Grant Number: 1R01AI079231-01 REVISED

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Risk of Viral Emergence from Bats

Aleksei A. Chmura
Executive Director, CCM
460 West 34th Street
17th Floor
New York, NY 10001

Budget Period: 09/18/2008 – 08/31/2009
Project Period: 09/18/2008 – 06/30/2013

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to WILDLIFE TRUST in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI079231 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Ann W. Devine
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AI079231-01 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$105,625
Fringe Benefits	$26,406
Personnel Costs (Subtotal)	$132,031
Equipment	$12,000
Supplies	$13,000
Travel Costs	$12,750
Other Costs	$5,000
Consortium/Contractual Cost	$304,672
Federal Direct Costs	$479,453
Federal F&A Costs	$55,536
Approved Budget	$534,989
Federal Share	$534,989
TOTAL FEDERAL AWARD AMOUNT	$534,989

AMOUNT OF THIS ACTION (FEDERAL SHARE) $0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$534,989	$534,989
2	$535,156	$535,156
3	$534,739	$534,739
4	$534,968	$534,968
5	$547,542	$547,542

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI079231A
Fiscal Year:	2008

IC	*CAN*	*2008*	*2009*	*2010*	*2011*	*2012*
AI	8472302	$534,989	$535,156	$534,739	$534,968	$547,542

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M34A / **OC**: 414A / **Processed**: (b) (6) 10/17/2008

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI079231-01 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AI079231-01 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.

e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI079231-01 REVISED

This revised award reflects the Office of Laboratory Animal Welfare (OLAW) approval of assurance(s) of compliance with the PHS Policy on Humane Care and Use of Laboratory Animals for the awardee and/or performance site(s) and removes the restrictive term on the Notice of Award (NoA) issued on 09/18/2008.

Supersedes NoA issued on 09/18/2008.
****
This award includes funds awarded for consortium activity with Columbia University in the amount of $217,732 ($135,828 direct costs + 81,904 facilities and administrative costs). Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.
****

This award includes funds awarded for consortium activity with CSIRO (The Australian Animal Health Laboratory) AAHL in the amount of $86,940 ($80,500 direct costs + $6,440 facilities and administrative costs). Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Howard A. England
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Eun-chung Park
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI079231-01 REVISED

INSTITUTION: WILDLIFE TRUST

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$105,625	$108,794	$112,058	$115,419	$118,882
Fringe Benefits	$26,406	$27,198	$28,014	$28,855	$29,720
Personnel Costs (Subtotal)	$132,031	$135,992	$140,072	$144,274	$148,602
Equipment	$12,000			$12,000	
Supplies	$13,000	$10,000	$5,000	$4,100	$17,000
Travel Costs	$12,750	$9,250	$6,250	$13,550	$13,550
Other Costs	$5,000	$5,000	$3,500	$3,000	$3,000
Consortium/Contractual Cost	$304,672	$333,091	$339,508	$314,999	$317,848
TOTAL FEDERAL DC	$479,453	$493,333	$494,330	$491,923	$500,000
TOTAL FEDERAL F&A	$55,536	$41,823	$40,409	$43,045	$47,542
TOTAL COST	$534,989	$535,156	$534,739	$534,968	$547,542

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	26.1%	26.1%	26.1%	26.1%	26.1%
F&A Cost Base 1	$212,781	$160,242	$154,822	$164,924	$182,152
F&A Costs 1	$55,536	$41,823	$40,409	$43,045	$47,542

PI: **DASZAK, PETER**	Title: Risk of Viral Emergence from Bats	
Received: 10/09/2007	FOA: PA07-246	Council: 05/2008
Competition ID:	FOA Title: NON-BIODEFENSE EMERGING INFECTIOUS DISEASES RESEARCH OPPORTUNITIES (R01)	
1 R01 AI079231-01	Dual:	Accession Number: 3030604
IPF: 4415701	Organization: ECOHEALTH ALLIANCE, INC.	
Former Number:	Department: CCM	
IRG/SRG: IRAP	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 399,304 Year 2: 395,764 Year 3: 381,276 Year 4: 391,092 Year 5: 407,481	Animals: Y Humans: N Clinical Trial: N Current HS Code: (b) (4) HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Peter Daszak	Wildlife Trust Inc	PD/PI
W. Lipkin	The Trustees of Columbia University in the City of New York	Co-PD/PI
Bruce Mungall	Commonwealth Science and Industry Organization	Co-PD/PI
Kate Jones	Zoolcgical Society of London	Co-PD/PI
Jonathan Epstein	Wildlife Trust	Co-PD/PI
Thomas Briese	The Trustees of Columbia University in the City of New York	Co-PD/PI
Gustavo Palacios	The Trustees of Columbia University in the City of New York	Co-PD/PI

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
10/05/2007	
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

❍ Pre-application ❍ Application
● Changed/Corrected Application

4. Federal Identifier
GRANT00349359

5. APPLICANT INFORMATION *** Organizational DUNS:**077090066

* Legal Name: Wildlife Trust Inc
Department: CCM
Division:
* Street1: 460 W34th Street
Street2: 17th Floor
* City: New York
County: New York
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 10001

Person to be contacted on matters involving this application

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	Peter		Daszak	

* Phone Number: (b) (6) Fax Number: 2123804475 Email: (b) (6)

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*:
311726494

7. * TYPE OF APPLICANT
M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

8. * TYPE OF APPLICATION: ● New
❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Other (Specify):

Small Business Organization Type
❍ Women Owned ❍ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

* Is this application being submitted to other agencies? ❍ Yes ● No
What other Agencies?

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Risk of Viral Emergence from Bats

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*
N/A

13. PROPOSED PROJECT:

* Start Date	* Ending Date
07/01/2008	06/30/2013

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
08	00-000

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	Peter		Daszak	

Position/Title: Executive Director
* Organization Name: Wildlife Trust Inc
Department: CCM
Division:
* Street1: 460 W34th Street
Street2: 17th Floor
* City: New York
County: New York
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 10001
* Phone Number: (b) (6) Fax Number: 2123804475 * Email: (b) (6)

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	$3,051,586.31
b. * Total Federal & Non-Federal Funds	$3,051,586.31
c. * Estimated Program Income	$0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
	Aleksei	Avery	Chmura	

* Position/Title: Program Assistant
* Organization Name: Wildlife Trust Inc
Department: CCM
Division:
* Street1: 460 West 34th Street
Street2: 17th Floor
* City: New York
County: New York
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 10001
* Phone Number: (b) (6)
Fax Number: 1.212.380.4475
* Email: (b) (6)

*** Signature of Authorized Representative**

Aleksei Chmura

*** Date Signed**

10/09/2007

20. Pre-application File Name: Mime Type:

21. Attach an additional list of Project Congressional Districts if needed.

File Name: Mime Type:

424 R&R and PHS-398 Specific Table Of Contents

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: Wildlife Trust

* Street1: 460 West 34th Street		Street2: 17th Floor
* City: New York	County:	* State: NY: New York
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 10001

Project/Performance Site Location 1

Organization Name: The Trustees of Columbia University

* Street1: 630 West 168th Street		Street2: Box 49
* City: New York	County:	* State: NY: New York
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 10032

Project/Performance Site Location 2

Organization Name: The Institute of Zoology

* Street1: Regent's Park		Street2:
* City: London	County:	* State:
Province:	* Country: GBR: UNITED KINGDOM	* Zip / Postal Code: NW14RY

Project/Performance Site Location 3

Organization Name: Australian Animal Health Laboratory (AAHL)

* Street1: 5 Portarlington Road		Street2:
* City: East Geelong	County:	* State:
Province: Victoria	* Country: AUS: AUS-TRALIA	* Zip / Postal Code: VIC 3219

	File Name	Mime Type
Additional Location(s)	7886-Other_performance_sites.pdf	application/pdf

Other Performance Sites

The research plan involves collection of serum and other samples from wildlife (bats) in regions around the world that are hotspots for emerging diseases. Our preliminary analyses (**See preliminary data, Section 4.1**) suggest that these countries will be largely in tropical regions, with high wildlife biodiversity, but where there are significant human population pressure. In the initial period of the project (years 1 and 2) these will include Bangladesh, China, Brazil, Mexico and Cameroon. It is possible that later on, as we refine our predictive modeling, we will also target other regions.

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved?	○ Yes	● No
1.a. If YES to Human Subjects		
Is the IRB review Pending?	○ Yes	○ No
IRB Approval Date:		
Exemption Number:	_ 1 _ 2 _ 3 _ 4 _ 5 _ 6	
Human Subject Assurance Number		
2. * Are Vertebrate Animals Used?	● Yes	○ No
2.a. If YES to Vertebrate Animals		
Is the IACUC review Pending?	● Yes	○ No
IACUC Approval Date:		
Animal Welfare Assurance Number	None	
3. * Is proprietary/privileged information included in the application?	○ Yes	● No
4.a. * Does this project have an actual or potential impact on the environment?	○ Yes	● No
4.b. If yes, please explain:		
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?	○ Yes	○ No
4.d. If yes, please explain:		
5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators?	● Yes	○ No
5.b. If yes, identify countries:	Australia	
5.c. Optional Explanation:	Please see attachment (item 11, below)	

6. * Project Summary/Abstract	6134-NIAID_abstract_Final.pdf	Mime Type: application/pdf
7. * Project Narrative	661-Project_Narrative.pdf	Mime Type: application/pdf
8. Bibliography & References Cited	5093-Bibliography.pdf	Mime Type: application/pdf
9. Facilities & Other Resources	3165-Resources_COMBINED.pdf	Mime Type: application/pdf
10. Equipment		
11. Other Attachments	1719-Justification_of_Work_at_Foreign_Site.pdf	Mime Type: application/pdf

Abstract (character limit)

Emerging zoonoses are a significant threat to global public health and our economies. The majority are caused by pathogens that emerge with increasing frequency from wildlife hosts (e.g. HIV-1 from chimpanzees, SARS CoV from bats and civets, Nipah virus from fruit bats). This group of diseases alone causes tens of thousands of deaths each year, and some outbreaks (e.g. SARS) have cost the global economy tens of billions of dollars. However, despite the huge social, demographic and economic impact of EIDs, there has been little advance in our understanding of the underlying process of how these wildlife zoonoses emerge, and in developing predictive approaches to prevent future emergence.

Developing predictive and proactive approaches to zoonotic emergence is a key challenge to medical science. New zoonoses emerge regularly from wildlife in a seemingly random way, from disparate regions of the globe, and from a wide diversity of wildlife species. Our ability to understand what drives this process is hampered by a lack of rigorous analyses of the processes that cause emergence; our lack of knowledge of the diversity of microbes in wildlife (the 'zoonotic pool') from which new zoonoses regularly emerge; and our poor understanding of pathogenic factors that explain why some viruses are able to cross the species barrier while others are not. In this proposal, we bring together a multidisciplinary team of emerging disease ecologists and modelers, viral bioinformaticists, and molecular virologists who are leaders in their fields, and who have already collaborated together to study zoonotic disease emergence. Building on preliminary data that demonstrates bats are a key wildlife reservoir, and that emergence is due to a range of anthropogenic drivers, this team will **1)** develop predictive models of global 'hotspots' for the future emergence of bat viruses; **2)** use a large repository of bat biological samples to conduct targeted surveillance in these 'hotspots' for known and undiscovered bat pathogens, elucidating the unknown diversity of the bat 'virome' and; **3)** using a range of *in vitro* techniques (including infection in bat cell culture), examine the pathogenesis of these new viruses, and a pool of available bat viruses which have not yet emerged in humans. **This multidisciplinary approach represents the first, concerted effort to understand the depth and breadth of the process of emergence within a key group of wildlife hosts associated with the recent emergence of SARS, Nipah, Hendra, Ebola and Marburg viruses.**

Emerging zoonoses (e.g. HIV/AIDS, Influenza) are a major threat to health globally, causing tens of thousands of deaths each year in the USA and abroad and a number of these have emerged from bats recently (SARS, Ebola, Nipah). This research provides a way to predict the regions where the next new emerging zoonosis from bats is most likely to emerge, and proposes targeted surveillance of these animals using state-of-the-art molecular techniques in those regions. It will characterize new viruses, and study the pathogenesis of these, and a bank of known bat viruses that have not yet emerged in the human population: It is therefore a predictive, proactive approach to combating the most high profile group of emerging pathogens.

Principal Investigator/Program Director (Last, First, Middle): Daszak, Peter

Consortium for Conservation Medicine (CCM) RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory: The Consortium for Conservation Medicine (CCM) has a fully equipped wet lab a their headquarters in Wildlife Trust, New York, designed for receiving, storing and aliquoting samples under BSL-2 conditions. All testing will be conducted at our collaborators, the Greene Lab and Australian Animal Health Lab (below)

Clinical: N/A

Animal: N/A

Computer:
The CCM is equipped with 20 PCs. Drs. Daszak, Epstein, and Jones have access to standard PC stations, 24-7 server and server support, and all required software including ArcGIS, MatLab, Microsoft Office, and Adobe CS

Office:
The CCM is based at Wildlife Trust in New York City with (b) (4) of office space including a meeting room and laboratory. The CCM is supported by administrative staff and two assistants who are available for work on this project and are part-funded through core foundation support.

Other:
N/A

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Principal Investigator/Program Director (Last, First, Middle): Daszak, Peter

Center for Infection and Immunity RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

The Center for Infection and Immunity occupies approximately 7,500 square feet on two floors in the Mailman School of Public Health of Columbia University. The center proper contains isolated areas for work with cultured mammalian cells, radioactivity, recombinant DNA and Biohazard Level (BL)-2 and BL-3 infectious agents, as well as a separate laboratory for molecular epidemiology using real time PCR. To minimize potential for spurious results, access to the latter laboratory is restricted; the room is positive pressure and equipped with overhead UV lamps; individual glove boxes are used for nucleic acid extraction and addition of reagents for PCR analyses.

The Center for Infection and Immunity is registered for 'Possession, Use, and Transfer of Select Biological Agents and Toxins'.

Clinical:

Not applicable

Animal:

Not applicable

Computer:

Computer equipment in the Center for Infection and Immunity includes personal computers and printers, and software for word processing, graphics, statistics, nucleic acid and protein sequence analysis. Computers are linked to larger systems on the Columbia campus that allow reference searches, computer mail and access to national and international protein and nucleic acid databases.

Office:

The Center for Infection and Immunity includes approximately 1,500 square feet of office and computer space.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

The Center for Infection and Immunity contains an ultracentrifuge and high speed preparative centrifuge, phosphorimager, on-line thermal cycler (ABI 7700), HPLC, flow cytometer for bead based immunologic and molecular assays (Luminex), automated sequencer (ABI 310), Agilent LC/MS 1D system, microfuges, MultiDrop plate dispenser station, CO_2 incubators for noninfected and infected cell lines, autoclave, scintillation counter, liquid nitrogen, dry ice, and darkroom with film developer incubators for bacterial plates, shaking incubator for plasmid preparation, freezers and refrigerators, thermal cyclers, cryostat, motorized sliding cryomicrotome suitable for cutting thick sections (Microm HM440E), brightfield and fluorescent microscope, inverted fluorescent microscope, water purification system, gel boxes and power supplies for nucleic acid and protein electrophoresis, gel dryer, water baths, pH meter, balances, tissue homogenizers, vacuum pumps, speedvac, vacuum oven, spectrophotometer, gel documentation system, UV transilluminator and gel documentation system, glassware, plasticware and pipetting aids. In close proximity are a confocal microscope, luminometer, FACS, amino acid analyzer, and DNA and protein sequencers.

Principal Investigator/Program Director (Last, First, Middle): Daszak, Peter

Australian Animal Health Laboratory RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

The PI's non-BSL4 laboratories are inside the secure area of AAHL which has approximately 30,000 square feet of BSL-3 lab space. There are 2 labs of approximately 400 square feet each, equipped with 2 CO_2 incubators for tissue culture, inverted, bright field microscopes, several high speed and ultracentrifuges, three biological safety cabinets, 2 PCR machines, an ELISA plate reader, luminometer, gel electrophoresis and western blot equipment and -20^{0}C and -80^{0}C storage facilities. A number of shared resources are also available including liquid handling robotics, fluorescence microscopes and image analysis software. Each laboratory is linked via LAN to a main frame and computers in offices outside the secure area.

The AAHL BSL-4 laboratory facility is approximately 400 square feet and is equipped with the necessary air supply systems, a CO2 incubator and large roller machine encased in an incubator, a low speed bench and high speed ultracentrifuge, -80^{0}C freezer, inverted microscope, dunk tank, class 1 And class II biohazard cabinets and computer linkage to the LAN. The laboratory has all the necessary ancillary facilities such as BSL-4 suits and Microchem Plus shower capacity required for BSL-4 facilities. Individuals working at BSL-4 wear head phones which permit instant communication with others outside the laboratory. An extensive training and safety program is in place to ensure operator safety. An engineering staff of about 30 ensure continuous and safe operation of the secure (BSL3 and BSL4) facilities at AAHL.

Clinical:

Animal:

AAHL's large animal facility has 28 large animal rooms, two of which (1000 and 400 square feet) have the capacity to operate at BSL-4. The animal rooms contain specially designed cages which have wire crushing mechanisms capable of pushing the animal to one side of the cage to permit easy and safe anaesthetization. Animal experiments done at BSL-4 are thoroughly planned by all scientific staff involved in the experiment with input form animal technicians, engineering, and microbiological security staff involved in the operation of the BSL-4 labs. Animals held at BSL-4 are under constant video surveillance and temperature is continuously monitored via radio telemetry.

Computer:

Pentium computers are present in both non-BSL-4 laboratories and the BSL-4 laboratory in the secure area and linked to the AAHL server by a LAN.

Office:

The PI has offices within and outside the secure area of AAHL and has access to full time library, records and IT personnel in addition to state of the art copying and printing facilities.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Common support facilities in the secure area of AAHL include cold rooms, dark rooms, fluorescent microscopes, ELISA facilities and image analysis facilities.

Principal Investigator/Program Director (Last, First, Middle): Daszak, Peter

Institute of Zoology (IOZ) - RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

N/A

Clinical: N/A

Animal: N/A

Computer:

Kate Jones' computer laboratory is based at IoZ and consists of 5 PCs and 1 Apple computer (desktops and laptops), running all the relevant software required for the project. There is also access to a number of different webservers in Cambridge for database development and hosting. There is also a separate agreement with the Data Management Center in Newcastle University who are contracted for additional technical database assistance and webserver hosting with one of their programmers is employed within the lab on a part-time basis.

Office:

The Institute of Zoology (IoZ) is scientific research department of The Zoological Society of London (ZSL) based in London, and is part of Cambridge University. IoZ has IT and administrative support both through ZSL and Cambridge University and access to webservers, technical assistance and the library through Cambridge University. The IT departments support a range of statistical software packages (e.g., R, SPSS, MatLab), geospatial programs (e.g. ArcView, ArcInfo, ArcGIS) as well as the standard applications.

