outside of well-protected ecosystems. **Conclusions.** The current lack of regulatory protection of cavernicolous bats and their roosts, increasing anthropogenic expansions and proximity to human settlements raises concerns about increased human-bat contact. Furthermore, uncontrolled roost visitation and vandalism are increasing, contributing to bat health risks and population declines, though the extent of roosts affected is yet to be quantified. In an era where pandemics are predicted to become more frequent and severe due to land-use change, our research is an urgent call for the formal protection of bat-inhabited caves to safeguard both bats and humans.

20. Establishment of a captive cave nectar bat (*Eonycteris spelaea*) breeding colony in Singapore.

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Introduction. Bats are special due to their capacity to host highly pathogenic zoonotic viruses, making them the ideal animal model for studying the regulation of infection, cancer, and longevity. A key factor that limits bat research is lack of breeding bat colonies. To address this need, a captive bat colony was established in Singapore with specialized diet, cages and husbandry practices. Conducting bat in vitro studies requires a continuous need to collect samples. To avoid the challenges when sampling wild bats such as unknown pathogen exposure history and disease status, we established a breeding colony of *Eonycteris spelaea* in Singapore for research. **Methods.** Regulatory approval for study was granted by authorities. Bats were captured, inspected and screened for known viruses. The facility features an anteroom, an ABSL-1 lab and a naturally ventilated animal holding area. Customized cages and diet were fabricated and formulated to house bats safely with enrichment. Bats were inspected daily and quarterly for pregnant and newborn bats. Bat's natural diet was replicated with fresh fruits, water, milk powder, pollen and commercial Wombaroo diet. **Results.** *Founding Population*. 19 bats were screened and introduced into facility and screen negative for rabies virus, coronavirus, filovirus and Nipah virus. A net weight gain was detected for both female and male bats after 9-24 months. Bats appear alert and responsive during handling and showed no signs of stress or injury. *Pregnancy.* ES 16 was found 9 months after introduction with weight gain and enlarged abdomen. Subsequently, 3 other female bats were found to have pups latching on 13 months introduction. Pregnant bats were more aggressive during handling while bats closer to parturition were more docile. A total of 35 bats were born and microchipped between May 2017 and December 2018. *Diet.* The final formula consists of Wombaroo Lorikeet and Honeyeater Food, saltwater, water, milk powder, pollen and fresh fruits. Formulation was palatable to bats as little leftover was recorded. **Conclusions.** A few factors are importing in establishing an *E. spelaea* breeding colony. These challenges include creating housing conditions that allowed the bats to breed, roost and feed. Our cages allow cages to be linked together to accommodate colony expansion. No injuries were detected indicating cage design is not detrimental to safety of juvenile and adult bats. Another challenge is to formulate a suitable diet to provide adequate nutrients for bats as there are no commercial diet for bats. Wombaroo diet contains the required nutrients for all nectar-eating birds and designed to recreate the texture and nutritional contents of nectar. Generally, fruit eating bats are polyestrous, generating 2 offspring per year. Our colony showed signs of pregnancy after 9 to 15 months in colony. A healthy fur coat and responsiveness were

used as markers of good health of bats. No signs of injury, joint inflammation or drastic loss of weight were seen. In the 16-month period from introduction of wild bats to the birth of new pups, no sign of trauma was observed in adult bats or pups. Taken together, the artificial housing and diet created a conducive environment for the bats to thrive. While hurdles still exist in bat research, this breeding colony represents a step forward in understand bat biology. This study concludes the successful establish of the first *E.spelaea* breeding colony in Asia. Bats produced in the colony and their samples are currently being used in ongoing studies.

21. Bat-borne viruses from an anthrozoological lens and community-based surveillance capacities in Mount Elgon, Uganda

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Introduction. In mountain areas like Elgon region, bat-borne virus disease outbreaks like Marburg have been reported threatening livelihoods within the area and at national scales. Occurrence of the Marburg virus disease outbreak was the first to be reported in the area. Small scale farming is the main source of livelihood within this area and has resulted into increased degradation of natural habitats that are critical in sustaining bat populations. Understanding of how humans interact with bats within such areas would be crucial in defining the human-bat interface. Unfortunately this is less explored within the areas. Additionally, surveillance capacities within such communities to detect and respond to bat borne viruses is less understood. **Methods**. This study sought to explore the surveillance capacities of communities as well as document practices and perceptions that influence human-bat interactions within Mount Elgon. Key informant interviews as well as Focus Group Discussions were conducted using Semi structured interview questionnaires. Key informant interviews were done with local chiefs at village level documenting practices around caves and household level that would increase human-bat interactions. Focus Group Discussions were held with village health team members documenting their knowledge in case definitions of Ebola and Marburg, their reporting and documentation abilities as well as platforms for communication to the communities. **Results**. Communities utilized caves as shelter during rainy days and as well used as shelter for themselves and their animals. They majorly did this during rainy seasons and some of them hunted bats for consumption and as a spot activity. The village health teams had less knowledge on the case definitions of the different bat-borne viruses and they had less capacities in using mobile phones for reporting. They also did not engage in any conservation related activities that would support bat populations. **Conclusions**. Community engagement in hard to reach areas ought to be strengthened to enhance potential spillover events of bat borne viruses. This should target bat conservation as well as surveillance for bat-borne viruses.