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Justification of Work at Foreign Site (AAHL)

The Australian Animal Health Laboratory (AAHL), a component of the CSIRO, conducts world leading research on a number of new and emerging zoonotic agents and has been a key sub-contractor of several recent and on-going NIH funded programs, including a previous R01 on bat viruses (Daszak, PI). In addition to providing fundamental expertise in bat virology and immunology, AAHL is one of the largest and most sophisticated biocontainment facilities in the world and houses a large repository of characterized and as-yet uncharacterized emerging viruses. Of relevance to the current proposal, a significant proportion of the bat samples collected by CCM are stored at AAHL (for biocontainment reasons) such that access to a world class biocontainment facility will be essential for the successful completion of this project. Additionally, a number of parallel projects supported by CSIRO investment in the area of Transformational Biology will significantly value add to the current proposal. We are the only group in the world with immortalized bat cell lines of relevance to this project, essential for enabling ongoing *in vitro* research activities (Eric French Fellowship 2007), and we are continuing to develop new cell lines that will be used by this project. We are also rapidly expanding our bat immunology and genetics programs with CSIRO Office of the Chief Executive funding and participation in the AB-CRC funded project to sequence the transcriptome of *Pteropus* and *Rhinolophus* spp. bats.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Peter		Daszak	

Position/Title: Executive Director
Department: CCM

Organization Name: Wildlife Trust Inc
Division:

* Street1: 460 W34th Street
Street2: 17th Floor

* City: New York
County: New York
* State: NY: New York Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 10001

*Phone Number	Fax Number	* E-Mail
(b) (6)	2123804475	(b) (6)

Credential, e.g., agency login: (b) (6)

*** Project Role:** PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	8982-Bio_Daszak.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	W.	Ian	Lipkin	

Position/Title: Professor
Department: Epidemiology

Organization Name: The Trustees of Columbia University in the City of New York
Division:

* Street1: 630 West 168 Street, Box 49
Street2:

* City: New York
County:
* State: NY: New York Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 10032

*Phone Number	Fax Number	* E-Mail
(b) (6)		(b) (6)

Credential, e.g., agency login (b) (6)

*** Project Role:** Co-PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	5696-Bio_Lipkin.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Bruce	Andrew	Mungall	

Position/Title: Research Scientist
Department: Australian Animal Health Lab

Organization Name: Commonwealth Science and Industry Organization
Division:

* Street1: Private Bag 24
Street2:

* City: Geelong
County:
* State:
Province: Victoria

* Country: AUS: AUSTRALIA	* Zip / Postal Code: VIC3220		
*Phone Number		Fax Number	* E-Mail
011-61-35227-5431			(b) (6)
Credential, e.g., agency login: (b) (6)			
* **Project Role:** Co-PD/PI		**Other Project Role Category:**	
		File Name	Mime Type
***Attach Biographical Sketch**		859-Bio_Mungall.pdf	application/pdf
Attach Current & Pending Support			

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Kate	Elizabeth	Jones	

Position/Title: Research Fellow		Department: Institute of Zoology	
Organization Name: Zoological Society of London		Division:	
* Street1: Regents Park		Street2:	
* City: London	County:	* State:	Province:
* Country: GBR: UNITED KINGDOM	* Zip / Postal Code: NW14RY		
*Phone Number		Fax Number	* E-Mail
(b) (6)			(b) (6)
Credential, e.g., agency login (b) (6)			
* **Project Role:** Co-PD/PI		**Other Project Role Category:**	
		File Name	Mime Type
***Attach Biographical Sketch**		2366-Bio_Jones.pdf	application/pdf
Attach Current & Pending Support			

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Jonathan	H.	Epstein	

Position/Title: Senior Research Scientist		Department: CCM	
Organization Name: Wildlife Trust		Division:	
* Street1: 460 West 34th Street		Street2: 17th Floor	
* City: New York	County:	* State: NY: New York	Province:
* Country: USA: UNITED STATES	* Zip / Postal Code: 10001		
*Phone Number		Fax Number	* E-Mail
(b) (6)			(b) (6)
Credential, e.g., agency login: (b) (6)			
* **Project Role:** Co-PD/PI		**Other Project Role Category:**	
		File Name	Mime Type
***Attach Biographical Sketch**		8568-Bio_Epstein.pdf	application/pdf
Attach Current & Pending Support			

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Thomas		Briese	

Position/Title: Associate Professor
Department: Epidemiology

Organization Name: The Trustees of Columbia University in the City of New York
Division:

* Street1: 630 West 168 Street, Box 49
Street2:

* City: New York
County:
* State: NY: New York Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 10032

*Phone Number	Fax Number	* E-Mail
(b) (6)		(b) (6)

Credential, e.g., agency login: (b) (6)

*** Project Role:** Co-PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	0031-Bio_Briese.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	Gustavo	F.	Palacios	

Position/Title: Assistant Professor
Department: Epidemiology

Organization Name: The Trustees of Columbia University in the City of New York
Division:

* Street1: 630 West 168 Street, Box 49
Street2:

* City: New York
County:
* State: NY: New York Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 10032

*Phone Number	Fax Number	* E-Mail
(b) (6)		(b) (6)

Credential, e.g., agency login: (b) (6)

*** Project Role:** Co-PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	5740-Bio_Palacios.pdf	application/pdf
Attach Current & Pending Support		

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Additional Senior/Key Person Form Attachments

When submitting senior/key persons in excess of 8 individuals, please attach additional senior/key person forms here. Each additional form attached here, will provide you with the ability to identify another 8 individuals, up to a maximum of 4 attachments (32 people).

The means to obtain a supplementary form is provided here on this form, by the button below. In order to extract, fill, and attach each additional form, simply follow these steps:

- Select the "Select to Extract the R&R Additional Senior/Key Person Form" button, which appears below.
- Save the file using a descriptive name, that will help you remember the content of the supplemental form that you are creating. When assigning a name to the file, please remember to give it the extension ".xfd" (for example, "My_Senior_Key.xfd"). If you do not name your file with the ".xfd" extension you will be unable to open it later, using your PureEdge viewer software.
- Using the "Open Form" tool on your PureEdge viewer, open the new form that you have just saved.
- Enter your additional Senior/Key Person information in this supplemental form. It is essentially the same as the Senior/Key person form that you see in the main body of your application.
- When you have completed entering information in the supplemental form, save it and close it.
- Return to this "Additional Senior/Key Person Form Attachments" page.
- Attach the saved supplemental form, that you just filled in, to one of the blocks provided on this "attachments" form.

Important: Please attach additional Senior/Key Person forms, using the blocks below. Please remember that the files you attach must be Senior/Key Person Pure Edge forms, which were previously extracted using the process outlined above. Attaching any other type of file may result in the inability to submit your application to Grants.gov.

1) Please attach Attachment 1
2) Please attach Attachment 2
3) Please attach Attachment 3
4) Please attach Attachment 4

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)

Filename

MimeType

Additional Biographical Sketch(es) (Senior/Key Person)

Filename

MimeType

Additional Current and Pending Support(s)

Filename

MimeType

Principal Investigator/Program Director (Last, First, Middle): Luby, Stephen Patrick

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Peter Daszak	Executive Director, Consortium for Conservation Medicine, Wildlife Trust
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bangor University (UK)	BSc. (hons)	1986	Zoology
University of East London (UK)	Ph.D	1994	Infectious Diseases

A. Positions and Honors

Positions and Employment

1989-1992 Research Assistant, University of East London
1993-1998 Senior Faculty Research Scientist, Kingston University
1999 Guest Researcher, Centers for Disease Control and Prevention (CDC)
1999-2001 Faculty Research Scientist, University of Georgia
2001- Adjunct Faculty, Tufts Univ. Sch. Veterinary Med.; Univ. Georgia; Columbia Univ.
2001- Executive Director, Consortium for Conservation Medicine
2007- Co-Director, Joint Institute for Wildlife & Zoonoses, ECNU, Shanghai, China

Other Experience and Professional Membership

2000 Keynote speaker Merieux Foundation Conference on Emerging paramyxoviruses, France
2002 National Academy of Sciences: gave evidence to panel on infectious disease & climate change
2002-2006 United Nations Millenium Ecosystem Assessment: Lead Author, human infectious diseases
2003- NIH: ad hoc member, ZRG1 IDM-G 90 study section: Virology, Biodefense & Emerg. Diseases
2005- NIH: ad hoc member, ZRG1 IRAP-Q study section (infectious diseases, epidemiology)
2004- Editorial Board, Conservation Biology (Blackwell); Founding Co-Editor *EcoHealth* (Springer)
2004-2005 National Research Council: Member, Committee on Future Needs in Veterinary Research
2004- Member of Scientific Committee (Treasurer 2007-), DIVERSITAS (UNESCO-ICSU).
2005- International Standing Advisory Committee, Australian Biosecurity Cooperative Research Center
2005 NIAID: Steering Committee, workshop on virus-host shifts & emergence of new pathogens
2006- Founding board of directors, Treasurer, International Association of Ecology and Health
2006 Keynote address, Pasteur Institute Shanghai annual conference on infectious diseases

Honors

1999 Meritorious service award, Centers for Disease Control and Prevention (CDC)
2000 Winner of the CSIRO silver medal for international collaborative research
2002 Daszak *et al.* (2000) *Science* paper cited by ISI as a "fast-breaking paper"
2003 Work on Nipah virus featured on CBS 60 Minutes
2003 6th Annual Lecturer in Medicine and Humanities, Texas A&M, 2003
2006 West Nile virus *PLOS Biology* paper cited as "editor's choice", *Science* 311: 1675
2007 Finalist, Director's Pioneer Award

B. Peer-reviewed publications (selected from 115)

*** = Corresponding author**

1. Ekbom A, **Daszak P**, Kraaz W & Wakefield AJ. Crohn's disease after *in utero* measles virus exposure. *Lancet* 1996; 348: 515-517.
2. Berger L, Speare R, **Daszak P**, et al. Chytridiomycosis causes amphibian population declines in the rain forests of Australia and Central America. *Proc. Natl Acad. Sci, USA* 1998; 95: 9031-9036.
3. **Daszak P**, Berger L, Cunningham AA, Hyatt AD, Green DE & Speare R Emerging infectious diseases & amphibian population declines. *Emerging Infectious Diseases* 1999; 5: 735-748.
4. **Daszak P**, Cunningham AA & Hyatt AD Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* 2000; 287: 443-449
5. **Daszak P**, Cunningham AA & Hyatt AD Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica* 2001; 78:103-116.
6. Mazzoni R, Cunningham AA, **Daszak P** et al. Emerging pathogen in frogs (*Rana catesbeiana*) farmed for international trade. *Emerging Infectious Diseases* 2003; 9: 995-998
7. Goldsmith CS, Whistler T, Rollin PE, Ksiazek TG, Rota PA, Bellini WJ, **Daszak P**, Wong KT, Shieh W-J & Zaki SR Elucidation of Nipah virus morphogenesis and replication using ultrastructural and molecular approaches. *Virus Research* 2003; 92: 89-98
8. Hyatt AD, **Daszak P**, Cunningham AA, Field H & Gould AR Henipaviruses: Gaps in the knowledge of emergence. *Ecohealth* 2004; 1: 25-38.
9. Field HE, Mackenzie J & **Daszak P** Novel viral encephalitides associated with bats (Chiroptera) – host management strategies. *Archives of Virology* 2004; S18: 113-121.
10. Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR & **Daszak P**. Emerging infectious diseases of plants. *Trends in Ecology and Evolution* 2004; 19: 535-544.
11. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, **Daszak P**, Eaton BT, Zhang S & Wang L-F Bats are natural reservoirs of SARS-like coronaviruses. *Science* 2005; 310: 676-679.
12. Olival KJ & **Daszak P** The ecology of emerging neurotropic viruses *J. Neurovirology* 2005; 11: 441-446
13. Kilpatrick AM, Kramer LD, Campbell S, Alleyne EO, Dobson AP & **Daszak P** West Nile virus risk and the bridge vector paradigm. *Emerging Infectious Diseases* 2005; 11: 425-429.
14. Wolfe ND, **Daszak P**, Kilpatrick AM & Burke DS. Bushmeat hunting, deforestation and prediction of zoonotic disease emergence. *Emerging Infectious Diseases* 2005; 11: 1822-1827.
15. Pulliam J, Field H, Olival KJ & the Henipavirus Ecology Research Group (**Daszak P.**). An alternative explanation of Nipah virus strain variation. *Emerging Infectious Diseases* 2005; 11: 1978-1979
16. Epstein JH, Rahman SA, Halpin K, Meehan G, Jamaluddin AA, Hassan SS, Field HE, Hyatt AD, **Daszak P** Feral cats (*Felis catus*) and risk for Nipah virus. *Emerging Infectious Diseases* 2006;12: 1178-1179.
17. Kilpatrick AM, **Daszak P**, Jones MJ, Marra PP & Kramer LD Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society: Biological Sciences* 2006; 273: 2327-2333.
18. Epstein JH, Field HE, Luby S, Pulliam JRC & **Daszak P.** Nipah Virus: Impact, Origins and Causes of Emergence. *Current Infectious Disease Reports* 2006; 8: 59-65
19. **Daszak P**, Plowright R, Epstein JH, Pulliam J, Abdul Rahman S, Field HE, Smith CS, Olival KJ, Luby S et al. The emergence of Nipah and Hendra virus: pathogen dynamics across a wildlife-livestock-human continuum. In: Collinge S & Ray S, Eds. *Disease Ecology*. Oxford Univ. Press 2006; 186-201
20. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP & **Daszak P** West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biology* 2006; 4: 606-610.
21. Mendelson JR, Lips KR, Gagliardo RW, Rabb GB, Collins JP, Diffendorfer JE, **Daszak P** *et al.* Policy Forum: Confronting amphibian declines and extinctions. *Science* 2006; 313: 48.
22. Wang L-F, Shi Z, Zhang S, Field H, **Daszak P** & Eaton BT. A review of bats and SARS: virus origin and genetic diversity. *Emerging Infectious Diseases* 2006; 12: 1834-1840.
23. Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP & **Daszak P**. Predicting the global spread of H5N1 avian influenza. *Proc. Natl. Acad. Sci., USA* 2006;103: 19368-19373.
24. Halpin K, Hyatt AD, Plowright RK, Epstein JH, **Daszak P**, Field HE, Wang L, Daniels PW and HERG. Emerging viruses: Coming in on a wrinkled wing and a prayer. *Current Infectious Disease Reports* 2007; 44: 711-717

25. Rodríguez JP, Taber AB, **Daszak P**. et al. Policy Forum: The globalization of conservation: A view from the South. *Science*; 317: 755-756.
26. Field HE, Mackenzie J & **Daszak P** Henipaviruses: Emerging paramyxoviruses associated with fruit bats. *Current Topics Microbiol. Immunol.* 2007; 315: 133-159.
27. **Daszak P**, Epstein JH, Kilpatrick AM, Aguirre AA, Karesh WB & Cunningham AA (2007). Collaborative research approaches to the role of wildlife in zoonotic disease emergence. *Current Topics Microbiol. Immunol.* 2007; 315: 463-475.
28. McLaughlin AB, Epstein JH, Prakash V, Smith CS, Field HE, **Daszak P** & Cunningham AA. Plasma biochemistry and hematological values for wild-caught flying foxes (*Pteropus giganteus*) in India. *J. Zoo. Wildl. Med.* 2007; 38: 446-452.
29. Cui J, Han N, Streicker D, Li G, Tang X, Shi Z, Hu Z, Zhao G, Fontanet A, Yi G, Wang L, Jones G, .Field HE, **Daszak P* (Corresponding Author)** & Zhang, S. Evolutionary relationships between bat coronaviruses and their hosts. *Emerging Infectious Diseases* 2007;13: 1526-1533
30. (b) (4)
31.
32.
33.

C. Research Support

ONGOING RESEARCH SUPPORT

N01 AI-25490 Kramer (PI) 10/01/02 - 10/01/09
NIH/NIAID
West Nile & pox viruses: ecology, pathogenesis & immunity
This subcontract provides partial salary for a postdoc to conduct field studies, mathematical modeling and analysis of the ecology of West Nile virus in the USA.
Role: PI on a subcontract, oversee research on WNV ecology.

NSF EF-062239 Kilpatrick (PI) 09/01/06 - 08/30/11
National Science Foundation/National Institutes of Health: Ecology of Infectious Diseases program
Predicting spatial variation in West Nile virus transmission
This project is to assess the interaction between vector populations, reservoir host populations and West Nile virus across an urban-to-rural human density gradient in the northeastern USA.
Role: Co-PI, planning and executing research on WNV ecology

NSF RCN Charles Perrings (PI) 02/01/07 – 01/31/10
NSF Research Coordination Network
Biodiversity and Ecosystem Services Training Network (BESTNet)
This project is to provide interdisciplinary research and training among diverse disciplines including ecologists and health scientists.
Role: Co-PI, responsible for program on biodiversity and infectious diseases

COMPLETED RESEARCH SUPPORT (during last 3 years)

R01 TW05869 Daszak 08/01/02 - 05/31/07
NIH/Fogarty International Center
Anthropogenic change & emerging zoonotic paramyxoviruses

This project investigated anthropogenic factors that drove the emergence of Nipah and Hendra viruses in Malaysia and Australia.
Role: PI, directed all research on Nipah and Hendra virus ecology, virology and pathology

DEB 02133851 Collins (PI) 10/01/03 - 09/30/06
National Science Foundation
Emerging diseases of wildlife: Threats to amphibian conservation
This project was to assess the role of environmental factors and emerging diseases on the global decline of amphibian populations.
Role: PI on subcontract, directed research on disease ecology and pathogenesis

HSD 0525216 Daszak (PI) 10/15/05-10/14/06
National Science Foundation: Human and Social Dynamics
Collaborative Research: Socio-Economic and Environmental Drivers of Emerging Diseases
National Science Foundation
This project was to analyze patterns of disease emergence globally and produce a broad risk assessment.
Role: PI, directed research on global patterns of disease emergence.

Principal Investigator/Program Director (Last, First, Middle): Lipkin, W. Ian

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
W. Ian Lipkin	Professor
eRA COMMONS USER NAME (b) (6)	

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Sarah Lawrence College, Bronxville, NY	B.A.	1974	Liberal Arts
Rush Medical College, Chicago, IL	M.D.	1978	Medicine

A. Positions and Honors.

Positions and Employment

1977-78 Clinical Clerk, Institute of Neurology, Queen Square, London, UK
1978-79 Intern in Medicine, Presbyterian Hospital, University of Pittsburgh, Pittsburgh, PA
1979-81 Resident in Medicine, University of Washington, Seattle, WA
1981-84 Resident in Neurology, University of California, San Francisco, CA
1984-90 Postdoctoral Fellow (Michael Oldstone & Floyd Bloom), Scripps Research Institute, La Jolla, CA
1990-02 Asst. Professor (1990-93), Assoc. Professor (1993-96), Professor (1996-02), Neurology; Anatomy & Neurobiology; Microbiology & Molecular Genetics, University of California Irvine, Irvine, CA
1996-97 Sabbatical Professor, Institut für Virologie und Immunbiologie, Universität Würzburg, Germany
1996-02 Adjunct Professor, Neuropharmacology, The Scripps Research Institute, La Jolla, CA
2000-02 Louise Turner Arnold Professor of Neuroscience, University of California Irvine, Irvine, CA
2002-07 Jerome L. and Dawn Greene Professor of Epidemiology; Director, Jerome L and Dawn Greene Infectious Disease Laboratory, Mailman School of Public Health; Professor of Neurology and Pathology, College of Physicians & Surgeons; Columbia University, New York, NY
2003- Principal Investigator and Scientific Director, Northeast Biodefense Center, Region II NIAID Regional Center of Excellence for Biodefense and Emerging Infectious Diseases
2003- Dalldorf Research Physician, Wadsworth Center, New York State Dept of Health
2007- Professor of Epidemiology, Neurology and Pathology; Director, Center for Infection and Immunity; Mailman School of Public Health and College of Physicians & Surgeons; Columbia University, New York, NY

Other Experience and Professional Memberships

Amer Bd of Internal Medicine, 1981; Amer Bd of Psychiatry and Neurology, 1986; National MS Soc Advisory Com on Fellowships, 1991-94; PI, UCI-Markey Program in Human Neurobiology, 1994-99; Founding Chair, Scientific Advisory Bd, Cure Autism Now Fdn, 1998-2000; Advisory Bd, 1st Intl Conf on Emerging Zoonoses, 1996; Organizer, Keystone Symp on Infections of the Nervous System, 1998; NCI/NIAID Blue Ribbon Panel on New Approaches to Identifying Infectious Etiologies of Chronic Disease, 1999; Bio-Centric Operations, US Joint Warfighting Center (bioterrorism), 1999; Organizer, NIAID Blue Ribbon Panel on Neurovirology, 2000; Organizer, Banbury Conf on Microbiology, Immunology and Toxicology of Autism and Other Neurodevelopmental Disorders, 2000; Organizer, Infectious Etiologies of Neuropsychiatric Disorders, World Congress Biol Psychiatry, Berlin, 2001; Organizer, FASEB Conf Microbial Pathogenesis, 2002; NCI Blue Ribbon Panel, Microbial Infection and Human Cancer, 2002; Scientific Advis Bd, 454 Life Sciences Corp, 2003; WHO SARS Lab Network, 2003; External Reviewer, Bd of Scientific Counselors, NIMH, 2003; Founding Chair, Emerging Infectious Diseases Discussion Group, NY Acad of Sciences, 2003; WHO Lab Network, 2004.