22. Serologic evidence of human exposure to known and unknown henipaviruses, Cambodia

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Introduction. Emerging zoonotic viruses in the genus *Henipavirus* (family *Paramyxoviridae*) can cause severe and often fatal disease in livestock and humans and are listed in the WHO priority pathogen blueprint. Nipah virus (NiV), the prototypical henipavirus, has been isolated from flying foxes (genus *Pteropus*) in the Battambang province of western Cambodia, and surveys suggest bat foraging behaviors occur near human settlements. Despite this, there have been no documented human cases of NiV infection in Cambodia. **Methods**. Convalescent serum samples from patients with undiagnosed febrile illness from 10 provinces in Cambodia were screened for antibodies reactive with the receptor binding proteins (RBP) of four henipaviruses and paired acute samples from the convalescent positives were screened for IgG reactivity. **Results**. Cedar virus (CedV) specific antibodies were detected in three convalescent samples and further were screened for neutralizing antibodies. Of which, one serum sample was able to neutralize recombinant CedV. Additionally, forty convalescent serum samples reacted with Ghana virus RBP, and twenty-three paired acute samples demonstrated similar reactivity. We did not find an association between provinces with flying fox roosts and serological prevalence. **Conclusions**. Here, we have demonstrated evidence of cryptic henipavirus infections in humans in Cambodia, with a majority of the samples demonstrating reactivity with Ghana virus, suggesting infection with a yet-described ancestral henipavirus that shares antigenic-similarity with Ghana virus. Lastly, we observed exposure to a CedV-like virus that can use human ephrin-B2 as an entry mechanism.

23. Characterization of *Eptesicus fuscus* splenic immune cell landscape following rabies infection

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Introduction. Big brown bats (*Eptesicus fuscus*) are the North American bat species most frequently associated with rabies. Big brown bats are also one of the most widely distributed bat species and therefore play a pivotal role in the sylvatic maintenance of the single rabies virus, RABV, that circulates in North America. Serological and experimental evidence suggest that, like the common vampire bat (*Desmodus rotundus*), *E. fuscus* have some inherent resistance to rabies infection. The host-pathogen interactions that lead to this resistance remain unclear and how host response affects transmission dynamics are unknown. Despite the evidence of novel host-pathogen dynamics and the role this species plays in endemic RABV in North America, there is a lack of basic knowledge of the *E. fuscus* immune system, especially the adaptive immune system. **Methods**. In this study, we use 10x single cell transcriptomics to characterize the splenic immune cell composition of *E. fuscus* individuals after surviving rabies vaccination and infection. We also use this approach to develop species-specific tools to characterize the B-cell receptor (BCR) and T-cell receptor (TCR) repertoires of individual bats. **Conclusions**. These data and tools will lay the foundation for a more in-depth characterization of the adaptive immune responses to rabies infection and vaccination in this species with the goal of understanding the mechanisms of RABV resistance, informing transmission dynamics and effective vaccinate strategies.

24. The role of Indian flying foxes (*Pteropus medius*) in propagating antimicrobial resistance in the environment

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Introduction. Climate change induced extreme weather has created conservation challenge for *Pteropus* species in many parts of the Old World. Extreme heat can affect thermoregulatory behavior of fruit bats and influence their gut microbial fauna. However, the role of extreme heat as a driver of Antimicrobial Resistant exposure through change in thermoregulatory behaviors is unknown. It is also hypothesized that gut microbiota of flying foxes modulates their defense mechanisms in response to heat stress that also renders them resistant to antibiotics. **Methods.** We collected noninvasive fecal samples (n = 1150) of Indian flying foxes over a period of one year from seven different roosting sites, in the central and northern areas of Pakistan. First, we isolated *E. coli* using biochemical tests and performed Antimicrobial Sensitivity Testing on selected *E. coli*, using the Kirby-Bauer Disk Diffusion method. to check their resistance profile against 12 different antibiotics. Second, *E. coli* were further tested using Double Disk Diffusion test and Double Disk Synergy test of identify any ESBL *E. coli*, an indicator of Antimicrobial Resistance acquired from environment. Similarly, rifampicin was included in the tested antibiotic to test rifampicin resistant *E.coli* mutation at codon 572 of rpoB gene, as an indicator of heat stress induced Antimicrobial Resistance. PCR will also be performed to identify ESBL *E.coli* producing *bla*CTX-M, *bla*SHV, *bla*TEM genes. **Results.** To date, we have isolated *E. coli* (120/230 (52%)) form fecal samples collected during the summer months samples. The range of antimicrobial resistance shown by *E. coli* isolates was 0–90%. All the *E. coli* isolates recovered during the study showed 90% resistance to rifampicin (class Ansamycin). Other *E. coli* resistance profiles include Cephradine (Cephalosporin) 30%, Oxytetracycline (Tetracycline) 32.5% and Ampicillin (Penicillin) 25%. The overall (46%) multidrug resistant *E.coli* were detected in the fecal samples. We also noted one possible XDR *E. coli* isolate in processed isolates. **Conclusions.** Detection of high levels of multidrug resistant *E. coli* in fecal samples from Indian flying foxes is a serious One Health concern. Knowledge about the internal and external drivers of antimicrobial resistance propagation from fruit bats will provide foundation for environmental antimicrobial stewardship, as a One Health priority.

25. Isolation of antimicrobial resistant *Salmonella* from Indian flying foxes (*Pteropus medius*) in Pakistan.

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Introduction. Pakistan is experiencing outbreaks of extensively drug-resistant (XDR) *Salmonella enterica* serotype Typhi. This food borne pathogen of One Health importance is developing resistance against last resort antibiotics such as the β-lactam family of antibiotics, causing the current outbreaks. XDR *S. enterica* can spread to frugivorous bats through contaminated wastewater as these bats tend to live in proximity to humans and livestock. Subsequently, fruit bats can act as a source of this enteric pathogen to people and livestock after being infected. No research work has been done to investigate the role of Indian flying foxes in *Salmonella* transmission and their susceptibility to antibiotics. **Methods.** In total 233 fecal samples were collected non-invasively from Indian flying foxes. Seven roosting sites with (N=3) and without (N=3) proximity to running water bodies were sampled between June 2021 and August 2021, to examine the impact of running water on *Salmonella* isolation and resulting antimicrobial resistance in fruit bat feces. Suspected *Salmonella* colonies were identified and confirmed using biochemical tests and PCR for *invA* gene. The antimicrobial susceptibility of *Salmonella* isolates against 12 different antibiotics (from 8 different antimicrobial groups) was performed using Kirby-Bauer Disk Diffusion method. **Results.** In total 26 (11.15%) *Salmonella* isolates were recovered from 233 fecal samples. *Salmonella* isolates recovered during the study showed resistance to Imipenem (Carbapenems) 60% and Cephradine (Cephalosporin) 30%,

Salmonella Isolates also showed resistance against Azithromycin (Macrolides) 20%. Relative humidity in the environment was a significant predictor (p = 0.08) in terms of salmonella isolation, while environmental temperature was negatively correlated (r = -0.03). Presence of running water channel was not significant (p = 0.19) in terms of *Salmonella* isolation. The range of antimicrobial resistance shown by *Salmonella* isolates were 0–100%. A total of 30% of isolated *Salmonella* showed multi drug-resistance (MDR) during this study. **Conclusions.** The study indicated that the bats are the carriers of AMR *Salmonella* and may be responsible for the transmission to the environment and humans. Presence of high levels of MDR *Salmonella spp*. as well as resistance to last resort antimicrobial (Carbapenems and Cephalosporin) is a serious One Health concern.

26. *Pteropus vampyrus* TRIM40 is an interferon stimulated gene that antagonizes RIG-I-like receptors

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Introduction. Henipaviruses (HNV; family *Paramyxoviridae*), including Nipah virus (NiV), naturally infect Old World fruit bats (family *Pteropodidae*) without causing overt disease. Conversely, NiV infection in humans and other mammals can be lethal. Despite understanding the ecology of HNV infection in pteropidid bats, bats' immune systems remains under-studied. Comparing bat antiviral responses with those of humans may illuminate the mechanisms that facilitate bats' tolerance. Tripartite motif proteins (TRIMs), a large family of E3-ubiquitin ligases, fine-tune innate antiviral immune responses, and two human TRIMs interact with HNV proteins. We hypothesize that NiV infection induces the expression of an immunosuppressive TRIM in bat, but not human, cells to promote tolerance. **Methods**. Here, we infected *Pteropus vampyrus* and human cells with NiV to evaluate the expression of TRIM family members, including TRIM40 a suspected bat-specific interferon stimulated gene (ISG). We then characterized bat TRIM40 to compare its role in regulating innate antiviral responses to human. **Results**. TRIM40 was upregulated in bat, but not human, cells within 16 hours following NiV infection. We also found that type I interferon (IFN-I) signaling is efficiently suppressed during NiV infection in bat cells, while IFN-I signaling is induced late in infection in human cells. Knockdown of bat TRIM40 increases gene expression of IFNβ, ISGs, and pro-inflammatory cytokines following poly(I:C) transfection. Analogous to human TRIM40, the bat ortholog negatively regulates IFN-I induction through ubiquitin-mediated antagonism of RIG-I and MDA5. **Conclusions**. Bats may have evolved to express TRIM40 in response to viral infections to control immunopathogenesis.