Honors

National MS Soc Postdoc Fellow, 1984; Silver Medal for Claret (Amateur) Sonoma County Fair, 1985; NINDS Clinical Investigator Development Award, 1987; National Alliance for Research in Schizophrenia and Depression Young Investigator, 1991; Pew Scholar Biomedical Sciences, 1991; State-of-the-Art Lecturer, American Soc Virology, 1997; Lecturer, XXIst Collegium Internationale Neuropsychopharmacologicum, 1997; Lecturer, 50th Anniversary NIAID/NIH, 1998; Visiting Professor, Japanese Health Sci Fdn, 1999; Visiting Bruenn Professor, Columbia Univ 2000; Millenium Commencement Speaker, Sarah Lawrence College, 2000; American Soc for Microbiol/Waksman Fdn Lecturer, 2001; Ellison Medical Fdn Senior Scholar in Global Infectious Diseases, 2002; Distinguished Lecturer, Institute of Genomics and Bioinformatics, UC Irvine, 2003; Special Advisor for Ministry of Science & Technology, People's Republic of China, 2003; Advisory Board, Guangzhou Ctr Biomedicine and Health, 2003; Dalldorf Res Physician NYS Dept of Health, 2003; Advisory Board, Institut Pasteur de Shanghai; Fellow, NY Academy of Sciences, 2003; CDC Distinguished Lecturer,

2005; Honorary Director, Beijing Infectious Disease Ctr, 2005; Visiting Professor Beijing University; Fellow, American Soc for Microbiol, 2006; Alumnae Citation for Achievement and Service, Sarah Lawrence College, 2006.

B. Selected peer-reviewed publications (in chronological order).

1. **Lipkin WI**, Parry G, Kiprov D, Abrams D (1985) Inflammatory neuropathy in homosexual men with lymphadenopathy. Neurology 35, 1479
2. Panitch HS, Francis GS, Hooper CJ, Messing RO, **Lipkin WI** (1985) Immunologic studies in patients with acquired immune deficiency syndrome. Ann NY Acad Sci 437, 413
3. **Lipkin WI**, Battenberg ELF, Bloom FE, Oldstone MBA (1988) Viral infection of neurons can depress neurotransmitter mRNA levels without histologic injury. Brain Res 451, 333
4. **Lipkin WI**, Carbone KM, Duchala CS, Narayan O, Oldstone MBA (1988) Neurotransmitter abnormalities in Borna disease. Brain Res 475, 366
5. **Lipkin WI**, Travis GH, Carbone KM, Wilson MC (1990) Isolation and characterization of Borna disease agent cDNA clones. Proc Natl Acad Sci USA 87, 4184
6. Briese T, de la Torre JC, Lewis A, Ludwig H, **Lipkin WI** (1992) Borna disease virus, a negative-strand RNA virus, transcribes in the nucleus of infected cells. Proc Natl Acad Sci USA 89, 11486
7. Briese T, Schneemann A, Lewis AJ, Park Y, Kim S, Ludwig H, **Lipkin WI** (1994) Genomic organization of Borna disease virus. Proc Natl Acad Sci USA 91, 4362
8. Schneider PA, Schneemann A, **Lipkin WI** (1994) RNA splicing in Borna disease virus, a non-segmented, negative-strand RNA virus. J Virol 68, 5007
9. Schwemmle M, De B, Shi L, Banerjee A, **Lipkin WI** (1997) Borna disease virus P-protein is phosphorylated by protein kinase Cε and casein kinase II. J Biol Chem 272, 21818
10. Schwemmle M, Salvatore M, Shi L, Lee C, **Lipkin WI** (1998) Interactions of the Borna disease virus P, N, and X proteins and their functional implications. J Biol Chem 273, 9007
11. Hatalski CG, Hickey WF, **Lipkin WI** (1998) Evolution of the immune response in the central nervous system during experimental Borna disease. J Neuroimmunol 90, 137
12. Hornig M, Weissenböck H, Horscroft N, **Lipkin WI** (1999) An infection-based model of neurodevelopmental damage. Proc Natl Acad Sci USA 96, 12102
13. Briese T, Jia X-J, Huang C, Grady LJ, **Lipkin WI** (1999) Identification of a Kunjin/West Nile-like flavivirus in brains of New York encephalitis patients. Lancet 354, 1261
14. Jia X-J, Briese T, Jordan I, Rambaut A, Chi HC, Mackenzie JS, Hall RA, Scherret J, **Lipkin WI** (1999) Genetic analysis of the West Nile New York 1999 encephalitis virus. Lancet 354, 1971
15. Walker MP, Schlaberg R, Hays AP, Bowser R, **Lipkin WI** (2001) Absence of echovirus sequences in brain and spinal cord of ALS patients. Ann Neurol 49, 249
16. Briese T, Rambaut A, Pathmajeyan M, Bishara J, Weinberger M, Pitlik S, **Lipkin WI** (2002) Phylogenetic analysis of a human isolate from the 2000 Israel West Nile virus epidemic. Emerg Infect Dis 8, 528
17. Zhai J, Briese T, Dai E, Wang X, Pang X, Du Z, Liu H, Wang J, Wang H, Guo Z, Chen Z, Jiang L, Zhou D, Han Y, Jabado O, Palacios G, **Lipkin WI**, Tang R (2004) Real-time polymerase chain reaction for detecting SARS coronavirus, Beijing, 2003. Emerg Infect Dis 10, 300
18. Hoffman KL, Hornig M, Yaddanapudi K, Jabado O, **Lipkin WI** (2004) A murine model for neuropsychiatric disorders associated with group A β-hemolytic streptococcal infection. J Neurosci 24,1780
19. Qiao M, Mundrigi A, Bernard KA, Palacios G, Zhou ZH, **Lipkin WI**, Jake Liang TJ (2004) Induction of sterilizing immunity against West Nile virus by immunization with West Nile virus-like particles produced in insect cells. J. Infectious Dis 190, 2104-2108
20. Briese T, Palacios G, Kokoris M, Jabado O, Liu Z, Renwick N, Kapoor V, Casas I, Pozo F, Limberger R, Perez-Brena P, Ju J, **Lipkin WI** (2005). Diagnostic system for rapid and sensitive differential detection of pathogens. Emerg Infect Dis 11, 310-313
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25. Briese T, Bird B, Kapoor V, Nichol ST, **Lipkin WI** (2006) Batai and Ngai virus: M- segment reassortment and association with severe disease in East Africa. J Virol 80, 5627-5630
26. Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St. George K, Briese T, **Lipkin WI** (2006) MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, causing influenza-like illness in New York State, 2004-2005/ J Infect Dis, Nov 15; 194 (10): 1398-402
27. Yaddanapudi K, Palacios G, Towner JS, Nichol ST, Sariol C, **Lipkin WI** (2006) Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. FASEB, 20, 2519
28. Jabado OJ, Palacios G, Kapoor V, Hui J, Renwick N, Zhai J, Briese T, **Lipkin WI** (2006) Greene SCPrimer: a rapid comprehensive tool for designing degenerate primers from multiple sequence alignments. *Nucleic Acids Res*, 34, 6605
29. Zhai J, Palacios G, Towner JS, Jabado O, Kapoor V, Venter M, Grolla, A, Briese T, Paweska J, Swanepoel R, Feldman H, Nichol ST, **Lipkin WI** (2006) A rapid molecular strategy for filovirus detection and characterization. J Clin Microbiol, 45, 224
30. Palacios G, Quan P-L, Jabado OJ, Conlan S, Hirschberg DL, Liu Y, Zhai J, Renwick N, Hui J, Hegyi H, Grolla A, Strong JE, Towner JS, Geisbert TW, Jahrling PB, Büchen-Osmond C, Ellerbrok H, Sanchez-Seco MP, Lussier Y, Formenty P, Nichol ST, Feldmann H, Briese T, **Lipkin WI** (2007) Panmicrobial oligonucleotide array for diagnosis of infectious diseases. *Emerg Infect Dis*, 13, 73, http://www.cdc.gov/ncidod/EID/13/1/73.htm
31. Quan P-L, Palacios G, Jabado OJ, Conlan S, Hirschberg DL, Richt J, Pozo F, Casas I, Perez-Breña P, Drysdale A, Hui J, Cisterna D, Baumeister E, Savy V, Garcia-Sastre A, Briese T, **Lipkin WI** (2007) Detection of respiratory viruses and subtype identification of influenza viruses by GreeneChipResp oligonucleotide microarray. *J Clin Microbiol*, 45, 2359.
32. (b) (4)
33.
34.

C. Research Support.

Ongoing Research Support

(b) (4) Lipkin (PI) 10/01/01 to 09/30/07
(b) (4)

Establish and implement new high throughput molecular methods for microbial surveillance.

U54 AI1057158 Lipkin (PI) 09/04/03 to 02/29/08
Northeast Biodefense Center
Establish a Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research.

U01 NS047537 Lipkin (PI) 09/30/03 to 05/31/08
Gene:Environment Interactions in an Autism Birth Cohort
Establish a 100,000 child prospective birth cohort in Norway, collect clinical data and samples, map the natural trajectory of neurodevelopmental disorders, and establish a foundation for determining the role of gene-environment interactions in pathogenesis of neurodevelopmental disorders.

UC1 AI062705 Lipkin (PI) 09/30/04 to 08/31/08
MassTag PCR Detection of Respiratory Pathogens
Establish a multiplex PCR platform for differential diagnosis of acute respiratory disease.

HL083850 Lipkin (PI) 05/08/06 to 04/30/10
Pathogen Discovery in Chronic Lung Disease by Mass Tag PCR and Microarrays
Employ high throughput molecular diagnostic tools to survey for pathogen discovery in idiopathic pulmonary fibrosis, pulmonary arterial hypertension and bronchiolitis obliterans syndrome.

1U01AI070411 Lipkin (PI) 09/01/06 to 08/31/11
Viral Arrays for Biodefense
Establish and validate a viral sequence database and its complementary oligonucleotide array technology for detection and differentiation of influenza viruses and hemorrhagic fever viruses.

1 R24 EY017404 Hageman (PI, Univ of Iowa) 08/01/06-07/31/11
Subcontract to Columbia (Lipkin) from the Univeristy of Alabama
Development of Complement Modulating Therapeutics for AMD
The sub-contract will survey clinical samples (eyes and blood) for evidence of infection using two novel molecular diagnostic platforms, Mass Tag PCR and GreeneChips.

HHSN266200400036C Lefkowitz (PI, Univ Alabama) 06/30/06 to 06/28/09
Subcontract to Columbia (Lipkin) from the Viral Bioinformatics Resource Center
ICTVdB: A Virus Database for Biodefense and Emerging Infectious Disease Research
Curate and improve the user interface of the electronic database of the International Committee for Taxonomy of Viruses.

Completed Research Support

R01 AI51292 Lipkin (PI) 07/01/02 to 06/30/07
A Staged Strategy for Virus Identification and Discovery
Establish an integrated program in bioinformatics and molecular diagnostics focused on investigating the role of infection in neurologic diseases and cancer.

CDC/American Academy of Pediatrics Lipkin (PI) 09/30/02 to 09/29/06
MV Sequences in Children with Autistic Disorders
Determine whether autism is associated with the presence of measles virus sequences in gastrointestinal tract through blinded analysis in three laboratories (Columbia, CDC, Coombe Women's Hospital).

HD37546 Lipkin (PI) 05/01/00 to 04/30/06
A Developmental Model for Autism Based on CNS Infection

AI55466 Rewers (PI, Univ Colorado) 10/01/02 to 09/30/04
Subcontract to Columbia (Lipkin)
Viral Triggers of Type I Diabetes

NS29425 Lipkin (PI) 07/01/98 to 06/30/03
Molecular Analysis of a Neurotropic Agent, Borna Virus

MH57467 Lipkin (PI) 07/01/99 to 06/30/03
Borna Disease Virus and Neuropsychiatric Disease

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Bruce Andrew Mungall	Research Scientist
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Queensland	B.Sc.	1991	Biological Science
University of Queensland	B.Sc. (Hons)	1992	Physiology & Pharmacology
University of Queensland	Ph.D.	2000	Veterinary Science

A. Positions

1996-2000	Doctoral Candidate, Department of Companion Animal Medical Science, School of Veterinary Science, University of Queensland, St. Lucia, Queensland, Australia
2000-2001	Post Doctoral Fellow, Department of Pediatrics, Division of Infectious Diseases, Epidemiology and Immunology, Emory University School of Medicine, Atlanta, Georgia.
2001-2002	Post Doctoral Fellow, Strain Surveillance Section, Influenza Branch, Division of Viral and Rickettsial Diseases, Centres for Disease Control and Prevention, Atlanta, Georgia.
2003	Post Doctoral Fellow, Metabolic Research Unit, School of Science, Deakin University, Waurn Ponds, Victoria, Australia
2004	Post Doctoral Fellow, Pituitary Research Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia
2004-2006	Research Scientist, Australian Animal Health Laboratory, Commonwealth Science and Industry Organisation, Geelong, Victoria, Australia
2007-present	Project Leader, Henipavirus Therapeutics, Australian Animal Health Laboratory, Commonwealth Science and Industry Organisation, Geelong, Victoria, Australia

Honours

2006	Smart Geelong Network Researcher of the Year (Bruce Mungall, Mark Rechenberg, Rob Hensel and Dayna Johnson) in the category of Animal Health for the development of radio telemetry monitoring systems for temperature monitoring of animals at BSL4.
2007	Recipient of the Eric French Fellowship (awarded by CSIRO Livestock Industries) to enable the acquisition and establishment of primary cell line transformation technology from US collaborators.
2007	Smart Geelong Network Researcher of the Year (Nick Schopman, Terry Wise, Tim Doran and Bruce Mungall) in the category of Biotechnology for the application of RNAi toward a therapeutic intervention for Nipah and Hendra virus.

B. Selected Publications.

1. **Mungall, B.A.**, Shinkel, T.A., Sernia, C. (1995) Immunocytochemical localization of angiotensinogen in the fetal and neonatal rat brain. Neuroscience. 67(2):505-24.

2. Wright, J.W., Clemens, J.A., Panetta, J.A., Smalstig, E.B., Weatherly, L.A., Kramar, E.A., Pederson, E.S., **Mungall, B.A.**, Harding, J.W. (1996) Effects of LY231617 and angiotensin IV on ischemia-induced deficits in circular water maze and passive avoidance performance in rats. Brain Res. 717(1-2):1-11.

3. **Mungall, B.A.**, Pollitt, C.C., Collins, R. (1998) Localisation of gelatinase activity in epidermal hoof lamellae by in situ zymography. Histochem Cell Biol.110(5):535-40.

4. **Mungall, B.A.**, Pollitt, C.C. (1999) Zymographic analysis of equine laminitis. Histochem Cell Biol. 112(6):467-72.

5. **Mungall, B.A.**, Kyaw-Tanner, M., Pollitt, C.C. (2001) In vitro evidence for a bacterial pathogenesis of equine laminitis. Vet Microbiol. 79(3):209-23.

6. **Mungall, B.A.**, Pollitt, C.C. (2001) In situ zymography: topographical considerations. J Biochem Biophys Methods. 47(3):169-76.

7. **Mungall, B.A.**, Pollitt, C.C. (2002) Thermolysin activates equine lamellar hoof matrix metalloproteinases. J Comp Pathol. 126(1):9-16.

8. Xu, X., Smith, C.B., **Mungall, B.A.**, Lindstrom, S.E., Hall, H.E., Subbarao, K., Cox, N.J., Klimov, A. (2002) Intercontinental circulation of human influenza A(H1N2) reassortant viruses during the 2001-2002 influenza season. J Infect Dis. 186(10):1490-3.

9. Loukopoulos, P., **Mungall, B.A.**, Straw, R.C., Thornton, J.R., Robinson, W.F. (2003) Matrix metalloproteinase-2 and -9 involvement in canine tumors. Vet Pathol. 40(4):382-94.

10. **Mungall, B.A.**, Xu, X., Klimov, A. (2003) Assaying susceptibility of avian and other influenza A viruses to zanamivir: comparison of fluorescent and chemiluminescent neuraminidase assays. Avian Dis. 47(3 Suppl):1141-4.

11. Xu, X., Lindstrom, S.E., Shaw, M.W., Smith, C.B., Hall, H.E., **Mungall, B.A.**, Subbarao, K., Cox, N.J., Klimov, A. (2004) Reassortment and evolution of current human influenza A and B viruses. Virus Res. 103(1-2):55-60.

12. **Mungall, B.A.**, Xu, X., Klimov, A. (2004) Surveillance of influenza isolates for susceptibility to neuraminidase inhibitors during the 2000-2002 influenza seasons. Virus Res. 103(1-2):195-7.

13. Loukopoulos, P., O'Brien, T., Ghoddusi, M., **Mungall, B.A.**, Robinson, W.F. (2004) Characterisation of three novel canine osteosarcoma cell lines producing high levels of matrix metalloproteinases. Res Vet Sci.77(2):131-41.

14. Bossart, K.N., Crameri, G., Dimitrov, A.S., **Mungall, B.A.**, Feng, Y.R., Patch, J.R., Choudhary, A., Wang, L.F., Eaton, B.T., Broder, C.C. (2005) Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. J Virol. 79(11):6690-702.

15. Bonaparte, M.I., Dimitrov, A.S., Bossart, K.N., Crameri, G., **Mungall, B.A.**, Bishop, K.A., Choudhry, V., Dimitrov, D.S., Wang, L.F., Eaton, B.T., Broder, C.C. (2005) Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. Proc. Natl. Acad. Sci. U.S.A. 26;102(30):10652-7.

16. Bossart, K.N., **Mungall, B.A.**, Crameri, G., Wang, L.F., Eaton, B.T., Broder, C.C. (2005) Inhibition of Henipavirus fusion and infection by heptad-derived peptides of the Nipah virus fusion glycoprotein. Virol J. 18;2:57.

17. Zhu. Z., Dimitrov, A.S., Bossart, K.N., Crameri, G., Bishop, K.A., Choudhry, V., **Mungall, B.A.**, Feng, Y.R., Choudhary, A., Zhang, M.Y., Feng, Y., Wang, L.F., Xiao, X., Eaton, B.T., Broder, C.C., Dimitrov, D.S. (2006) Potent neutralization of hendra and nipah viruses by human monoclonal antibodies. J Virol.80(2):891-9.

18. **Mungall, B.A.**, Middleton, D., Crameri, G., Bingham, J., Halpin, K., Russell, G., Green, D., McEachern, J., Pritchard, L.I., Eaton, B.T., Wang, L.F., Bossart, K.N., Broder, C.C. (2006) Feline model of acute nipah

virus infection and protection with a soluble glycoprotein-based subunit vaccine. J. Virol. 80(24): 12293-302.

19. **Mungall, B.A.**, Middleton, D., Crameri, G., Halpin, K., Bingham, J., Eaton, B.T. and Broder, C.C. (2007) Vertical transmission and fetal replication of Nipah virus in an experimentally infected cat. J.I.D. 196(6): 812-6.

20. Porotto, M., Carta, P., Deng, Y., Kellogg, G.E., Whitt, M., Lu, M., **Mungall, B.A.**, Moscona, A. (2007) Molecular determinants of antiviral potency of paramyxovirus entry inhibitors. *J Virol.* Jul 25 (Epub).

21. Halpin, K. and **Mungall, B.A.** (2007) Recent progress in henipavirus research. Comparative Immunology, Microbiology & Infectious Diseases Jul 13 (Epub).

C. Research Support
Previous None

Current None

Completed (last 3 years)
"Nipah Virus and Hendra Virus Peptide Therapeutics"
Principal Investigator: Christopher C. Broder, Ph.D.
Agency: NIH/NIAID, Type 1 U01 AI056423-01 Period: September 15, 2003 to February 28, 2007
Major goals: 1. Establish virus infection, lethal dose, and detection parameters of Nipah virus in the cat model. 2. Design and synthesize second-generation, capped, heptad peptides and develop assay procedures for peptide detection in cat blood/plasma. 3. Evaluate the pharmacokinetics and determine the serum half-life of the peptide in the cat. 4. Determine the efficacy of heptad peptide in Nipah virus infected cats. Bruce Mungall PhD. Research Scientist responsible for performing and achieving all activities listed. (No overlap).

Principal Investigator/Program Director (Last, First, Middle):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kate Elizabeth Jones	Research Fellow, Institute of Zoology, Zoological Society of London
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Leeds (UK)	B.Sc.	1993	Zoology (with honors)
Roehampton University (UK)	Ph.D.	1998	Zoology
Imperial College (UK)	Post Doc	1999-2000	Biodiversity
University of Virginia (US)	Post Doc	2000-2003	Biodiversity

A. Positions and Honors

Positions and Employment

1999	London Conservation Officer, Bat Conservation Trust (UK)
1999-2000	Postdoctoral Research Assistant, Imperial College (UK)
2000-2003	Postdoctoral Research Asscciate, University of Virginia (USA)
2003-2005	Research Fellow, Earth Institute, Columbia University (US)
2005-	Research Fellow, Institute of Zoology, Zoological Society of London (UK)
2005-	Honorary Research Fellow, University College London (UK)
2005-	Adjunct Faculty, University of Cambridge (UK)
2006-	Associate, Consortium for Conservation Medicine (US)

Other Experience and Professional Membership

2006-	Journal Editor - Global Ecology and Biogeography
2006-	Member of IUCN Species Specialist Conservation Group, Chiroptera
2006-	Chair of IUCN Advisory Group on Historical Extinctions
2006-	Trustee of The Bat Conservation Trust (UK)

Workshop participant for the following:

- *Research Coordination Network: TraitNet.* Columbia University, New York, US (2007-2010)
- *Research Coordination Network: Integrating Macroecological Pattern and Processes across Scales.* University of New Mexico, US (2007-2010).
- *Global Biodiversity Indicators Development.* Zoological Society of London & Imperial College, UK (2006-2009).
- *Infectious Disease and Host Behavior.* National Center for Ecological Analysis and Synthesis, University of California, US (2001-2003).
- *Role of Pathogens in the Conservation of Biological Diversity.* Conservation International, US, (2002-2006).

Honors

- Nominated in 2006 for the Philip Leverhulme Prize for recognition of original and significant contributions to knowledge in zoology.
- My research has been widely reported in the local, national and international press (including BBC, CNN, The Daily Telegraph UK, Discover Magazine, BBC Wildlife, The Economist, Radio 4 UK, Radio Scotland UK, National Public Radio USA, New Scientist UK, New York Times USA; The Sun UK, Washington Post USA).

B. Peer-reviewed publications (selected from 55)

1. Publications: Peer Reviewed Journals

1. 1997. ***Jones K.E.** and A. Purvis. An optimum body size for mammals? Comparative evidence from bats. *Functional Ecology* 11:751-756.
2. 2000. Purvis A., **K.E. Jones**, and G. Mace. Extinction. *Bioessays* 22:1123-1133.
3. 2001. Gittleman J.L., M.E. Gompper and **K.E. Jones**. Extinction: complexity of assessing risk. *Science* 292:217-218.
4. 2001. Hosken D., **K.E. Jones**, K. Chipperfield and A. Dixson. Is the bat os penis sexually selected? *Behavioural Ecology and Sociobiology* 50:450-460.
5. 2001. ***Jones K.E.** and A. MacLarnon. Bat life-histories: testing models of mammalian life history evolution. *Evolutionary Ecology Research* 3:465-476.
6. 2001. ***Jones K.E.**, K.E. Barlow, N. Vaughan, A. Rodriguez-Duran and M. Gannon. Short-term impacts of extreme environmental disturbance on the bats (Chiroptera) of Puerto Rico. *Animal Conservation* 4:56-66.
7. 2002. Hewitt G., A. MacLarnon and **K.E. Jones**. The functions of laryngeal air sacs in primates: a new hypothesis. *Folia Primatologica* 73:70-94.
8. 2002. ***Jones K.E.**, A. Purvis, A. MacLarnon, O.R.P. Bininda-Emonds and N.B. Simmons. A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biological Reviews* 77:223-259.
9. 2003. Altizer S., C.L. Nunn, P.H. Thrall, J.L. Gittleman, J. Antonovics, A.A. Cunningham, A.P. Dobson, V. Ezenwa, **K.E. Jones**, A.B. Pedersen, M. Poss and J.R.C. Pulliam. Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution and Systematics* 34:517-547.
10. 2003. Bininda-Emonds O.R.P., **K.E. Jones**, S.A. Price, R. Grenyer, M. Cardillo, M. Habib, A. Purvis and J.L. Gittleman. Supertrees are a necessary not-so-evil: A comment on Gatesy *et al*. *Systematic Biology* 52:724-729.
11. 2003. ***Jones K.E.**, A. Purvis and J.L. Gittleman. Biological correlates of extinction risk in bats. *American Naturalist* 161:601-614.
12. 2003. Nunn C.L., S. Altizer, **K.E. Jones** and W. Sechrest. Comparative tests of parasite species richness in primates. *American Naturalist* 162:597-614.
13. 2003. Purvis A., A. Webster, P-M. Agapow, **K.E. Jones** and N.J.B. Isaac. Primate life histories and phylogeny. In: *Primate Life Histories and Socioecology* (eds. P.M . Kappeler and M. Pereira), pp 25-40. Chicago: University of Chicago Press.
14. 2003. Smith F.A., S.K. Lyons, S.K.M. Ernest, **K.E. Jones**, D.M. Kaufman, T. Dayan, P.A. Marquet, J.H. Brown, and J.P. Haskell. Body mass of late Quaternary mammals. *Ecology* 84: 3403.
15. 2004. Bininda-Emonds O.R.P., **K.E. Jones**, S.A. Price, M. Cardillo, R. Grenyer, and A. Purvis. Garbage in, garbage out: Data issues in supertree construction. In: *Phylogenetic supertrees: Combining information to reveal the Tree of Life* (ed. O.R.P. Bininda-Emonds). Computational Biology Series, Vol 4, pp 267-280. Kluwer Academic Publishers, Dordrecht, The Netherlands.
16. 2004. Blackburn T.M., **K.E. Jones**, P. Cassey and N. Losin. The influence of spatial resolution on macroecological patterns of range size variation: a case study using parrots (Psittaciformes) of the world. *Journal of Biogeography* 31:285-293.
17. 2004. Cassey P., T.M. Blackburn, G.J. Russell, **K.E. Jones** and J.L. Lockwood. Influences in the transport and establishment of traded bird species: a comparative analysis of the parrots (Psittacidae) of the world. *Global Change Biology* 10:417-426.

18. 2004. Cassey P., T.M. Blackburn, **K.E. Jones** and J.L. Lockwood. Mistakes in the analysis of exotic species establishment: source pool designation and correlates of introduction success among parrots (Psittaciformes) of the world. *Journal of Biogeography* 31:277-284
19. 2004. Gittleman J.L., **K.E. Jones** and S.A. Price. Supertrees: using complete phylogenies in comparative biology. In: *Phylogenetic supertrees: combining information to reveal the Tree of Life* (ed. O.R.P. Bininda-Emonds). Computational Biology Series, Vol 4, pp 439-460. Kluwer Academic Publishers, Dordrecht, The Netherlands.
20. 2004. ***Jones K.E.** and A.M. MacLarnon. Affording larger brains: testing hypotheses of mammalian brain evolution on bats. *American Naturalist* 164:20-31.
21. 2004. Maurer B.A., J.H. Brown, T. Dayan, B.J. Enquist, S.K.M. Ernest, E.A. Hadly, J.P. Haskell, D. Jablonski, **K.E. Jones**, D.M. Kaufman, S.K. Lyons, K.J. Niklas, W.P. Porter, K. Roy, F.A. Smith, B. Tiffney and W.R. Willig. Similarities in body size distributions of small-bodied flying vertebrates. *Evolutionary Ecology Research* 6:783-797.
22. 2004. Nunn C.L., S. Altizer, W. Sechrest, **K.E. Jones**, R.A. Barton and J.L. Gittleman. Parasites and the evolutionary diversification of primate clades. *American Naturalist* 164: 90-103.
23. 2004. Smith F.A., J.H. Brown, J.P. Haskell, S.K. Lyons, J. Alroy, E.L. Charnov, T. Dayan, B.J. Enquist, S.K.M. Ernest, E.A. Hadly, **K.E. Jones**, D.M. Kaufman, P.A. Marquet, B.A. Maurer, K.J. Niklas, W.P. Porter, B. Tiffney and M.R. Willig. Similarity of mammalian body size across the taxonomic hierarchy and across space and time. *American Naturalist* 163:672-691.
24. 2005. Cardillo M., G.M. Mace, **K.E. Jones**, J. Bielby, O.R.P. Bininda-Emonds, W. Sechrest, C.D.L. Orme and A. Purvis. Multiple causes of high extinction risk in large mammal species. *Science* 309:1239-1241.
25. 2005. Isaac N.J.B., **K.E. Jones**, J.L Gittleman and A. Purvis. Correlates of species richness in mammals: Body size, life-history and ecology. *American Naturalist* 165:600-607.
26. 2005. ***Jones K.E.**, O.R.P. Bininda-Emonds and J.L. Gittleman. Bats, clocks and rocks: diversification patterns in Chiroptera. *Evolution* 59:2243-2255.
27. 2005. ***Jones K.E.**, W. Sechrest and J.L. Gittleman. Age and area revisited: identifying global patterns and implications for conservation. In: *Phylogeny and Conservation* (eds. A. Purvis, J.L. Gittleman and T. Brooks), pp 141-165. Cambridge: Cambridge University Press.
28. 2006. Cruz-Neto A.P. and **K.E. Jones**. Exploring the evolution of the basal metabolic rates of bats. In: *Functional and Evolutionary Ecology of Bats* (eds. A. Zubaid, G.F. McCracken and T.H. Kunz), pp 56-89. New York: Oxford University Press.
29. 2006. Grenyer, R., C. D.L. Orme, S.F. Jackson, G.H. Thomas, R.G. Davies, T.J. Davies, **K.E. Jones**, V.A. Olson, R.S. Ridgely, P.C. Rasmussen, T-S Ding, P.M. Bennett, T.M. Blackburn, K.J. Gaston, J.L. Gittleman and I.P.F. Owens. The global distribution and conservation of rare and threatened vertebrates. *Nature* 444:93-96.
30. 2006. Pitnick S., **K.E. Jones** and G.S. Wilkinson. Mating system and brain size in bats. *Proceedings of the Royal Society of London, Series B*. 273:719-724.
31. 2007. Bielby J.N., G.M. Mace, O.R.P. Bininda-Emonds, M. Cardillo, J.L. Gittleman, **K.E. Jones**, D. Orme and A. Purvis,. The fast-slow continuum in mammalian life history: an empirical re-evaluation. *American Naturalist* 169:748-757.
32. 2007. Bininda-Emonds, O.R.P., M. Cardillo, **K.E. Jones**, R.D.E. MacPhee, R.M.D. Beck, R. Grenyer, S.A. Price, R. Vos, J.L. Gittleman & A. Purvis. The delayed rise of present-day mammals. *Nature* 446:507-512.
33. 2007. Davies, R.G., C. D.L. Orme, A.J. Webster, **K.E. Jones**, T.M. Blackburn and K.J. Gaston. Environmental predictors of global parrot (Aves: Psittaciformes) species richness and phylogenetic diversity. *Global Ecology and Biogeography* 16:220-233.
34. 2007. Lindenfors, P, J.L. Gittleman and **K.E. Jones**. Sexual size dimorphism in mammals. In: *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism* (eds. Fairbairn, D.J., W.U. Blanckenhorn, and T. Szekely), pp 16- 26. Oxford: Oxford University Press.
35. 2007. Lindenfors, P., C.L. Nunn, **K.E. Jones**, A.A. Cunningham, W. Sechrest and J.L. Gittleman. Parasite species richness in carnivores: Effects of host body mass, latitude, geographic range and population density. *Global Ecology and Biogeography*16:496-509.
36. 2007. Pedersen, A.B., **K.E. Jones**, C.L. Nunn and S. Altizer. Infectious diseases and extinction risk in wild mammals. *Conservation Biology* 21:1269-1279.

Principal Investigator/Program Director (Last, First, Middle):

37. (b) (4)

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*** = Corresponding author**

C. Research Support

ONGOING RESEARCH SUPPORT

Jones & Chatterjee (Co-PIs) Oct 2007-Oct 2010
(b) (4)
Evolution of echolocation in bats – PhD studentship.
Role: Co-supervisor

Jones (PI) June 2007- June 2008
(b) (4)
Bats of the Steppe: monitoring bat biodiversity in Mongolia.
This is a scoping award to set up a bat monitoring program in Mongolia.
Role: PI

Jones (PI) May 2006- May 2009
(b) (4)
Monitoring bat biodiversity: indicators of sustainable development in Eastern Europe.
This is 3 year project to set up bat monitoring programs in Romania, Hungary, Bulgaria and Moldova.
Role: PI

COMPLETED RESEARCH SUPPORT (during last 3 years)

HSD 0525216 Daszak (PI) Jones (Co-PI) Oct 2005 – Oct 2006
National Science Foundation: Human and Social Dynamics
Collaborative Research: Socio-Economic and Drivers of Emerging Diseases
This project was to analyse patterns of disease emergence globally and produce a broad risk assessment.
Role: Co-PI, analyzed data and co-wrote resulting papers.

Jones (PI) June 2003- June 2005
Earth Institute, Columbia University
Predicting Extinction: Models of Global Priority Setting for Conservation.
This was a 2 year fellowship to use spatial and biological and ecological trait data to model extinction risk in mammals and predict future extinction under different scenarios of global change.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jonathan H. Epstein	POSITION TITLE Senior Research Scientist Veterinary Epidemiology, Emerging Zoonoses
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Brandeis University, MA	BA	1996	Biology
Tufts University, Sch. Vet. Med., Grafton, MA	DVM	2002	Wildlife Med., Intl. Med.
Tufts University, School of Medicine, Boston, MA	MPH	2002	Epidemiology
Tufts University, Sch. Vet. Med., Grafton, MA	Cert Intl Med	2002	Zoonotic Diseases

Please refer to the application instructions in order to complete sections A, B, and C of the Biographical Sketch.

A. Positions and Honors

Positions and Employment

2002 Public Health Externship, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, GA

2002-2003 Veterinary Internship, Small animal emergency and critical care, Ocean State Vet. Spec., RI

2003- Senior Research Scientist, Consortium for Conservation Medicine, Columbia University, NY

2003- Adjunct Faculty, Ecology, Columbia Univ., NY & Tufts University Cummings School of Veterinary Medicine, MA.

2006- Adjunct Faculty, Mailman School of Public Health, Columbia Univ, NY

2007- Adjunct Asst. Clinical Professor, Public Health & Family Med, Tufts Univ School of Medicine, MA

Other Experience and Professional Memberships

1998- Member: American Veterinary Medicinal Association, American Association of Zoo Vets, Wildlife Disease Association, New York Academy of Sciences,

2003- Appointed Member, IUCN Veterinary Specialist Group

2004 Invited speaker, WHO, Emerging Zoonotic Diseases Working Group meeting

2004 Invited speaker, Merieux Foundation Conference on Emerging Viral Respiratory Pathogens

2004 Invited speaker, Swiss Re Executive Roundtable on Emerging Diseases

2004 Invited speaker, Royal Swedish Academy of Forestry and Agriculture: Ecology of Henipaviruses

2004 Invited speaker, Swedish University of Agricultural Sciences: Disease Emergence

2006- Member, IUCN Chiroptera Species Specialist Group; Advisory committee, Suffolk Country Board of Public Health; Delta Omega Public Health Honors Society, International Assoc. Ecology and Health

Honors

2002 First recipient, Certificate of International Veterinary Medicine, Tufts University Sch. Vet. Med.

2002 Hills award for excellence in veterinary clinical nutrition

2002 Sylvia Mainzer award for outstanding achievement in the field of public health

2006 Inducted into Delta Omega Honor Society for Public Health (Alpha Rho Chapter – 1st alumni inductee; 1st Inaugural Keynote Speaker)

B. Peer-reviewed publications (in chronological order)

1. McCall, B.J., **Epstein, J.H.** & Annette, N., Potential human exposure to Australian bat Lyssavirus, Queensland, 1996-1999. *Emerging Infectious Diseases* 2000; 6: 259-264

2. Kaufman, G.E., Else, J., Bowen, K., Anderson, M. & **Epstein, J.H.** Conservation medicine in the veterinary curriculum. *EcoHealth* 2004; 1: S43-S49.

3. Daszak, P., Tabor, G.M., Kilpatrick, A.M., **Epstein, J.** & Plowright, R. Conservation Medicine and a new agenda for emerging diseases. *Annals of the New York Academy of Sciences* 2004; 1026: 1-11

4. Patz, J.A., Daszak, P., Tabor, G.M., Aguirre, A.A., Pearl, M., **Epstein, J.**, Wolfe, N.D., Kilpatrick, A.M., Foufopoulos, J., Molyneux, D., Bradley, D.J. & Members of the Working Group Land Use Change and Disease Emergence. Unhealthy Landscapes: Policy Recommendations on Land Use Change and Disease Emergence. *Environmental Health Perspectives* 112: 1092-1098

5. Newman SH, **Epstein JH**, Schloegel LM. The nature of emerging zoonotic diseases: ecology, prediction, and prevention. *Medical Laboratory Observer* 2005 37:10-19.

6. Li W, Shi Z, Yu M, Ren W, Smith C, **Epstein JH**, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S & Wang L-F Bats are natural reservoirs of SARS-like coronaviruses. *Science* 2005; 310: 676-679.

7. Pulliam J, Field H, Olival KJ & the Henipavirus Ecology Research Group (**Epstein**). An alternative explanation of Nipah virus strain variation. *Emerging Infectious Diseases*. 2005; 11: 1978-1979

8. Daszak, P., Plowright, R., **Epstein, J.H.**, Pulliam, J., Abdul Rahman, S., Field, H.E., Smith, C.S., Olival, K.J., Luby, S., Halpin, K., Hyatt, A.D. & the Henipavirus Ecology Research Group (HERG). The emergence of Nipah and Hendra virus: pathogen dynamics across a wildlife-livestock-human continuum. In: Collinge, S.K. & Ray, C. (Eds.), Disease Ecology: Community Structure and Pathogen Dynamics Oxford University Press 2006; pp 186-201.

9. **Epstein, J.H.**, Field, H.E., Luby, S., Pulliam, J., and Daszak, P. Nipah Virus: Impact, Origins, and Causes of Emergence. *Current Infectious Disease Reports* 2006; 8: 59-65.

10. **Epstein, J.H.**, Rahman, S.A., Zambriski, J.A., Halpin, K., Meehan, G., Jamaluddin, A.A., Hassan, S.S., Field, H.E., Hyatt, A.D., Daszak, P. & HERG. Feral cats (*Felis catus*) as possible vectors for Nipah virus. *Emerging Infectious Diseases*. 2006; 12: 1178-1179.

11. Breed, A.C., Field, H.E., **Epstein, J.H.**, Daszak, P. Emerging henipaviruses and flying foxes - conservation and management perspectives. *Biological Conservation* 2006;131: 211-220.

12. **Epstein, J.H.,** McKee, J., Shaw, P., Hicks, V., Micalizzi, G., Daszak, P., Kilpatrick, A.M. & Kaufman, G.The Australian white ibis (*Threskiornis molucca*) as a reservoir of zoonotic and livestock pathogens.*EcoHealth*. 2006; 3: 290-298.