27. Isolation and identification of mammalian orthoreoviruses from bats in the United States

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Introduction. Mammalian orthoreovirus (MRV) infects many mammalian species including humans, bats and domestic animals. MRVs have been identified in humans, pigs and bovine in the USA; however, the MRVs in bats remain poorly understood in the USA. We hypothesize different MRVs circulating in bats that can be the mixing vessel to generate novel genotypes of viruses potential spillover to other species. **Methods**. In this study, we collected approximately one thousand bats of different species from eight states in the USA from 2015 to 2019 that were screened by RT-qPCR and then performed virus isolation for MRV-positive samples. The isolates were further characterized by molecular analysis, in vitro and in vivo. **Results**. Thirteen MRVs were isolated and sequenced. Sequence and phylogenetic analysis revealed these isolates belonging to four different genotypes of viruses in serotypes 1 or 2, which contain genes showing the highest homology to those of MRVs detected in humans, bats, bovine and deer. Further characterization showed that these four different genotypes of MRVs replicated efficiently on human, canine, monkey and swine cell lines. The MRV1/040/Bat/USA/2018 isolate belonging to serotype 1 demonstrated the ability to infect and transmit in pigs. **Conclusions**. Taken together, different reassortant MRVs are circulating in bats in the USA that could be the mixing vessel of MRVs and provide MRVs to infect other species. Our results warrant the significance of MRV-surveillance in bats and other mammals.

28. Ecology and biosurveillance methods for bats and their viral pathogens in Uganda

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Introduction. Emerging and reemerging viral pathogens present a continual threat to global health security, and often wildlife reservoirs involved in spillover events are left understudied. Substantial work remains to develop sustainable and effective biosurveillance programs in regions of interest to global health. **Methods**. To strengthen active research on bat ecology and biosurveillance methods in Uganda, we developed partnerships between US and Ugandan academic institutions and government agencies to characterize public health risks at the human-bat interface. **Results**. In May 2021 we began seasonal sampling of bat communities residing in cave systems in Eastern Uganda. This ongoing research includes the use of global positioning systems (GPS), acoustic monitoring, and infrared camera technologies to describe cave community composition, bat dispersal patterns, and bat interactions with both wild and domestic animal communities. Pathogen screening of bat samples via RT-PCR and next-generation sequencing (NGS) has revealed virus circulation within *Rhinolophus* bat populations. Camera trap observations provide evidence of cave use by humans and wild animal species implicated in previous spillover events. **Conclusions**. The preliminary data gathered to date reinforce the importance of ecological research and sustainable biosurveillance programs for the rapid response to future outbreak events. Additional project aims to evaluate the impact of bat dispersal patterns on seasonal virus distribution, the role of citizen science in bat biosurveillance, and the human risk of exposure to bat-borne viruses are underway.

29. Comparative nasal anatomy and goblet cell distribution reveal possible adaptation to viruses in bats

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Introduction. Bats are established reservoirs for coronaviruses and other respiratory pathogens, but do not manifest symptoms of infection and minimal immune response. Bat viral tolerance must be explained by mechanisms of infection without invasion, but little is known about entry routes of respiratory viruses in bats and how the mucus-lining cells and overall morphology may facilitate viral tolerance or hinder viral tropism. **Methods**. We focus on goblet cells in particular, which were long thought to be mucus-producing bystanders, but now are known as entry points for SARS-CoV-2 and their implications in differential severity of COVID-19 infections. We used various creative imaging approaches of several bat species, including horseshoe bats and neotropical bats, and characterized goblet cell distribution throughout the nasal cavity. We then mapped these distributions in 3D using contrast-enhanced μCT-scanning. **Results**. We discovered that despite most bats having a continuous nasal passage with olfactory and respiratory epithelia, horseshoe bats have significantly rearranged their nasal cavity, including significant expansion anteriorly of the nasopharynx and complete separation and reduction of goblet cell distribution in their respiratory epithelia relative to the olfactory epithelia. **Conclusion**. This rearrangement may contribute to viral deposition and entry, isolating ways in which the virus can spread throughout the head and potentially facilitating viral tolerance rather than a strong immune response and viral clearing.