13. Halpin, K., Hyatt, A.D., Plowright, R.K., **Epstein, J.H.**, Daszak, P., Field, H.E., Wang, L., Daniels, P., and the Henipavirus Ecology Research Group. 2007 Emerging viruses – coming in on a wrinkled wing and a prayer. *Clinical Infectious Diseases* 2007; 44: 711-17.

14. [redacted] (b) (4)

15. (b) (4)

C. Research Support
Ongoing Research Support

1K08AI067549 - 01A2 Epstein (PI) 07/01/2007 – 6/30/2011
NIH/NIAID
Understanding the ecology of Nipah virus in Bangladesh. The study will conduct cross-sectional and longitudinal Nipah virus surveillance in *Pteropus giganteus* across Bangladesh and analyze data alongside human outbreak data to model the drivers of emergence.
Role: PI

(b) (4) Epstein (PI) 01/01/2007 – 12/31/2007
The study is designed to train Bangladeshi health care professionals including veterinarians and physicians in Nipah virus surveillance and to develop intervention techniques that prevent infection.
Role PI

Principal Investigator/Program Director (Last, First, Middle):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Thomas Briese	Associate Professor
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Freie Universität Berlin, Germany	M.S.	1983	Biology
Freie Universität Berlin, Germany	Ph.D.	1987	BIOLOGY

A. Positions and Honors.

Positions and Employment

1987-1988 Postdoctoral Fellow, Dept. T. A. Trautner, Max-Planck-Institut für molekulare Genetik, Berlin, Germany
1989-1990 Postdoctoral Fellow, Institute of Virology, Freie Universität Berlin, Germany
1991-1992 Assistant Researcher, Institute of Virology, Freie Universität Berlin, Germany
1992-1994 Visiting Assistant Researcher, Dept. of Neurology, University of California, Irvine, CA
1994-1995 Assistant Researcher, Institute of Virology, Freie Universität Berlin, Germany
1995-1996 Researcher, Institute of Microbiology, BFA für Viruskrankheiten der Tiere, Tübingen, Germany
1997-2002 Assistant Adjunct Professor, Dept. of Neurology, University of California, Irvine, CA
2002- Associate Professor, Dept. of Epidemiology, Columbia University, NY

Honors

1987 Postdoctoral grant of the Max-Planck Society
1993 NARSAD Young Investigator Award

B. Selected peer-reviewed publications (in chronological order).

1. **Briese T** and Hakenbeck R (1985) Interaction of the pneumococcal amidase with lipoteichoic acid and choline. Eur J Biochem 146, 417-427
2. Hakenbeck R, Ellerbrok H, **Briese T**, Handwerger S and Tomasz A (1986) Penicillin-binding proteins of penicillin-susceptible and penicillin-resistant pneumococci: immunological relatedness of altered proteins and changes in peptides carring the beta-lactam binding site. Antimicrob Agents Chemother 30, 553-558
3. Hakenbeck R, **Briese T** and Ellerbrok H (1986) Antibodies against the benzyl-penicilloyl moiety as a probe for pencillin-binding proteins. Eur J Biochem 157, 101-106
4. Hakenbeck R, **Briese T**, Chalkley L, Ellerbrok H, Kalliokoski R, Latorre C, Leinonen M and Martin C (1991) Variability of penicillin-binding proteins from penicillin-sensitive *Streptococcus pneumoniae*. J Inf Dis 164, 307-312
5. Hakenbeck R, **Briese T**, Chalkley L, Ellerbrok H, Kalliokoski R, Latorre C, Leinonen M and Martin C (1991) Antigenic variation of penicillin-binding proteins from penicillin-resistant clinical strains of *Streptococcus pneumoniae*. J Inf Dis 164, 313-319
6. Hakenbeck R, **Briese T**, Laible G, Martin C and Schuster C (1991) Penicillin-binding proteins in *Streptococcus pneumoniae*: Alterations during development of intrinsic penicillin resistance. J Chemother 3, 86-90
7. Martin C, **Briese T** and Hakenbeck R (1992) Nucleotide sequences of genes encoding penicillin-binding proteins from *Streptococcus pneumoniae* and *Streptococcus oralis* with high homology to *Escherichia coli* penicillin-binding proteins 1A and 1B. J Bacteriol 174, 4517-4523
8. **Briese T**, de la Torre JC, Lewis A, Ludwig H and Lipkin WI (1992) Borna disease virus, a negative-strand RNA virus, transcribes in the nucleus of infected cells. Proc Natl Acad Sci U.S.A. 89, 11486-11489
9. Schneider PA., **Briese T**, Zimmermann W, Ludwig H and Lipkin WI (1994) Sequence conservation in field and experimental isolates of Borna disease virus. J Virol 68, 63-68
10. **Briese T**, Schneemann A, Lewis AJ, Park Y, Kim S, Ludwig H and Lipkin WI (1994) Genomic organization of Borna disease virus. Proc Natl Acad Sci U.S.A. 91, 4362-4366
11. Stoyloff R, **Briese T**, Borchers K, Zimmermann W and Ludwig H (1994) N-glycosylated protein(s) are important for the

infectivity of Borna disease virus (BDV). Arch Virol 137, 405-409
12. Kliche S, **Briese T**, Henschen AH, Stitz L and Lipkin WI (1994) Characterization of a Borna disease virus glycoprotein, gp18. J Virol 68, 6918-6923
13. **Briese T**, Hatalski CG, Kliche S, Park Y and Lipkin WI (1995) Enzyme-linked immunosorbent assay for detecting antibodies to Borna disease virus-specific proteins. J Clin Microbiol 33, 348-351
14. Kliche S, Stitz L, Mangalam H, Shi L, Binz T, Niemann H, **Briese T** and Lipkin WI (1996) Characterization of the Borna disease virus phosphoprotein, p23. J Virol 70, 8133-8137
15. Jordan I, **Briese T**, Averett D.R. and Lipkin WI (1999) Inhibition of Borna disease virus replication by ribavirin. J Virol 73, 7903-7306
16 **Briese T**, Jia XY, Huang C, Grady LJ and Lipkin WI (1999) Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis. Lancet 354, 1261-1262
17. Evengård B, **Briese T**, Lindh G, Lee S and Lipkin WI (1999) Absence of evidence of Borna disease virus infection in Swedish patients with chronic fatigue syndrome. J NeuroVirol 5, 495-499
18. Jia XY, **Briese T**, Jordan I, Rambaut A, Chi HC, Mackenzie JS, Hall RA, Scherret J and Lipkin WI (1999) Genetic analysis of West Nile New York 1999 encephalitis virus. Lancet 354, 1971-1972
19. Portlance Walker M, Jordan I, **Briese T**, Fischer N and Lipkin WI (2000) Expression and Characterization of the Borna disease virus polymerase. J Virol 74, 4425-4428
20. **Briese T**, Glass WG and Lipkin WI (2000) Detection of West Nile virus sequences in cerebrospinal fluid. Lancet 355, 1614-1615
21. Jordan I, **Briese T**, Fischer N, Lau JY-N and Lipkin WI (2000) Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. J Inf Dis 182, 1214-1217
22. Solbrig M V, Koob GF, Parsons LH, Kadota T, Horscroft N, **Briese T** and Lipkin WI (2000) Neurotrophic factor expression after CNS viral injury produces enhanced sensitivity to psychostimulants: Potential mechanism for addiction vulnerability. J Neurosci 20, RC104, U1- U6
23. Scherret JH, Poidinger M, Mackenzie JS, Broom AK, Deubel V, Lipkin WI, **Briese T**, Gould EA and Hall RA (2001) The relationships between West Nile and Kunjin viruses. Emerging Inf Dis 7, 697-705
24. **Briese T**, Rambaut A, Pathmajeyan M, Bishara J, Weinberger M, Pitlik S and Lipkin WI (2002) Phylogenetic analysis of a human isolate from the 2000 Israel West Nile virus epidemic. Emerging Inf Dis 8, 528-531
25. Solbrig MV, Schlaberg R, **Briese T**, Horscroft N and Lipkin WI (2002) Neuroprotection and reduced microglial proliferation in Ribavirin treated Bornavirus infected rats. Antimicrob. Agents Chemother 46, 2287-2291
26. Zhai J, **Briese T**, Dai E, Wang X, Pang X, Du Z, Liu H, Wang J, Wang H, Guo Z, Chen Z, Jiang L, Zhou D, Han Y, Jabado O, Palacios G, Lipkin WI, and Tang R (2004) Real-time polymerase chain reaction for detecting SARS coronavirus, Beijing, 2003. Emerg Infect Dis 10, 300-303
27. **Briese T**, Rambaut A, and Lipkin WI (2004) Analysis of the medium (M) segment sequence of *Guaroa virus* and its comparison to other orthobunyaviruses. J Gen Virol 85, 3071-3077
28. **Briese T**, Palacios G, Kokoris M, Jabado O, Liu Z, Renwick N, Kapoor V, Casas I, Pozo F, Limberger R, Perez-Brena P, Ju J, and Lipkin WI (2005) Diagnostic system for rapid and sensitive differential detection of pathogens. Emerg Infect Dis 11, 310-313
29. Palacios G, Jabado O, Renwick N, **Briese T**, and Lipkin WI (2005) Severe acute respiratory syndrome coronavirus persistence in Vero cells. Chin Med J (Engl) 118, 451-459
30. Hirsch AJ, Medigeshi GR, Meyers HL, DeFilippis V, Fruh K, **Briese T**, Lipkin WI, and Nelson JA (2005) The Src family kinase c-Yes is required for maturation of West Nile virus particles. J Virol 79, 11943-11951
31. Macdonald J, Tonry J, Hall RA, Williams B, Palacios G, Ashok MS, Jabado O, Clark D, Tesh RB, **Briese T**, and Lipkin WI (2005) NS1 protein secretion during the acute phase of West Nile virus infection. J Virol 79, 13924-13933
32. Palacios G, **Briese T**, Kapoor V, Jabado O, Liu Z, Venter M, Zhai J, Renwick N, Grolla A, Geisbert TW, Drosten C, Towner J, Ju J, Paweska J, Nichol ST, Swanepoel R, Feldmann H, Jahrling PB, Lipkin WI (2006) MassTag polymerase chain reaction for differential diagnosis of viral hemorrhagic fevers. Emerg Infect Dis 12, 692-695
33. **Briese T**, Bird B, Kapoor V, Nichol ST, and Lipkin WI (2006) Batai and Ngari viruses: M-segment reassortment and association with severe febrile disease outbreaks in East Africa. J Virol 80, 5627-5630
34. Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St George K, **Briese T**, Lipkin WI (2006) MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, causing influenza-like illness in New York State, 2004-2005. J Infect Dis, Nov 15; 194 (10): 1398-402
35. Zhai J, Palacios G, Towner JS, Jabado O, Kapoor V, Venter M, Grolla A, **Briese T**, Paweska J, Swanepoel R, Feldmann H, Nichol ST, Lipkin WI (2006) A rapid molecular strategy for filovirus detection and characterization. J Clin Microbiol, 2006 Nov 1
36. Palacios G, Quan P-L, Jabado OJ, Conlan S, Hirschberg DL, Liu Y, Zhai J, Renwick N, Hui J, Hegyi H, Grolla A, Strong JE, Towner JS, Geisbert TW, Jahrling PB, Büchen-Osmond CV, Ellerbrok H, Sanchez-Seco MP, Lussier Y, Formenty P, Nichol ST, Feldmann H, **Briese T**, Lipkin WI (2007) Panmicrobial oligonucleotide array for diagnosis of infectious diseases. Emerg Infect Dis, 13, 73 http://www.cdc.gov/ncidod/EID/13/1/73.htm

37. Jabado OJ, Palacios G, Kapoor V, Hui J, Renwick N, Zhai J, **Briese T**, Lipkin WI (2006) Greene SCPrimer: a rapid comprehensive tool for designing degenerate primers from multiple sequence alignments. Nucleic Acids Res, 34, 6605

38. Quan P-L, Palacios G, Jabado OJ, Conlan S, Hirschberg DL, Richt J, Pose F, Casas I, Perez-Breña P, Drysdale A, Hui J, Cisterna D, Baumeister E, Savy V, García-Sastre A, **Briese T**, and Lipkin WI (2007) Detection of respiratory viruses and subtype identification of influenza viruses by GreeneChipResp oligonucleotide microarray. J. Clin Microbiol. 45, 2359.

39. Medigeshi GR, Lancaster AM, Hirsch AJ, **Briese T**, Lipkin WI, DeFilippis V, Früh K, Mason PW, Nikolich-Zugich J, and Nelson JA (2007) West Nile virus infection activates the unfolded protein response leading to CHOP 1 induction and apoptosis. J Virol. published online ahead of print on 8 August 2007.

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C. Research Support

Ongoing Research Support

(b) (4) Lipkin (PI) 10/01/01 to 09/30/07
Pandora's Box Project
Establish and implement new high throughput molecular methods for microbial surveillance.

U54 AI05715801 Lipkin (PI) 09/04/03 to 02/29/08
NIH/NIAID
Northeast Biodefense Center
Establish a Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research.

U01 NS047537 Lipkin (PI) 12/01/03 to 11/30/08
NIH/NINDS
Gene:Environment Interactions in an Autism Birth Cohort
Establish a 100,000 child prospective birth cohort in Norway, collect clinical data and samples, map the natural trajectory of neurodevelopmental disorders, and establish a foundation for determining the role of gene-environment interactions in pathogenesis of neurodevelopmental disorders.

R01 HL083850 Lipkin (PI) 05/08/06-04/30/10
NIH
Pathogen Discovery in Chronic Lung Disease by Mass Tag PCR and Microarrays
Employ high throughput molecular diagnostic tools to survey for pathogen discovery in idiopathic pulmonary fibrosis, pulmonary arterial hypertension and bronchiolitis obliterans syndrome.

1 U01 AI070411-01 Lipkin (PI) 09/01/06 to 08/31/11
NIH
Viral Arrays for Biodefense
Establish and validate a viral sequence database and its complementary oligonucleotide array technology for detection and differentiation of influenza viruses and hemorrhagic fever viruses.

1 R24 EY017404 Hageman (PI, Univ of Iowa) 08/01/06-07/31/11
NIH/NEI
Development of Complement Modulating Therapeutics for AMD

Sub-Contract
The sub-contract will survey clinical samples (eyes and blood) for evidence of infection using two novel molecular diagnostic platforms, Mass Tag PCR and GreeneChips.

HHSN266200400036C Lefkowitz (PI, Univ Alabama) 06/30/06 to 06/28/09
Subcontract to Columbia (Lipkin) from the Viral Bioinformatics Resource Center
ICTVdB: A Virus Database for Biodefense and Emerging Infectious Disease Research
Curate and improve the user interface of the electronic database of the International Committee for Taxonomy of Viruses.

Completed Research Support

R01 AI51292 Lipkin (PI) 07/01/02 to 06/30/07
NIH/NIAID
A Staged Strategy for Virus Identification and Discovery
Establish an integrated program in bioinformatics and molecular diagnostics focused on investigating the role of infection in neurologic diseases and cancer.

CDC/American Academy of Pediatrics Lipkin (PI) 09/30/02 to 09/29/06
MV Sequences in Children with Autistic Disorders
Determine whether autism is associated with the presence of measles virus sequences in gastrointestinal tract through blinded analysis in three laboratories (Columbia, CDC, Coombe Women's Hospital).

HD37546 Lipkin (PI) 05/01/00 to 04/30/06
A Developmental Model for Autism Based on CNS Infection

AI55466 Rewers (PI, Univ Colorado) 10/01/02 to 09/30/04
Subcontract to Columbia (Lipkin)
Viral Triggers of Type I Diabetes

NS29425 Lipkin (PI) 07/01/98 to 06/30/03
Molecular Analysis of a Neurotropic Agent, Borna Virus
MH57467 Lipkin (PI) 07/01/99 to 06/30/03
Borna Disease Virus and Neuropsychiatric Disease

Principal Investigator/Program Director (Last, First, Middle):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Gustavo Palacios	Assistant Professor
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Buenos Aires University, Buenos Aires, Argentina	B.S.	1990	Microbiology
Buenos Aires University, Buenos Aires, Argentina	M.S.	1992	Medical Virology
Buenos Aires University, Buenos Aires, Argentina	Ph.D.	2002	Virology

A. Positions and Honors

Positions and Employment

1996-2001 Graduate Research Assistant of the Neurovirosis Division in the Department of Virology, of the National Institute of Infectious Diseases, Buenos Aires, Argentina
1995-2001 Assistant Professor of the Chair of Applied Chemistry I and II of the Forensic Sciences Department of the Instituto Universitario de la Policia Federal Argentina
1994-2002 Research Assistant at the Virology Department, School of Pharmacy and Biochemistry, University of Buenos Aires
2002-2004 Postdoctoral Research Scientist, Epidemiology Department, MSPH, Columbia University
2004-2006 Associate Research Scientist, Epidemiology Department, MSPH, Columbia University
2007-present Assistant Professor, Epidemiology Department, MSPH, Columbia University

B. Selected peer-reviewed publications (in chronological order)

1. Lopez JL, Telenta P, **Palacios Poggio G,** Alonso A, Gonzalez J, Lemberg A, y Campos R. Detección caracterización de mutantes pre-core del virus de la hepatitis B (HBV) en pacientes crónicamente infectados (Detection and characterization of HBV pre-core mutants in chronically infected Patients) (1995). *Act Gastr Latinoam*; 25:85-90.
2. Telenta P, **Palacios Poggio G**, López JL, González J, Lemberg A, Campos R. Increased prevalence of genotipo F Hepatitis B virus isolates in Buenos Aires, Argentina (1997). *J Clin Microbio*l; 35(7);1873-5.
3. Beltramino JC, Freire MC, Cisterna D, Almitrani H, Karakachoff M, Battagliotti C, Meneghetti F, Ara P, **Palacios Poggio G**, Rodriguez C. Manifestaciones neurológicas del virus de la parotiditis en niños sin paperas. Neurological manifestations of Mumps virus in children without parotitis inflammation (1998). *Arch Arg Pediatr*; 96:376-80.
4. Mbayed VA, Lopez JL, Telenta P, **Palacios G**, Badia I, Ferro A, Galoppo C, Campos R. Distribution of Hepatitis B virus genotypes in two different pediatric populations from Argentina (1998). *J Clin Microbiol*; 36(11): 3362-65.
5. **Poggio GP**, Rodriguez C, Cisterna D, Freire MC, Cello J. Nested PCR for rapid detection of mumps virus in cerebrospinal fluid from patients with neurological diseases (2000). *J Clin Microbiol*; 38(1): 274-8.
6. **Palacios G**, Cisterna D, Freire MC, Cello J. RT-Nested PCR for the detection of enterovirus in biological samples from patients with suspected enteroviral infections (2000). *Rev Argent Microbiol*; 32(4): 165-72.
7. Casas I, **Palacios G**, Cisterna D, Trallero G, Freire MC, Tenorio, A. Molecular characterization of human enteroviruses in clinical samples by three different RT nested-PCR assays and direct sequencing of amplified products (2001). *J Med Virol*; 65:138-48.
8. Bok K, **Palacios G,** Sijvarger K, Matson D, Gomez J. Emergence of G9 P[6] human rotaviruses in Argentina: phylogenetic relationships among G9 strains (2001). *J Clin Microbiol*; 39(11): 4020-25.
9. **Palacios G**, Casas I, Cisterna D, Trallero G, Tenorio, Freire C. Molecular epidemiology of Echovirus 30: temporal circulation and prevalence of single lineages (2002). *J Virol*; 76(10): 4940-9.

10. **Palacios G**, Casas, Tenorio A, Freire C. Molecular identification of enterovirus analysing a partial VP1 genomic region with different methods (2002). *J Clin Microbiol*; 40(1): 182-92.
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C. Research Support

Ongoing Research Support

(b) (4) 09/01/2007-07/31/2010
(b) (4)

Environmental Triggers of Type 1 Diabetes Mellitus
Role: Principal Investigator

R24 EY017404 (Hageman, PI) 08/01/2006-07/31/2011
National Institute of Health
Development of Complement Modulating Therapeutics for AMD
The sub-contract will survey clinical samples (eyes and blood) for evidence of infection using two novel molecular diagnostic platforms, Mass Tag PCR and GreeneChips.
Role: Co-Investigator

R01 HL 083850 (Lipkin, PI) 05/08/2006-04/30/2010
National Institute of Health
Pathogen Discovery in Chronic Lung Disease by Mass Tag PCR and Microarrays
The project will investigate the contributions of viruses to the pathogenesis of chronic lung diseases.
Role: Co-Investigator

U01 AI070411 (Lipkin, PI) 09/01/2006-08/31/2011
National Institute of Health

Viral Arrays for Biodefense
The objective of this program is to establish stable and sensitive viral microarray assays to enable differential diagnosis of infection by select NIAID priority agents.
Role: Co-Investigator

Completed Research Support

U54 AI057158 (Palacios, PI) 03/01/2006-2/28/2007
Northeast Biodefense Center
Identification of interferon-antagonists encoded by new world arenavirus
The major goal of this project is to characterize the activity of a domain found in the Z arenavirus protein that presents structural and positional similarity to the protein inhibitor of stat1 activation (PIAS1).
Role: Principal Investigator

U54 AI07158 (Palacios, PI) 03/01/2005-8/30/2006
Northeast Biodefense Center
Immunosuppression in filovirus infections
The major goal of this project is to characterize the activity of a domain found in the Ebola virus glycoprotein that presents structural and positional similarity to an immunosuppressive domain found in the retroviral envelope.
Role: Principal Investigator

R01 AI51292 (Lipkin, PI) 07/01/04 to 06/30/06
National Institute for Allergies and Infectious Disease
Underrepresented Minority Supplement to AI51292
The major goal of this project is to establish an integrated program in bioinformatics and molecular diagnostics focused on investigating the role of infection in neurologic diseases and cancer.
Role: Co-Investigator

(b) (4) (Palacios, PI) 09/01/2000-09/01/2001
Fundación Alberto J. Roemmers, Buenos Aires, Argentina.
Molecular Epidemiology of the Mumps Virus In Argentina
The major goal of this project was to design a method to genotype mumps virus and to characterize the strains of mumps virus circulating in Argentina
Role: Principal Investigator

(b) (4) Freire (PI) 09/01/1997-09/01/1998
Fundación Alberto J. Roemmers, Buenos Aires, Argentina.
Viral Infections of the central nervous system
The major goal of this project was to design, develop and validate diagnostic and characterization systems for virus causing neurological disease.
Role: Co-Investigator

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates						(b) (4), (b) (6)
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b)
						Total Salary, Wages and Fringe Benefits (A+B)	**79,933.84**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	20,000.00
Total Travel Cost	**20,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	25,542.17
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	2,000.00
5. Subawards/Consortium/Contractual Costs	371,871.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**399,413.17**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**499,347.01**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Total Direct Costs	22.16	499,347.00	110,665.30
		Total Indirect Costs	**110,665.30**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**610,012.31**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 8155-Justification_CCM.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates						(b) (4), (b) (6)
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**82,331.85**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	9,000.00
Total Travel Cost	**9,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	7,367.15
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	2,000.00
5. Subawards/Consortium/Contractual Costs	398,302.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**407,669.15**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**499,001.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Total Direct Costs	22.16	499,001.00	110,578.62
		Total Indirect Costs	**110,578.62**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**609,579.62**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 8155-Justification_CCM.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates						(b) (4), (b) (6)
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**84,801.30**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	5,000.00
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	5,077.35
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	2,000.00
5. Subawards/Consortium/Contractual Costs	403,087.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**410,164.35**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**499,965.65**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Total Direct Costs	22.16	499,966.16	110,792.50
		Total Indirect Costs	**110,792.50**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**610,758.15**

J. Fee	**Funds Requested ($)**

K. * Budget Justification	File Name: 8155-Justification_CCM.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates						(b) (4), (b) (6)
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**87,345.86**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		*** Funds Requested ($)**
Total funds requested for all equipment listed in the attached file		
	Total Equipment	
Additional Equipment:	File Name:	Mime Type:

D. Travel		**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)		
2. Foreign Travel Costs		12,000.00
	Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)**
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	25,388.14
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	2,000.00
5. Subawards/Consortium/Contractual Costs	372,979.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**400,367.14**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**499,713.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Total Direct Costs	22.16	499,713.00	110,736.40
		Total Indirect Costs	**110,736.40**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**610,449.40**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 8155-Justification_CCM.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Peter		Daszak		PD/PI							(b) (4), (b) (6)
2.	Dr.	Jonathan	H.	Epstein		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates						(b) (4), (b) (6)
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**89,966.24**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	12,000.00
Total Travel Cost	**12,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

* ORGANIZATIONAL DUNS: 0770900660000

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: Wildlife Trust Inc

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	22,491.77
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	2,000.00
5. Subawards/Consortium/Contractual Costs	373,539.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**398,030.77**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**499,997.01**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Total Direct Costs	22.16	499,997.00	110,799.34
		Total Indirect Costs	**110,799.34**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**610,796.35**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 8155-Justification_CCM.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification – NIH R01

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**113,795.14**
Section B, Other Personnel		**310,583.95**
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		**424,379.09**
Section C, Equipment		
Section D, Travel		**58,000.00**
1. Domestic	0.00	
2. Foreign	58,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**2,015,644.58**
1. Materials and Supplies	85,866.58	
2. Publication Costs		
3. Consultant Services	0.00	
4. ADP/Computer Services	10,000.00	
5. Subawards/Consortium/Contractual Costs	1,919,778.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**2,498,023.67**
Section H, Indirect Costs		**553,572.16**
Section I, Total Direct and Indirect Costs (G + H)		**3,051,595.83**
Section J, Fee		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Walter	Ian	Lipkin		co-PD/PI							(b) (4), (b) (6)
2.	Dr.	Thomas		Briese		co-PD/PI							
3.	Dr.	Gustavo		Palacios		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sean Conlan, Rsch. Scientist						(b) (4), (b) (6)
1	**Total Number Other Personnel**				**Total Other Personnel**		(b) (4), (b) (6)
					Total Salary, Wages and Fringe Benefits (A+B)		**53,728.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	67,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. sequencing, courier, maintemnance contracts, dishwasing	27,000.00
Total Other Direct Costs	**94,000.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**147,728.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. federal	61	147,729.00	90,115.00
		Total Indirect Costs	**90,115.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**237,843.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 6878-Justification_CU.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Walter	Ian	Lipkin		co-PD/PI							(b) (4), (b) (6)
2.	Dr.	Thomas		Briese		co-PD/PI							
3.	Dr.	Gustavo		Palacios		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Sean Conlan	1.20					(b) (4), (b) (6)
0	**Total Number Other Personnel**				**Total Other Personnel**		(b) (4), (b) (6)
					Total Salary, Wages and Fringe Benefits (A+B)		**54,985.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	70,170.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. sequencing, courier, maintenance contracts, dishwashing	27,810.00
Total Other Direct Costs	**97,980.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**152,965.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. federal	61	152,965.00	93,309.00
		Total Indirect Costs	**93,309.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**246,274.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 6878-Justification_CU.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Walter	Ian	Lipkin		co-PD/PI							(b) (4), (b) (6)
2.	Dr.	Thomas		Briese		co-PD/PI							
3.	Dr.	Gustavo		Palacios		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Sean Conlan Rsch. Scientist	1.20					(b) (4), (b) (6)
0	**Total Number Other Personnel**				**Total Other Personnel**		(b) (4), (b) (6)
					Total Salary, Wages and Fringe Benefits (A+B)		**56,278.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	61,375.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. sequencing, courier, maintenance contracts, dishwashing	58,644.00
Total Other Direct Costs	**122,019.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**178,297.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. federal	61	178,297.00	108,762.00
		Total Indirect Costs	**108,762.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**287,059.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 6878-Justification_CU.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Walter	Ian	Lipkin		co-PD/PI							(b) (4), (b) (6)
2.	Dr	Thomas		Briese		co-PD/PI							
3.	Dr.	Gustavo		Palacios		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Sean Conlan Rsch. Scientist						(b) (4), (b) (6)
0	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**57,612.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	42,616.00
2. Publication Costs	2,060.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. sequencing, courier, maintenance contracts, dishwashing	59,504.00
Total Other Direct Costs	**104,180.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**161,792.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. federal	61	161,792.00	98,693.00
		Total Indirect Costs	**98,693.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**260,485.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 6878-Justification_CU.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Walter	Ian	Lipkin		co-PD/PI							(b) (4), (b) (6)
2.	Dr.	Thomas		Briese		co-PD/PI							
3.	Dr.	Gustavo		Palacios		co-PD/PI							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	Sean Conlan Rsch. Scientist						(b) (4), (b) (6)
0	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	58,983.00

RESEARCH & RELATED Budget (A-B) (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 6218898150000

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	43,895.00
2. Publication Costs	2,122.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. sequencing, courier, maintenance contracts, dishwashing	30,389.00
Total Other Direct Costs	**76,406.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**135,389.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. federal	61	135,389.00	82,588.00
		Total Indirect Costs	**82,588.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**217,977.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name: 6878-Justification_CU.pdf	Mime Type: application/pdf
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**236,408.00**
Section B, Other Personnel		**45,178.00**
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		**281,586.00**
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**494,585.00**
1. Materials and Supplies	285,056.00	
2. Publication Costs	6,182.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	203,347.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**776,171.00**
Section H, Indirect Costs		**473,467.00**
Section I, Total Direct and Indirect Costs (G + H)		**1,249,638.00**
Section J, Fee		

Personnel

W. Ian Lipkin, MD, Principal Investigator and Director, Center for Infection and Immunity (b) (4), (b) (6) Lipkin is a physician scientist with expertise in molecular microbiology and high throughput methods for pathogen surveillance and discovery. He is principal investigator and scientific director of the Northeast Biodefense Center, and a member of the WHO laboratory network. Lipkin will be responsible for coordination of the research to be conducted at Columbia, for experimental design, analysis and reporting of results and fiscal oversight.

Thomas Briese, Associate Director, Center for Infection and Immunity (b) (4), (b) (6) Briese is a molecular microbiologist with experience in high throughput methods for pathogen surveillance and discovery including bioinformatics, phylogenetic analysis, real time PCR, differential display and MassTag PCR. He will direct fellows and technicians responsible for extracting nucleic acids, and performing MassTag and real time PCR assays.

Gustavo Palacios, Assistant Professor (b) (4), (b) (6) Palacios is a molecular microbiologist with experience in PCR, DNA microarrays, phylogenetic analyses, and tissue culture. Palacios will direct fellows and technicians in GreeneChip and tissue culture experiments.

Sean Conlan, Research Scientist (b) (4), (b) (6) Conlan is an expert in bioinformatics. Under Lipkin's direction Conlan will be responsible for database creation and management, and design of software for MassTag and GreeneLAMP analyses.

Note: Fringe benefits are calculated at 27.1% in years 1 thru 5. Salary increases at a rate of 3% per year.

Supplies

Photocleavable mass tags coupled to primers: $25,000 (year 01 only)

Microarrays:
The current unit cost for each slide (either single-plex or 24-plex array format) is $400. In year 02 we will need 75 arrays ($30,000), in year 03- 50 arrays ($20,000).

Reagents for molecular biology and biochemistry: $20,000
Sequencing kits and columns, oligonucleotide primers (including fluorescence labeled probes for real time PCR), modification and restriction enzymes, Tri-Reagent (RNA extraction), vectors, competent cells, columns for the purification of nucleic acids, size markers (DNA, RNA), dry ice, liquid nitrogen, columns for chromatography, salts, acids, bases, buffers, alcohols, phenol, chloroform, acetone, agarose, agar, yeast extract, tryptone, acrylamide, formamide, sepharose, membranes.

Plastics/Glassware: $7,000
Plates and tubes for real time PCR, flasks, beakers, glass plates for protein and nucleic acid electrophoresis, centrifuge tubes, syringes, microfuge tubes, pipettes, tips, columns for chromatography, gloves, biohazard waste bags.

Chemicals: $10,000

Salts, acids, bases, buffers, alcohols, phenol, chloroform, acetone, B-gal, scintillation fluid, acrylamide, formamide, sepharose (includes costs for hazardous waste disposal).

Pipetting equipment: $3,000 (year 01 only)
Gilson pipetmen-two sets (1000 mcl, 200 mcl, 20 mcl, 10 mcl) dedicated for RNA work, and two Pipet-Aid-pipettors.

Software and Licenses: $2,000
Microarray, statistical, and data management programs; shared cost for access.

Other Expenses
Conventional (DNA) sequencing of PCR products and plasmid clones: $15,000
Sequencing of isolates, standards and hybridized nucleic acid eluted from arrays.

Metagenomic high throughput sequencing (HTS)/ 454:
Year 03- $30,000, year 04- $30,000.

Courier/Import Fees: $3,000
Includes cost of hazardous goods/biohazard shipping containers, dry ice, handling, import fees.

Publications:
We anticipate publications in the years 03-05 of the project:
Year 03- $2,000, year 04- $2,060, year 05- $2,122.

Equipment maintenance/Maintenance contracts: $4,000
This project will require 20% usage allocation of core equipment, and BSL-2 and BSL-3 maintenance, mass spectrometer, autoclave, laminar flow hoods, cell culture incubators, stationary and shaking bacterial incubators, DNA sequencer, microscopes, liquid nitrogen, freezers, centrifuges, spectrophotometer, thermal cyclers, gel documentation system.

Dishwashing/Autoclave: $5,000

Note: Supplies and other expenses increase at a rate of 3% per year.

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Bruce		Mungall		co-PD/PI							(b) (4), (b) (6)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician (Molecular Genetics)						(b) (4), (b) (6)
1	Research Technician (Cell Culture)						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**75,000.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		*** Funds Requested ($)**
Total funds requested for all equipment listed in the attached file		
	Total Equipment	
Additional Equipment:	File Name:	Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	46,100.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**46,100.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**124,100.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Facilities and Administration	8	124,100.00	9,928.00
		Total Indirect Costs	**9,928.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**134,028.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name:	Mime Type: application/pdf
	6351-NIAID_Bat_viruses_Budget_Justification_(AAHL).pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Bruce		Mungall		co-PD/PI							(b) (4), (b) (6)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Technician (Molecular Genetics)						(b) (4), (b) (6)
1	Research Technician (Cell Culture)						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**75,000.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2009 *** End Date:** 06-30-2010 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	46,100.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**46,100.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**124,100.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Facilities and Administration	8	124,100.00	9,928.00
		Total Indirect Costs	**9,928.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**134,028.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name:	Mime Type: application/pdf
	6351-NIAID_Bat_viruses_Budget_Justification_(AAHL).pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1. Dr.	Bruce		Mungall		co-PD/PI							(b) (4), (b) (6)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Molecular Genetics)						(b) (4), (b) (6)
1	Research Assistant (Cell Culture)						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	75,000.00

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2010 *** End Date:** 06-30-2011 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	46,100.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**46,100.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**124,100.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Facilities and Admininstration	8	124,100.00	9,928.00
		Total Indirect Costs	**9,928.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**134,028.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name:	Mime Type: application/pdf
	6351-NIAID_Bat_viruses_Budget_Justification_(AAHL).pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Bruce		Mungall		co-PD/PI							(b) (4), (b) (6)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Molecular Genetics)						(b) (4), (b) (6)
1	Research Assistant (Cell Culture)						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**75,000.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		*** Funds Requested ($)**
Total funds requested for all equipment listed in the attached file		
	Total Equipment	
Additional Equipment:	File Name:	Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2011 *** End Date:** 06-30-2012 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	46,100.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**46,100.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**124,100.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Facilities and Administration	8	124,100.00	9,928.00
		Total Indirect Costs	**9,928.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**134,028.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name:	Mime Type: application/pdf
	6351-NIAID_Bat_viruses_Budget_Justification_(AAHL).pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Bruce		Mungall		co-PD/PI							(b) (4), (b) (6)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** (b) (4), (b) (6)

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Molecular Genetics)						(b) (4), (b) (6)
1	Research Assistant (Cell Culture)						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4), (b) (6)
						Total Salary, Wages and Fringe Benefits (A+B)	**75,000.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
	Equipment Item	*** Funds Requested ($)**
Total funds requested for all equipment listed in the attached file		
	Total Equipment	
Additional Equipment:	File Name:	Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** 7543079570000

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: CSIRO

*** Start Date:** 07-01-2012 *** End Date:** 06-30-2013 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	46,100.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**46,100.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**124,100.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Facilities and Administration	8	124,100.00	9,928.00
		Total Indirect Costs	**9,928.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**134,028.00**

J. Fee	Funds Requested ($)

K. * Budget Justification	File Name:	Mime Type: application/pdf
	6351-NIAID_Bat_viruses_Budget_Justification_(AAHL).pdf	
	(Only attach one file.)	

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**0.00**
Section B, Other Personnel		**375,000.00**
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		**375,000.00**
Section C, Equipment		
Section D, Travel		**15,000.00**
1. Domestic	0.00	
2. Foreign	15,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**230,500.00**
1. Materials and Supplies	230,500.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**620,500.00**
Section H, Indirect Costs		**49,640.00**
Section I, Total Direct and Indirect Costs (G + H)		**670,140.00**
Section J, Fee		

Bruce Mungall, Ph.D. Consortium (b) (4), (b) (6) is a Project Leader at the Australian Animal Health Laboratory in the field of Henipavirus Therapeutics. He will coordinate and oversee the efforts of all personnel in addition to performing much of the *in vitro* viral characterization experimental work. Dr. Mungall has expertise in most of the technical areas directly related to this project and has developed the current proposal with Dr. Daszak (PD/PI) and Dr. Lipkin (PI). Dr. Mungall has established key assays relating to viral quantitation *in vitro*.

To Be Appointed, B.Sc. Research Technician (b) (4), (b) (6) will be an infectious disease technician with considerable experience in molecular virolcgy. Under the supervision of Dr. Mungall, the technician will assist during reverse genetics studies and all *in vitro* experiments and will assist with the laboratory characterization of experimental samples.

To Be Appointed, B.Sc. Research Technician (b) (4), (b) (6) will be an infectious disease technician with experience in the routine culture of viruses. Under the supervision of the Dr. Mungall, the technician will perform much of the *in vitro* assessment of viral samples.

Supplies:

Supplies budgeted are in keeping with the types of experiments and number of persons engaged in the research to be conducted in Dr. Mungall's laboratories. A significant portion of the initial work involves *in vitro* characterization of viral infection, such that considerable funds are requested to support tissue culture activities.

(Itemized by category)

Tissue Culture (sera, media etc.)	$16,100
Disposable Plasticware, gloves, gowns	$10,000
Taqman PCR reagents	$20,000
	$46,100

Travel:

Funds are requested for Dr. Mungall to travel to the US to meet with Dr's Daszak and Lipkin once each year ($3,000).