30. Molecular basis of *Rhinolophus* ACE2 receptor association with Cambodian bat sarbecovirus RshTT200 spike glycoprotein

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Introduction. Twice in recent history sarbecoviruses have crossed the species barrier transferring from their *Rhinolophus* bat hosts into human populations. Little is known however about the promiscuity of sarbecoviruses across Rhinolophus species and the role of polymorphism of ACE2 receptors in *Rhinolophus* bat susceptibility to infection by these viruses. A Cambodian sarbecovirus, RshTT200, with close relation to SARS-CoV-2 was discovered in 2010 and represents the earliest discovery of a SARS-CoV-2-like-sarbecovirus clade however little is known about the promiscuity of this virus with respect to its ability to infect various *Rhinolophus* bats. **Methods**. To understand receptor promiscuity in *Rhinolophus* bats, we performed binding assays with the receptor binding domain and cell entry assays with spike pseudotyped VSV to make comparisons between RshTT200, and human-infecting SARS-CoV-1, SARS-CoV-2. To understand the structural and molecular basis of receptor usage of the RshTT200 spike glycoprotein, we solved the spike structure by cryo-EM which revealed remodeling of key antigenic sites. **Results**. We determined broader promiscuity of human-infecting sarbecoviruses SARS-CoV-1 and SARS-CoV-2 compared to RshTT200 and identified key molecular interactions that drive receptor association. Polymorphism of bat ACE2 plays a strong role in the susceptibility of each species to sarbecovirus infection. Additionally, unlike SARS-CoV-1 and SARS-CoV-2, the RshTT200 spike was only observed to adopt a closed conformation in which the receptor-binding domains cannot bind to ACE2. **Conclusions**. The predominance of closed conformations of the RshTT200 spike glycoprotein, which can also be seen in endemic coronaviruses in humans, may reflect the endemic nature of sarbecoviruses in bats. Polymorphism of *Rhinolophus* bat ACE2 within species is one mechanism of protection against these viruses. Differences in ACE2 between species

both drives the evolution of sarbecoviruses, but also can be protective as in the case of *R. shameli* bats for which in our panel only RshTT200 and SARS-CoV-2 is capable of receptor association.

31. Viral surveillance in *Rhinolphus* and *Hipposideros* bats from eastern Uganda

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Introduction. Bats are known to harbor a variety of viruses with considerable capability for co-circulation and impact on medical and veterinary health worldwide. Thus, surveillance of bat-associated viruses is crucial to developing novel strategies for public health response. Previously, we have reported the presence of several viral families detected and isolated from bats in Eastern Uganda. Bat species documented to co-roost in caves of this regionality include *Rhinolophus* and *Hipposideros* bats, which are known to harbor coronaviruses, yet the full extent of viral diversity in these genera is uncharacterized. Caves where these bats have been observed frequently experience human encroachment from the local population and tourists. Taken together, we hypothesize that if *Rhinolophus* and *Hipposideros* bats are reservoirs for multiple pathogens, then longitudinal surveillance will uncover the dynamic bat/virus relationships between across cave structures. **Methods.** Oral and fecal samples from bats were collected from bats captured in four caves in Eastern Uganda during May 2021 (wet season), January 2022 (dry season), and May 2022. Samples were subjected to RNA extraction and viral nucleic acid detection using consensus PCR assays targeting six viral families: *Coronaviridae*, *Filoviridae*, *Flaviviridae*, *Peribunyavidae*, *Paramyxoviridae*, and *Rhabdoviridae*. **Results.** To date, coronavirus nucleic acid has been detected in the rectal swab or fecal simples from 3 *Hipposideros ruber* bats, and 1 *Rhinolophus fumigatus* bat. Sanger sequencing of the coronavirus amplicon from *R. fumigatus* confirmed detection of a *Betacoronavirus*. Testing and sequence confirmation of additional positive samples is ongoing, as are attempts to isolate virus from positive samples. **Conclusions.** Overall, this work will contribute to our understanding of the capacity for bats to harbor pathogens and aid in discovery of novel virus strains.