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Peter
Middle Name:
* Last Name: Daszak
Suffix:

* New Investigator? ● No ○ Yes

Degrees: Ph.D. | BSC (HON) |

2. Human Subjects

Clinical Trial? ● No ○ Yes

* Agency-Defined Phase III Clinical Trial? ○ No ○ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Dr.
* First Name: Peter
Middle Name:
* Last Name: Daszak
Suffix:

* Phone Number: (b) (6)
Fax Number: 2123804475
Email: (b) (6)

* Title: Executive Director, CCM

* Street1: 460 West 34th Street
Street2: 17th Floor
* City: New York
County:
* State: NY: New York
Province:
* Country: USA:
* Zip / Postal Code: 10001

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ●No ○Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp . Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

OMB Number: 0925-0001
Expiration Date: 9/30/2007

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

● New ○ Resubmission ○ Renewal ○ Continuation ○ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	4103-SPECIFIC_AIMS.pdf
3. Background and Significance	1143-BACKGROUND.PDF
4. Preliminary Studies / Progress Report	7901-PRELIMSTUDIES.PDF
5. Research Design and Methods	8817-RESEARCHDESIGNMETHODS.pdf
6. Inclusion Enrollment Report	
7. Progress Report Publication List	

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects	
9. Inclusion of Women and Minorities	
10. Targeted/Planned Enrollment Table	
11. Inclusion of Children	

Other Research Plan Sections

12. Vertebrate Animals	4896-vertebrate_animals.pdf
13. Select Agent Research	
14. Multiple PI Leadership	
15. Consortium/Contractual Arrangements	3161-Consortium_Contractual_Arrangements.pdf
16. Letters of Support	6720-Support.pdf
17. Resource Sharing Plan(s)	

18. Appendix

Attachments

IntroductionToApplication_attDataGroup0

File Name	Mime Type

SpecificAims_attDataGroup0

File Name	Mime Type
4103-SPECIFIC_AIMS.pdf	application/pdf

BackgroundSignificance_attDataGroup0

File Name	Mime Type
1143-BACKGROUND.PDF	application/octet-stream

ProgressReport_attDataGroup0

File Name	Mime Type
7901-PRELIMSTUDIES.PDF	application/octet-stream

ResearchDesignMethods_attDataGroup0

File Name	Mime Type
8817-RESEARCHDESIGNMETHODS.pdf	application/pdf

InclusionEnrollmentReport_attDataGroup0

File Name	Mime Type

ProgressReportPublicationList_attDataGroup0

File Name	Mime Type

ProtectionOfHumanSubjects_attDataGroup0

File Name	Mime Type

InclusionOfWomenAndMinorities_attDataGroup0

File Name	Mime Type

TargetedPlannedEnrollmentTable_attDataGroup0

File Name	Mime Type

InclusionOfChildren_attDataGroup0

File Name	Mime Type

VertebrateAnimals_attDataGroup0

File Name	Mime Type
4896-vertebrate_animals.pdf	application/pdf

SelectAgentResearch_attDataGroup0

File Name	Mime Type

MultiplePILeadershipPlan_attDataGroup0

File Name	Mime Type

ConsortiumContractualArrangements_attDataGroup0

File Name	Mime Type
3161-Consortium_Contractual_Arrangements.pdf	application/pdf

LettersOfSupport_attDataGroup0

File Name	Mime Type
6720-Support.pdf	application/pdf

ResourceSharingPlans_attDataGroup0

File Name **Mime Type**

Appendix

File Name **Mime Type**

2. Specific Aims.

A key challenge to understanding emerging zoonosesis their unpredictability. They emerge in a seemingly random way, from disparate regions on the globe, from a wide diversity of wildlife and domestic animals. Our ability to understand this process is hampered by three major issues: **1)** the lack of rigorous analyses of the processes that cause emergence; **2)** the large diversity of microbes in wildlife (the 'zoonotic pool') from which new zoonoses regularly emerge; and **3)** our poor understanding of why some viruses are able to cross the species barrier and others not. Despite this, a number of key studies have begun to analyze the rules that govern zoonotic disease emergence. There are also a growing number of molecular tools for discovering potential zoonoses, and assessing their capacity to infect humans. In this proposal, we will use a novel, multidisciplinary approach to examine the basic rules of emergence for zoonoses from a key wildlife group - bats. Bat-borne viruses are a significant, and expanding group of emerging pathogens that include viruses with high mortality rates in people (e.g. Rabies virus, Nipah virus, Ebola virus, Hendra virus), and which have caused pandemic outbreaks (e.g. bat SARS-like coronaviruses). There are also a variety of bat viruses with unknown potential to become zoonotic. We will address these significant challenges to global health in three aims:

Aim 1. Predictive modeling of bat viral diversity and risk of future emergence. We have just published an important new tool for the prediction of pathogen emergence. (PI Daszak, Co-PI Jones)

1.1. Prediction of global hotspots for bat viral biodiversity, and for the emergence of new zoonoses from bats. We will use our database of bat pathogens and GAP analysis to predict the global biodiversity of bat viruses (the bat 'virome'). We will test the hypothesis that bats are reservoirs of a disproportionate number of zoonotic and potentially zoonotic pathogens. We will then use spatial multivariate logistic regression models to examine the correlation between bat-borne zoonotic EIDs (from our Human EID database) and a range of socio-economic, demographic and ecological factors. This will provide spatial prediction of EID emergence from bats ('hotspot' maps).

1.2. Targeted surveillance in EID 'hotspots' to expand our current knowledgebase of bat viruses. We will target collection of bat biological samples at the sites predicted to be high bat viral diversity and at high risk of an EID emergence in *Aim 1.1*,.

1.3. Risk assessment of future viral emergence from bats. In years 04 and 05, we will expand our hotspot modeling to incorporate global, gridded datasets on projected future changes in EID drivers, and on international travel and trade. This will provide a refined, more accurate risk assessment for future viral emergence from bats.

Aim 2. Bat viral pathogen identification using a staged strategy. We will implement an efficient, staged strategy for microbial surveillance and discovery. In year -01 MassTag PCR panels will be used to rapidly screen bat samples for the presence of known and closely related paramyxoviruses, lyssaviruses, and coronaviruses. In years -02 and -03 GreeneChips will be used to survey all vertebrate virus taxa. In years -03 and -04 a selected subset of samples from the CCM collection will be subjected to HTS analysis to identify microbes not captured by MassTag PCR or GreeneChip and to profile microflora. (Co-PIs Lipkin & Briese)

2.1. MassTag PCR assays for detection of paramyxoviruses, lyssaviruses and coronaviruses. We will establish bat housekeeping gene controls for MassTag PCR assays and optimize MassTag PCR assays for bat feces, saliva, urine and serum. We will then Implement MassTag PCR assays for paramyxoviruses, lyssaviruses and coronaviruses, confirm identity of viruses identified by MassTag PCR analysis and establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance

2.2. GreeneChip Microarray assays for detection of vertebrate viruses We will optimize and implement GreeneChip assays to screen bat feces, saliva, urine and serum for any known vertebrate viruses, confirm the identity of those viruses identified by GreeneChip analysis, and establish and implement specific real time PCR assays for the quantitation of pathogen burden and surveillance.

2.3. Metagenomic sequence analysis of bat feces, saliva, urine and serum. We will design and implement software for subtraction of bat sequences, implement HTS assays of bat

feces, saliva, urine and serum, confirm identity of microbes identified in metagenomic assays, and establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance

2.3. Metagenomic sequence analysis of bat feces, saliva, urine and serum. We will design and implement software for subtraction of bat sequences, implement HTS assays of bat feces, saliva, urine and serum, confirm identity of microbes identified in metagenomic assays, and establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance

Aim 3. Bat viral pathogenesis. We will use a multi-platform *in vitro* approach to investigate the likelihood of known, non-select agent bat viruses, and of new viruses discovered and sequenced in Aim 2 emerging in people.

3.1. In vitro evaluation of bat derived paramyxoviruses, coronaviruses and lyssaviruses in Vero and bat cell lines to determine correlates of infection. Routine cell culture systems (Vero cells or BHK cells) or primary and/or continuous bat cell lines (developed in a previous CSIRO project) will be utilized to evaluate a number of recently emerged, non-biodefence related paramyxoviruses (MenV, TPMV, SalV, Tioman, Mapuera and PoRV Virus), coronaviruses (bat SARS CoV, bat CoV, and a number of bat coronavirus isolates from Hong Kong) in addition to several lyssaviruses (and related rhabdoviruses) for determining molecular correlates of infection via whole genome, rapid pyrosequencing.

3.2. Evaluation of paramyxovirus correlates of infection using reverse genetics. Once infection correlates have been determined *in vitro*, we will reverse engineer specific molecular correlates into, or out of, wild type paramyxoviruses and assess their phenotypes in suitable *in vitro* systems.

3.3. Identification of viral or host correlates of infection. Using a range of cell lines and the NHBE cell as a surrogate model for human respiratory infection, we will evaluate the cellular pathogenicity of novel viruses discovered through Aims 1 and 2. Incorporating rapid, whole virus genome sequencing technologies, we will rapidly evaluate quasispecies changes relevant to host adaptation. By comparison of data for these newly discovered viruses to well characterised, but closely related viruses, we expect to determine potential viral or host correlates of infectivity and pathogenicity, or both.

3. Background and Significance.

3.1. Emerging zoonoses from wildlife and their drivers.

Emerging infectious diseases (EIDs) are a key threat to global health [1, 2]. They are caused by pathogens that emerge on a pandemic scale (e.g. HIV/AIDS) or through smaller outbreaks that have high case fatality rates or lack effective therapies or vaccines (e.g. Ebola virus, multi-drug resistant TB). Recent work using large databases of human pathogens have shown that around three-fourths of the pathogens that have emerged in people originate in wildlife [3, 4]. These 'wildlife EIDs' include the most significant and highly threatening EIDs to have emerged so far (e.g. HIV/AIDS, SARS Coronavirus, Ebola virus, Nipah virus). This group of EIDs alone causes tens of thousands of deaths each year, and some outbreaks (e.g. SARS) have cost the global economy tens of billions of dollars. However, despite the huge social, demographic and economic impact of EIDs, there has been little advance in our understanding of the underlying process of how these wildlife zoonoses emerge, and in developing predictive approaches to prevent future emergence[5-8].

This unpredictability is a key challenge to medical science. New zoonoses emerge from wildlife regularly, and in a seemingly random way, from disparate regions on the globe, and from a wide diversity of wildlife and domestic animals. Our ability to understand what drives this process is hampered by three major issues: **1)** a lack of rigorous analyses of the processes that cause emergence; **2)** our lack of knowledge of the diversity of microbes in wildlife (the 'zoonotic pool'[9, 10]) from which new zoonoses regularly emerge; and **3)** our poor understanding of pathogenetic factors that explain why some viruses are able to make the species jump, and others are not. Despite these problems, a number of recent studies have begun to analyze the rules that govern zoonotic disease emergence[3, 4, 11-15] (**Fig. 1**), to develop molecular techniques to assess the dimensions of the 'zoonotic pool' [16, 17], and to work with animal models and in vitro approaches to examine viral pathogenesis and host-jumping [18, 19].

Previous research on how anthropogenic drivers cause disease emergence has either reviewed broad trends without detailed analyses[1, 8, 9, 20], or concentrated on specific diseases and the role of single anthropogenic driver (e.g. deforestation, climate and malaria [21-25]). Other work has studied how specific changes in travel and trade may facilitate the spread of specific diseases (e.g. SARS[7, 26-28] and H5N1 avian influenza[29]). The critical need for research in this field has led to the National Research Council listing 'Infectious Disease and the Environment' as their 5th of 6 "Grand Challenges in Environmental Sciences"[30]. In this proposal, we will refine our recently-published approach to predicting EID 'hotspots'[13], and test the predictive ability of the hotspots map through targeted, enhanced surveillance of a key group of wildlife reservoirs (bats) within high-risk geographic locations.

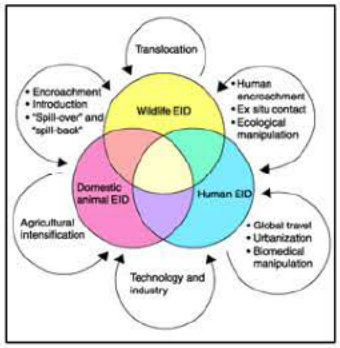


Figure 1. Factors that promote disease emergence[31, 32]. This figure from[15] illustrates that anthropogenic factors (socio-economic, demographic or environmental changes) drive pathogen emergence [8, 33]. For example, agricultural intensification led to the emergence of Nipah virus in 1998[34, 35]. These factors alter the contact among humans, wildlife and livestock to promote pathogen 'spill-over' and emergence. There is a critical underlying relationship: **Zoonotic emergence is a product of the high diversity of pathogens in non-human animals, and a series of anthropogenic changes that bring our populations into contact.** This is a central theme to the current proposal.

3.2. Bats and Emerging Zoonoses.

While there are a considerable number of recently emerged zoonotic pathogens[36], of particular interest are those which appear to have emerged from mammalian reservoir hosts, particularly rodents, ungulates, primates, carnivores and, more recently, bats[3, 37]. More than 30 viruses have been isolated from bats including a number of zoonotic viruses [38] (**Table 3, Research design *5.2.1***). Bats have been implicated as the natural host of some of these zoonoses, and may be simply aberrant carriers of others. We believe that through targeted sampling of various bat populations in predicted EID hotspots, additional viruses with zoonotic potential will be discovered. Analyzing the diversity of pathogens in this group will inform our predictive models

and allow us to develop more generalizable strategies for combating future zoonotic EIDs. Statistically, RNA viruses are more likely to emerge, comprising 37% of all emerging and reemerging pathogens [36]. RNA viruses are also prominent among the subset of emerging pathogens that have apparently entered the human population only in the past few decades, such as HIV, SARS-CoV or Nipah virus. Among these, both paramyxoviruses and coronaviruses appear to infect the broadest range of host species, particularly mammalian species[39], and include a number of agents with bats as reservoir hosts. They also include a number of viruses related to emerging viruses in humans, but which have not been associated with human infections. Elucidating the likely pathogenesis of these viruses in human cells may have significance in understanding the future risk of them emerging into the human population.

3.2.1. Paramyxoviruses. Paramyxoviruses include common human pathogens which entered the human population (mumps, measles, parainfluenza 1-4) and a number of important emerging pathogens (Hendra and Nipah viruses). Currently, we have unique access to a set of related bat-derived paramxyoviruses that display either mild clinical signs during infection of swine, asymptomatic infections, or are as yet uncharacterised with respect to their pathogenicity in terrestrial mammals. Six new members of the paramyxovirus family have been described in the last decade:

1) *Hendra virus* (HeV), the cause of an outbreak of fatal respiratory disease resulting in the death of 14 horses and one human, was isolated in September 1994 [40, 41]. The virus is carried by fruit bats (genus *Pteropus*)[42, 43]. Recently the cellular receptor which HeV (and NiV) us to gain entry to vertebrate cells was discovered [44, 45] with an additional co-receptor discovered subsequently [46]; 2) *Menangle virus* (MenV) was isolated in 1997 from stillborn piglets at a commercial piggery in New South Wales, Australia[47]. The presence of neutralizing antibodies against MenV in several species of fruit bats (genus *Pteropus*) and their absence in a range of domestic and other wild animals within the vicinity of the piggery suggested that flying foxes are the probable natural host of this virus [47]; 3) *Nipah virus* (NiV), was the cause of an outbreak of viral encephalitis in Malaysia and Singapore which resulted in 105 human fatalities and the slaughter of over a million pigs once it became evident that swine were the source of human infection [48-53]. Malaysian fruit bats (*P. vampyrus* and *P. hypomelanus*) are the natural reservoirs of NiV [54, 55], and its emergence is thought linked to the intensification of pig production in the region[34]. NiV has now also emerged repeatedly in Bangladesh[56], where it has undergone chains of human-to-human transmission[57]. NiV is closely related to HeV (and uses the same cell receptor) and it has been proposed that these two viruses represent members of a new genus within the subfamily *Paramyxovirinae* [51, 58, 59]; The remaining three new viruses, in contrast, have an unknown propensity to cause disease: 4) *Tupaia paramyxovirus* (TPMV) was isolated from an apparently healthy Southeast Asian tree shrew [60]; 5) *Salem virus* (SalV), although identified while investigating the cause of an unknown equine illness, was not considered to be the etiological agent of the disease in question [61]. Although neither of these viruses has been classified taxonomically, initial phylogenetic comparisons suggested that the closest evolutionary relationships existed between TPMV, SalV, HeV, and members of the genus *Morbillivirus*, with TPMV more closely related to HeV, and SalV more closely related to the morbilliviruses; 6) *Tioman virus* (TiV), was isolated in Malaysia from urine samples collected from fruit bats in an attempt to identify the natural host of NiV [62] and cross-reacts with MenV-specific antisera which it appears to be closely related to genetically [62].

The first finding of a paramyxovirus in bats was described in 1966. A parainfluenza virus type 2 was isolated from the frugivorus bat *Rousettus leschenaulti* in India[63]. *Mapuera virus* was the second paramyxovirus isolated from the yellow-shouldered bat (*Sturnira lilium*) in Brazil in 1979 [64] and recent studies suggest Mapuera may be related to a porcine rubulavirus (PoRV, a.k.a. blue eye disease virus) [65] isolated from pigs with nervous disorders, pneumonia, corneal opacity and infertility [66]. Neither bat virus has been found to cause human infections. Two additional porcine paramyxoviruses have been identified including the Isolation of a cytopathogenic virus from a case of porcine reproductive and respiratory syndrome (PRRS) and its characterization as parainfluenza virus type 2 [67]. Janke and co-workers [68] reported a neurological and respiratory disease in a swine herd in the northern USA associated with Porcine

Paramyxovirus (PPMV). The clinical respiratory and neurological disease was similar to that observed with Nipah virus infection except the disease was milder.

Other paramyxoviruses with unknown human, bat or porcine involvement include phocine, dolphin, and porpoise morbilliviruses[69-72]; *Mossman virus* (MoV) isolated from wild rats trapped in Queensland, Australia, during the early 1970s [73]; *Nariva virus* (NaV) was isolated on four separate occasions from the forest rodent species *Zygodontomys brevicauda brevicauda,* trapped in Eastern Trinidad during 1962 and 1963 [74 75]; Jvirus (J-V) from moribund *Mus musculus* trapped in 1972 during a study of the pathology of feral rodents in North Queensland, Australia [76, 77]; Beilong virus (BeiPV), discovered as two putative novel cDNAs, termed Angrem 104 and Angrem 52 using BLASTx searches on the NCBI server [78], from human mesangial cells with significant homology to JPV genes [79 80, 81]. BeiPV was then isolated from the human mesangial cell line from which the cDNA sequences Angrem 104 and Angrem 52 were originally obtained [82]. Later, it was discovered that a rat mesangial cell line, from the same laboratory in which the human mesangial cells were cultured, carried BeiPV, which is believed to be the original source of the virus [82]. Whilst the ability of many of these new viruses to cause disease is unknown their isolation and characterization further illustrates the wide host range, distribution, and genetic diversity of recently emerged members of the subfamily *Paramyxovirinae*.

3.2.2. Coronaviruses. Coronaviruses are found in a wide range of animal species. In humans, coronaviruses are mainly respiratory pathogens, although they have been occasionally shown to be the cause of diarrhea. Before the SARS epidemic, only two human coronaviruses (HCoVs) had been characterized (HCoV- 229E and HCoV-OC43). Both of these usually cause a mild upper respiratory tract infection. In 2004, two novel human coronaviruses were identified in individuals with respiratory infections[83, 84]. HCoV-NL63 has since been detected in individuals with typical features of acute respiratory infection in Europe, Japan, China, Australia, and North America, and HCoV-HKU1 was isolated from individuals with pneumonia (Reviewed in [85]). HCoVs, including the previously known HCoV-229E and HCoV-OC43, may account for up to 30% of respiratory infections in the general population [83, 86]. Animal and human coronaviruses have been classified into three different serologically distinct groups based on their antigenicity [87, 88]: Group 1 contains HCoV- 229E and porcine [TGEV (transmissible gastroenteritis virus) and PDEV, (porcine diarrhea epidemic virus)], feline [FIPV (feline infectious peritonitis virus)] and canine coronaviruses; Group 2 contains HCoV-OC43 along with MHV (mouse hepatitis virus), bovine coronavirus and haemagglutinating encephalomyelitis virus; and Group 3 contains avian coronaviruses, including IBV (infectious bronchitis virus) of chickens and turkey coronavirus. SARS-CoV lies in a group of its own, based on gene sequencing studies [87, 88].