32. Innate immune responses of Jamaican fruit bat cells infected with Middle Eastern respiratory syndrome coronavirus

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Introduction. Middle East respiratory syndrome coronavirus (MERS-CoV) is a betacoronavirus that causes Middle East respiratory syndrome in humans. Bats are believed to be a natural reservoir because they are hosts for a variety of related coronaviruses. Thousands of coronavirus sequences have been identified in bat species spanning Asia, Africa, the Americas, and Europe; however, there are limited data on the interactions between host immunity and the virus. Previous studies have determined that deletion of MERS-CoV genes encoding for proteins 4a and 4b enhances the interferon response in infected human cell lines compared to wildtype MERS-CoV. This study aimed to determine what immune pathways are used by Jamaican fruit bats (*Artibeus jamaicensis*) when infected to wildtype MERS-CoV or the Δ4ab deletion mutant. **Methods**. MERS-CoV was passaged on primary kidney cells from Jamaican fruit bats. Expression of several innate immune response was evaluated by SYBR Green qPCR and genome sequencing performed on passage 10 virus. **Results**. Jamaican fruit bat kidney cells lines are susceptible and permissive to MERs-CoV wild type and the Δ4ab deletion mutant. Several genes had increased expression in the MERS-CoV Δ4ab but are lower in the WT infection. Furthermore, MERS-CoV WT and Δ4ab cause cytopathic effects after ten viral passages. Several mutations occurred in the viruses that suggest adaptation to a new host. **Conclusions**. MERS-CoV induces an innate response in Jamaican fruit bat cells but it is insufficient to protect the cells from death. Deletion of the 4ab locus, encode proteins with double-stranded RNA binding and phosphodiesterase activities, altered gene expression profiles, suggesting these pathways may be targets of 4ab accessory proteins.

33. Jamaican fruit bats as a potential model of Cedar virus infection

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Introduction. The viral family *Paramyxoviridae* has been the source of several human and veterinary pathogens such as Nipah virus, Hendra, measles virus, human parainfluenza viruses, canine distemper virus, and rinderpest virus. Bats are the known reservoir for henipaviruses and are the suspected reservoir of the ancestors of many other medically important paramyxoviruses. Understanding the relationship between viruses and their reservoir hosts is essential for spillover preparedness. **Methods**. In this study, we investigated the immunological and evolutionary relationship between Cedar virus (CedV), a henipavirus from Australian flying foxes that is not thought to be pathogenic, and a New World bat species, the Jamaican fruit bat (*Artibeus jamaicensis*). Using qPCR arrays, we

examined expression of 45 innate immune genes in AJK cells following infection with NiV, HeV, CedV, and CedV that had been serially passaged on AJK cells 10 times. Additionally, we performed a preliminary experimental challenge of Jamaican fruit bats with CedV to determine whether Jamaican fruit bats are susceptible to infection. **Results**. Our work demonstrates that primary Jamaican fruit bat kidney (AJK) cells are susceptible and permissive to CedV infection, that CedV is unable to suppress the innate immune response in those cells, whereas Nipah and Hendra viruses do, and that serial passage of CedV in AJK cells resulted in several mutations in viral protein coding regions. We also showed that Jamaican fruit bats develop transient viremia and an antibody response following experimental challenge with CedV. **Conclusions**. This work demonstrates that Jamaican fruit bats may serve as a surrogate bat model to study henipavirus infection and viral evolution.

34. Generation of Dendritic Cells and Macrophages from the Jamaican Fruit Bat (*Artibeus jamaicensis*)

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Introduction. New World fruit bats serve as reservoirs for rabies virus, H17N10 and H18N11 bat influenza A viruses, and coronaviruses. Because bat influenza A viruses were first detected in New World fruit bats in Guatemala, and subsequently Peru and Brazil, these species likely serve as the natural reservoir hosts. Unlike other Influenza A viruses that use sialic acid residues for cellular binding, H18N11 uses MHC class II beta chains for cellular entry. Because of the high sequence conservation of MHC molecules, the use of MHC class II molecules as an entry mediator leads to the possibility of broad vertebrate tropism. Therefore, the characterization and study of H18N11 infection in professional antigen presenting cells (APC) from New World fruit bats will lead to a greater understanding of the mechanisms by which these species control viral infections and how H18N11 infects MHC class II positive cells. **Methods**. APCs were generated with Jamaican fruit bat (*Artibeus jamaicensis)* bone marrow-derived hematopoietic stem cells (HSC) cultured in Jamaican fruit bat GM-CSF or Egyptian rousette fruit bat (*Rousettus aegyptiacus*) M-CSF. Cells were characterized using flow cytometry and challenged with H18N11. **Results.** Cultured dendritic cells were found to express MHC-II, which signifies their role as APCs. Despite their expression of MHC class II, the cells were not permissive to infection with H18N11, suggesting that another molecule may be required for successful H18N11 entry. **Conclusions**. Through culture of Jamaican fruit bat HSCs with GM-CSF and M-CSF, functional dendritic cells and macrophages were generated. These cells are important for the activation and augmentation of the adaptive immune response to viruses. The cells cultured can be used to define function and test viral susceptibility and permissibility. Cultured dendritic cells can also be used to stimulate T cells in culture with antigens of interest, in order to gauge the bat adaptive immune response to viruses of interest. It is important to understand the cellular characteristics of antigen presenting cells (APCs) in New World fruit bats, particularly in relation to bat influenza, which uses MHC II as an entry mediator.

35. Sosuga virus infection of Jamaican fruit bat primary kidney epithelial cells.

Shijun Zhan, Maggie Priore, Miles Eckley and Tony Schountz

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Introduction. Sosuga virus (SOSV) was first identified in a bat biologist with febrile disease that had returned from the South Sudan/ Uganda border region. Subsequent work determined that Egyptian fruit bats (*Rousettus aegyptiacus*) are a reservoir host of the newly discovered pararubulavirus, and infection of this species resulted in an innocuous infection. We were interested to determine whether SOSV can infect Jamaican fruit bats (*Artibeus jamaicensis*) or cells from this species. **Methods**. Wild-type and ZsGreen-1 (ZsG) infectious clones of SOSV and primary kidney cells from Jamaican fruit bats were used in these studies. For challenge of Jamaican fruit bats, three bats were inoculated with 10^3 $TCID_{50}$ of SOSV intraperitoneally and intranasally. On days 3, 6, 10, 14 and 21, rectal and oral swabs, opportunistic urine, blood, and were collected from each bat and terminal sera on day 21. Real-time RT-PCR was used to detect virus in samples. For cell culture experiments, 0.1 MOI of virus was inoculated onto primary kidney cells from 6 different Jamaican fruit bats (Aj2-Aj7 cells) and monitored daily for CPE. SOSV was independently passaged on Aj4 and Aj6 cells 10 times to assess differences that might arise. **Results**. Only one bat had evidence of SOSV infection by qPCR in which virus was detected in the day 3 blood and urine. None of the swab samples had evidence of infection and none of the bats showed signs of disease. Primary kidney cells inoculated with the virus appeared healthy until day 6, when subtle CPE became apparent and by day 8 CPE with extensive syncytia was extensive. The virus was passaged on the primary kidney cells and by 5 passages CPE was occurring by day 4, suggesting adaptation to the primary kidney cells. Inoculation of Aj4 cells with the ZsG-expressing SOSV showed that some, but not all, cells became infected. SOSV passaged on Aj4 and Aj6 cells 10 times replicated to higher titers than the original wild-type virus. The Aj4 cell-10x passaged virus caused substantial CPE on both Aj4 and Aj6 cells; however, the Aj6 cell-10x passaged virus lost the ability to cause CPE on either Aj6 or Aj4 cells. **Conclusions**. The results of this study suggests that SOSV is capable of infecting cells from New World fruit bats and that it has the potential to adapt to infect Jamaican fruit bats. The Aj4 and Aj6 cell passaged viruses are currently being sequenced to determine what mutations may have occurred during passage. Future studies will passage SOSV in Jamaican fruit bats and inoculation of bats with the viruses passaged on their cells (i.e., 10x passaged viruses) to determine if an adapted SOSV can be generated to infect Jamaican fruit bats that could serve as a surrogate animal model for the study of the virus.

36. The synthesis of a self-regenerative monolayer of Jamaican fruit bat gastrointestinal epithelial cells.

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Introduction. Studies of bat H18N11 influenza A virus are limited by the permissibility of H18N11 virus has in cell culture, coupled with the small number of available bat cell lines. Two canine cell lines permit for H18N11 propagation but after two passages, mutations arise invariably in its neuraminidase gene segment that result in nonfunctional head domain of neuraminidase (frameshift, premature stop codon), impairing research into the functions of this protein. Previous work in our lab determined that the H18N11 virus primarily replicates in the enterocytes of the small intestine in putative reservoir Jamaican Fruit Bats (*Artibeus jamaicensis*). Within the small intestine, there are crypts amongst the microvilli, which harbor stem cells that continuously repopulate the epithelial lining of the gastrointestinal tract. By isolating the crypts that contain these stem cells, specific growth factors can be introduced to induce the production of a self-regenerative primary crypt stem cell lines that can then be differentiated into enterocytes. Culturing these primary cells will allow us to emulate an infection with H18N11 in vitro. Additionally, the use of stem cells eliminates the need to immortalize this cell line, because the cells are self-regenerative. To better understand the functions of the H18N11 neuraminidase in bats, it will be necessary to establish a permissive cell culture. Therefore, this project aims to generate primary cell lines using bat crypt cells and growth factors to establish a monolayer of gastrointestinal epithelial cells that will be permissive to H18N11. **Methods.** The sequences for Jamaican fruit bat growth factors, gastrin, epidermal growth factor (EDGF), noggin, R-spondin-2, and Wnt3a were de novo synthesized to contain the mouse Igk leader sequence, a C-terminal histidine tag, and codon-optimized for expression in human cells. The designed gene fragments were cloned into the pcDNA3.3-TOPO vector using the pcDNA 3.3-TOPO TA Cloning Kit from Invitrogen. The plasmid constructs were then transformed into OneShot TOP10 cells, and sequenced to ensure they were free of mutations. The plasmids were transfected into FreeStyle MAX CHO cells and selected with Geneticin to generate stable cell lines that express the growth factors. The transfected cell lines were then verified through PCR with primers specific for the gene fragment and Geneticin resistance. **Results**. Sanger sequencing verified the designed gene fragments with correct orientation within the pcDNA3.3-TOPO vector using a CMV forward primer and a TKpolyA reverse primer. **Conclusions**. After the growth factor producing CHO cell lines are established and confirmed with PCR, the proteins will be purified using the His-tags. This will allow for the generation of a sufficient media to induce the synthesis of a gastrointestinal epithelial monolayer. Then, the crypts of the small intestine can be isolated to generate monolayers. The monolayer will be maintained using a gentle 2-step dissociation technique with collagenase. Once established, the cell lines can be used screen for other gastrointestinal viruses that bats may harbor and in vitro studies of H18N11.

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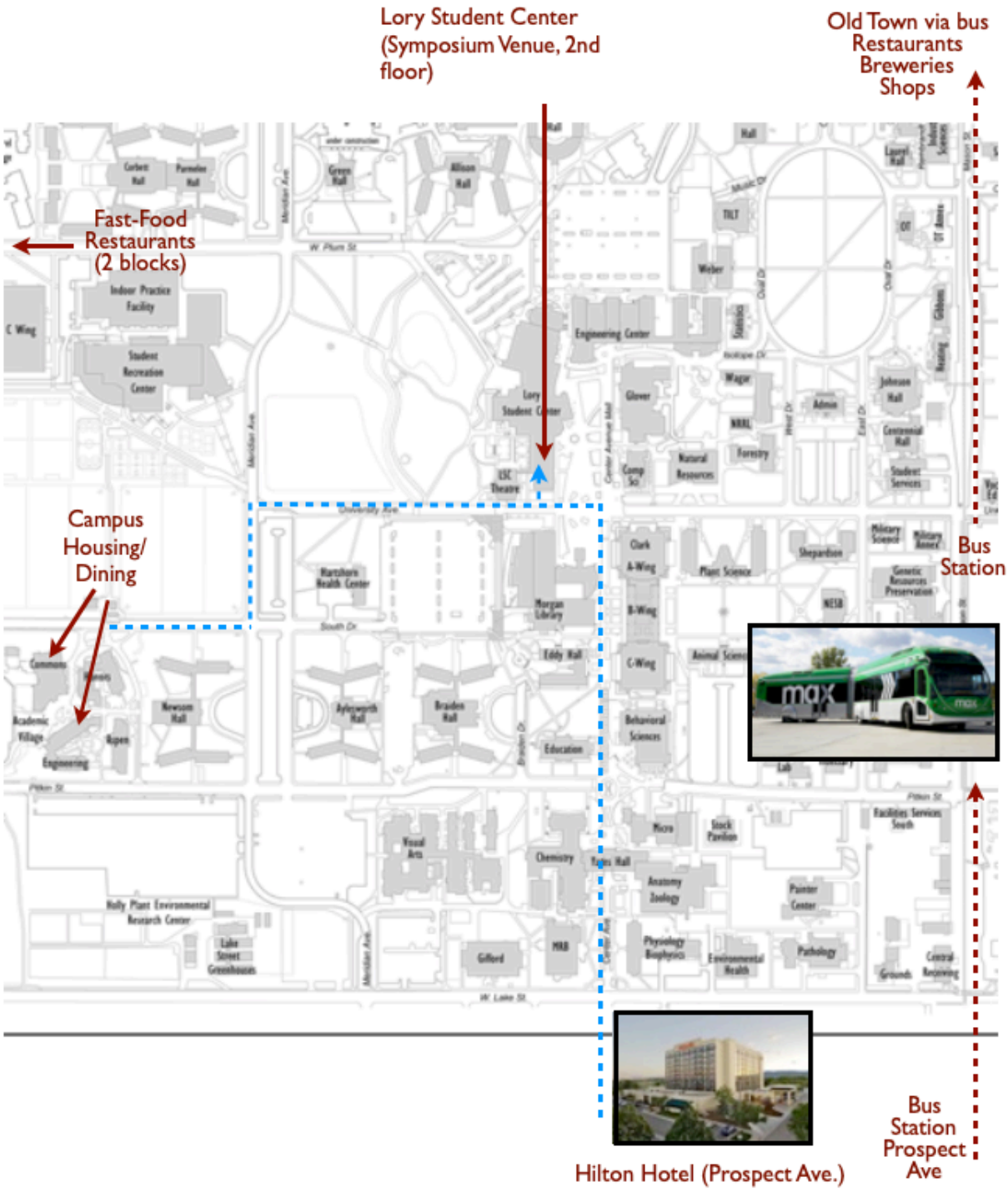
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