Coronaviruses have been shown experimentally and in nature, to undergo genetic recombination by a genomic template-switching mechanism and to generate genetic point mutations at a rate similar to that of other RNA viruses including influenza A viruses. This may explain the high degree of host switching and zoonotic transmission within the group [89, 90]. The origin of SARS-CoV host-switch to humans appears to be wildlife. Civets and related small carnivores were implicated in the emergence of SARS-CoV[91] and recent work by our group shows that Chinese horseshoe bats harbor SARS-like CoV that are the likely wildlife source of the SARS-CoV lineage[92, 93]. Since then, a new group 1 coronavirus (includes the human pathogens human coronaviruses 229E and NL63) was detected in three species of *Miniopterus* bats in Southeast Asia [94]. In Hong Kong, Coronaviruses were detected by RT-PCR of rectal swabs in 37 of 309 bats (12%) with the bat-SARS-CoV being detected in 21 of 118 (17.8%) *R. sinicus*[95]. Six other coronaviruses were also discovered in different bat species during a survey of Chinese bats[96]. Even more recently, antibodies bat-SARS-CoV have been detected in African bats[97], and a diverse assemblage of group 1 coronaviruses has been reported from North American bats[98].

3.2.3. Lyssaviruses. The Lyssavirus species include rabies virus, (genotype 1), Lagos bat virus (genotype 2), Mokola virus (genotype 3), Duvenhage virus (genotype 4), European bat lyssaviruses 1 and 2 (genotypes 5 and 6, respectively), Australian bat lyssavirus (genotype 7), and four newly described genotypes found in Eurasia, Aravan (isolated in 1991), Khujand (isolated in 2001), Irkut (isolated in 2002), and West Caucasian bat viruses (isolated in 2002) [71,

[99, 100]. These four Eurasian genotypes and the Lagos bat virus have not been shown to cause human infections to date [101]. There had only been one reported case of human infection caused by the Duvenhage virus to date which occurred in South Africa in 1970 [102, 103]. *Miniopterus schreibersii* was considered to be the host associated with the infection and the virus was isolated once from a *Nycteris thebaica* bat [103]. All the above lyssaviruses have been isolated from bats except Mokola virus which is mainly isolated from cats and occasionally from rodents and shrews [96]. For the bat-associated lyssaviruses, only rabies virus is also associated with other terrestrial animals (especially carnivores); all the others have bats as the sole natural reservoir.

Rabies virus has a worldwide distribution while the other lyssaviruses are relatively restricted geographically. A large number of animals are susceptible to infection by the classical rabies virus. However, only mammals of the orders Chiroptera and Carnivora transmit the virus efficiently in nature. In countries which are free from canine rabies, bats are the most important source for human rabies. Cross-species transmission of rabies virus still occurs today but all these incidents are the result of bat-to-terrestrial animals spillover not the reverse [104]. Phylogenetic division of bat rabies viruses was clearly shown to be associated with clustering of specific bat species in two studies, suggesting that some rabies viruses co-segregate with their bat hosts [105, 106]. In Canada, for example colonial and non-migratory *Myotis* bats are associated with rabies virus clades that are distinct from those associated with solitary, tree-dwelling and migratory red bats *(Lasiurus* spp.) [106]. From 1958 to 2000, bat rabies accounted for 32 of the 35 indigenous cases of rabies in the USA [107]. In 26 of the patients, there was no history of bat bites. Nineteen of these 26 'cryptic' rabies were associated with two species, *Lasionycteris noctivagans* and *Pipistrellus subflavus.* Similarly in Latin America, bat rabies is as important as canine rabies in causing disease in humans and livestocks. In Brazil, analysis showed that canine- and bat-related rabies viruses reside in distinct groups, reinforcing the hypothesis that different rabies virus strains are preferentially related to different mammalian hosts [108, 109]. Bat rabies viruses are associated with a large number of bat species, both frugivorous, insectivorous, and sanguivorous. Pteropus bats have been found to be infected with rabies virus in India [110], and with other lyssaviruses in Thailand, Bangladesh, and Australia[111, 112]. Australian bat lyssavirus was first recovered from *Pteropus alecto* in New South Wales, Australia, and later also found in other bat species [113]. Two fatal human infections in Australia have been reported, both had sustained bat-related injuries prior to onset of disease [114]. Post-exposure prophylaxis with rabies vaccine and immunoglobulin were given in subsequent potential exposures to Australian bat lyssavirus and no further human cases have ever been described [115, 116]. In contrast to other bat-associated pathogens, Australian bat lyssavirus can cause encephalitis in infected bats [115, 116].

3.3. Viral Discovery

In this proposed work, we will develop a three-phase viral discovery strategy to examine the viral diversity with bats. The first step is ***MassTag PCR***, an inexpensive high-throughput system which uses digital mass tags rather than fluorescent dyes to serve as reporters, and which was first implemented to distinguish 22 different viral and bacterial respiratory pathogens [117, 118]. Our next step is the use of ***DNA Microarrays (our GreeneChip)*** which have potential to provide a still broader platform for highly multiplexed microbial surveillance. The number of potential features far exceeds that with any other known technology. Furthermore, probes of up to 70 nt are not uncommon. Thus, unlike PCR where short primer sequences demand complementarity between probe and target, DNA arrays are less likely to be confounded by minor sequence mismatches. Despite these advantages, DNA arrays have not been widely employed because of limited sensitivity. This limitation has been addressed with GreeneChip technology through improvements in sample preparation, random amplification and labelling protocols. Thus, specimens found negative by MassTag PCR will be analysed using GreeneChips that comprise probes for all known vertebrate viruses. Finally, we will use ***High-throughput sequencing (HTS) to enable*** pathogen discovery. Unlike multiplex PCR or array methods where investigators are limited by known sequence information and must make choices regarding the range of pathogens to consider in a given experiment, HTS is unbiased and allows an opportunity to consider the entire tree of life: bacteria, viruses, fungi and parasites.

3.4. Viral Pathogenesis.

Both closely and distantly related animal hosts are potential sources of emerging human disease[3, 11]. However, the scope of the virus to solve novel molecular problems encountered in different hosts is likely a key factor in preventing emergence. If fewer natural evolutionary molecular barriers need to be overcome, whether in cell surface receptors, engagement of entry or fusion machinery, evasion of host interferon responses, or interaction with cell replication mechanisms, then emergence will be favoured assuming the novel host and agent are suitably juxtaposed. Thus, understanding viral pathogenesis is likely critical to understanding emergence.

3.4.1. Cell viability and apoptotic events. The ultrastructural characteristics of paramyxovirus-infected cells share common features including the generation of large syncytia and presence of viral nucleocapsids in cytoplasmic inclusion bodies and underlying electron dense areas of the serum membrane [119]. Under normal circumstances, once infected, cells then attempt pre-programmed cell death, apoptosis, in order to aid in virus elimination from the host. Some viruses, among them certain paramyxoviruses, actually suppress apoptosis in order to prolong viral replication cycles and enhance virus spread [120, 121]. Apoptosis and necrosis represent two extremes of a continuum of cell death. This continuum includes many variations. "Apoptosis-like programmed cell death" refers to a cell death process that has some of the hallmarks of apoptosis such as chromatin condensation and the appearance of PS on the outer leaflet of the cell membrane but does not necessarily require caspase activity [122]. "Necrosis-like programmed cell death" describes programmed cell death that does not include chromatin condensation and has varying degrees of other apoptotic features. Caspase-1 and caspase-8 have been implicated in some cases of this type of programmed cell death [123].

Apoptosis occurs via a complex signaling cascade that is tightly regulated at multiple points, providing many opportunities to evaluate the activity of the proteins involved. The initiator and effector caspases are particularly good targets for detecting apoptosis in cells. These ubiquitous enzymes exist as inactive zymogens in cells and are cleaved before forming active heterotetramers that drive apoptotic events. Luminescent and fluorescent substrates for specific caspases have allowed the development of homogeneous assays to detect their activity. The caspase family of cysteine proteases are the central mediators of the proteolytic cascade leading to cell death and elimination of compromised cells. As such, the caspases are tightly regulated both transcriptionally and by endogenous anti-apoptotic polypeptides, which block productive activation [124]. Furthermore, the enzymes involved in this process dictate distinct pathways and demonstrate specialized functions consistent with their primary biological roles [125]. Assays that directly measure caspase activity can provide valuable information about the mechanism of death in infected or dying cells.

3.4.2. Interferon function/blockade. The IFN system is one of the first lines of innate immune defense against infection in mammals, and is designed to limit the spread of microorganisms from the source of infection [126-129]. There are two types of IFN: i) Type I IFNs are produced in response to virus and bacterial infection and comprise a family of related IFNα proteins and IFNβ. The type II IFN, IFNγ, is synthesized only by certain cells of the immune system. The transcriptional activation of type I IFNα/β genes is a complex, bi-phasic process (Reviewed in [130]). The first phase, IFN induction, occurs in cells soon after infection and leads to the synthesis of IFNβ and a subset of IFNα proteins [129, 131]. The IFN induction pathway can be activated by double-stranded (ds)RNA [132] or by virus infection, in which viral components other than dsRNA might be responsible [133]. In the second phase, IFN signaling, the IFNs that are induced as a result of virus infection bind to type-I-IFN receptors on the surface of both infected and uninfected cells, and activate hundreds of IFN-inducible genes, some of which have antiviral activity [127-129].

Almost all viruses have evolved ways to evade the IFN-induced antiviral responses of their hosts [126, 131]. These mechanisms include the inhibition of host-cell transcription and translation and the consequent failure to synthesize IFN, inhibition of dsRNA-signalling and IFN-signalling pathways, and antagonizing the IFN-induced antiviral effecter proteins. The anti-IFN activities of many paramyxoviruses are encoded by the viral P gene. Products of the P gene inhibit both dsRNA signaling [134-137] and IFN signaling [138-140], but often by slightly different approaches.

3.5. Significance.

Understanding the natural wildlife reservoir, amplifying host, the routes of transmission, the type of susceptible human hosts, and the epicentres for zoonotic and human transmissions is crucial in the control of zoonotic infections. As an illustration, there have been multiple recent paramyxovirus emergence events, many of these involving bats. Some of these newly emerged viruses are highly pathogenic (henipaviruses), some are moderately pathogenic (Menangle, PoRV, PPMV) while many are of unknown pathogenicity (Tioman, Mapuera, SalV, TPMV). Significantly, members of these viruses are present on at least five continents (Australia, Asia, Africa, North and South America), suggesting a high global importance associated with paramyxovirus infections. Two of these newly emerged viruses, HeV and NiV, are not only novel discoveries, they are also BSL4 agents that possess several biological features that make them highly adaptable for use as bioterror agents. Firstly, unlike most notable viral agents of biodefense concern such as smallpox or ebola, NiV can be isolated from natural sources [141, 142], it can be readily grown in cell culture to high titers near $1x10^8$ $TCID_{50}$/ml [143], it is highly infectious and transmitted via the respiratory tract [144, 145], it can be amplified and spread in livestock serving as a source for transmission to humans, and recently it has been shown to be transmitted directly from person to person [57]. Clearly, there is a high level of public health importance attributed to Henipaviruses, and by inference, a considerable risk associated with new emergence events by other unknown or uncharacterised bat viruses.

Similarly, there have been multiple recent human coronaviruses identified. Some are mildly pathogenic (HCoV-229E and HCoV-OC43) causing mild upper respiratory tract infections that result in self-resolving common colds in otherwise healthy individuals or severe pneumonia in immunocompromised people [146, 147]. Others are moderately pathogenic (HCoV-NL63) causing conjunctivitis, croup, and, sometimes, serious respiratory infections in children [83, 148], while SARS-CoV infected patients presented with an influenza-like illness that began with headache, myalgia, and fever, often followed by acute atypical pneumonia, respiratory failure, and death [88]. While there are many lyssaviruses that have been isolated from bats (Reviewed in [96]), only rabies virus is commonly associated with other terrestrial animals (especially carnivores). All the others appear to have bats as the sole natural reservoir hosts. However, the recent fatal lyssavirus infections in humans resulting directly from bats, suggest the potential for sporadic lyssavirus infections is poorly understood [114].

EID hot spot modeling integrated with a proven strategy for microbial surveillance and discovery affords an unprecedented opportunity to identify new pathogens before they emerge to threaten human health. Viruses identified using a range of cutting edge PCR and microarray technologies will then be comparatively analyzed with closely related viruses via whole genome sequencing, combined with assessment of their relative pathogenicity and the nature of the induced host immune responses using a range of cell lines. Additionally, these studies will enhance our understanding of bat microbial ecology, an important reservoir of a significant and increasing number of emerging pathogens. We will also make use of our excellent experimental collections of paramyxoviruses, coronaviruses and lyssaviruses enabling comparative pathogenesis studies to be undertaken *in vitro*, and potentially *in vivo*. If novel paramyxoviruses are discovered, in addition to comparative pathogenomics, we will also employ manipulation of viral genomes using established reverse genetics technologies to evaluate potential molecular correlates of infection. Combining this knowledge with the epidemiology of emergent zoonoses and predictive modeling techniques, we will not only be able to more accurately predict future transmission events, but we will be able to rapidly identify the relative threat posed by newly emerged related viruses. Further, through the identification of conserved therapeutic targets, we will enhance our ability to respond quickly and effectively to mitigate these threats.

4. Preliminary Studies

4.1. Analyzing the process of zoonotic disease emergence. At the Consortium for Conservation Medicine (CCM) (PI Daszak, Co-PI Kate Jones), we specialize in research to understand and predict the process of disease emergence. Our recent work on the ecology of West Nile virus[149-152], Nipah virus[34, 35, 153-156] and SARS [157-160] has provided evidence that the process of disease emergence via 'host-jumping' can be mathematically modeled, and predicted. Disease emergence has been characterized as a three-part process: a) initial pathogen establishment, b) persistence, and c) spread to other host communities[161], or initial spill-over to a new host, limited host-to-host transmission, and larger-scale geographic spread [162]. Each of the steps in the emergence process (**Fig. 2**) has been analyzed and modeled for some individual pathogen systems[7, 27, 163-165], and we can use this to develop a predictive approach to zoonotic disease emergence.

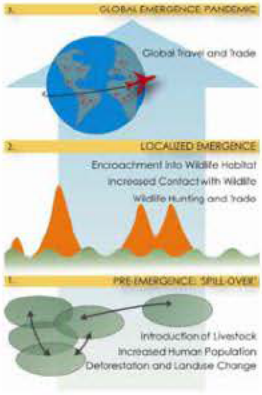


Figure 2. Widespread zoonotic disease emergence involves three critical phases: 1) cross species transmission (black arrows) where environmental changes cause animal populations to interact and share pathogens; 2) Spill-over and local establishment in humans. This relies on a high diversity of pathogens in wildlife hosts, the traits of these pathogens, phylogenetic similarity between hosts, and environmental conditions; 3) pandemic emergence depends on large-scale geographic contact networks established through trade and travel, leading to movement of hosts, pathogens and vectors.

To analyze disease emergence, we have spent the past 3 years studying the impact of human change on global disease emergence. To do this we developed our **Human Emerging Infectious Disease Event Database**, which includes data on all 338 diseases that have emerged in people from 1940 to 2004, referenced to the primary literature. We identified 375 EID 'events', defined as the first emergence of a new disease, or the first cluster of cases or outbreaks that represented a pathogen being listed as emerging, following definitions in the literature. We based our data collection on a previously-published list of emerging infectious diseases [4] updated to 2004. We added information on time, location, pathogen type, transmission mode, other hosts, and pathogen life history traits. We also listed the most commonly cited causes of emergence for each pathogen following published definitions [1, 2, 8, 15, 33]. The location of each EID event was digitized into ArcGIS [166] (**Figure 3**).

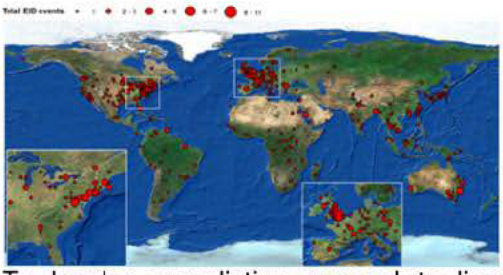


Figure 3. Human EID 'hotspots' 1940-2004. Global richness map of the origin of each emerging pathogen reported in humans 1940-2004. Points represent 1º grid cell centroids where # of EID events in the grid is proportional to the area of the point. The major EID hotspots are NE USA, W. Europe, Japan and SE Australia, albeit that these also likely represent regions where infectious disease surveillance and research is also high[13].

To develop a predictive approach to disease emergence, we first corrected for reporting biases in the dataset. We calculated the annual number of articles published in the leading infectious disease journal (*Journal of Infectious Diseases*) from 1945 to 2004, and the country of origin of each author (senior and co-authors, n=17,979). We were able to answer some significant questions regarding disease emergence. First, we showed that the number of EIDs has risen significantly with time after correcting for reporting bias (Generalised Linear Model with Poisson errors, offset by log(JID articles) ($GLM_{P,JID}$), $F_{1,57} = 86.4$, $p < 0.001$). We found that zoonotic diseases originating in wildlife (e.g. SARS, Ebola, Hantavirus) represent a large and increasing fraction of all emerging diseases, having increased significantly with time, controlling for reporting effort ($GLM_{P,JID}$ $F_{1,57} = 53.4$, $p < 0.001$), and constituted the majority (53.1%) of EID events in the most recent decade (1990-2000). This supports the suggestion that zoonotic EIDs are increasing and represent the most significant current EID threat to global health[1, 2, 167-169].

We then built a multivariate logistic regression model which included our correction for reporting bias. We found a significant correlation between zoonotic EID events originating in

wildlife and mammalian biodiversity (species richness)[13], supporting the hypothesis that regions with higher diversity of wildlife are more likely to foster new zoonotic EIDs. Zoonotic EIDs from wildlife are also significantly correlated with human population density. This factor acts as a 'proxy' for anthropogenic and socio-economic changes (e.g. alterations to agriculture, intensification of livestock production, deforestation). Thus, the key group of EIDs – those caused by zoonoses from wildlife – emerge in regions with high human population density and high wildlife biodiversity. In the current proposal, we will develop this approach further and test the correlations between other drivers (agriculture, deforestation, hunting etc), projected trends in these drivers, and the emergence of bat viruses.

Pathogen Type # EID event grid cells	All 356-366		Zoonotic: Wildlife 198-204	
	b	B	b	B
log(JID articles)	0.31-0.34***	1.36-1.40	0.29-0.31***	1.34-1.36
log(Human Pop. Density)	0.51-0.57***	1.67-1.77	0.42-0.48***	1.52-1.61
Human Pop. Growth	0.14-0.47	1.15-1.60	-0.12-0.24	0.89-1.27
Latitude (decimal degrees)	0.02-0.03**	1.02-1.03	0.01-0.02#	1.01-1.02
Rainfall (mm)	$0.27x10^{-3}$-$0.51x10^{-3}$***	1.00-1.00	$0.05x10^{-3}$-$0.29x10^{-3}$	1.01-1.01
Wildlife Host Richness	$0.16x10^{-2}$-$0.42x10^{-2}$	1.00-1.00	$0.67x10^{-2}$-$0.92x10^{-2}$**	1.00-1.00
Constant	-13.69--12.66***		-12.96--11.96***	
Pathogen Type **# EID event grid cells**	**Drug-Resistant** **64-68**		**Vector-Borne** **118-121**	
log(JID articles)	0.47-0.53***	1.60-1.69	0.16-0.22***	1.17-1.24
log(Human Pop. Density)	0.99-1.24***	2.69-3.45	0.35-0.50***	1.41-1.66
Human Pop. Growth	1.02-1.53***	2.76-4.62	-0.44-0.06	0.65-1.06
Latitude (decimal degrees)	0.05-0.06**	1.05-1.06	-0.01-0.00	0.99-1.00
Rainfall (mm)	$0.37x10^{-3}$-$0.62x10^{-3}$*	1.00-1.00	$0.02x10^{-3}$-$0.35x10^{-3}$	1.00-1.00
Sub-national GDP	0.25-0.49***	1.29-1.55	0.18-0.34**	1.19-1.40
Constant	-27.03--23.33***		-12.20--10.11***	

Table 1: Multivariable logistic regressions for EID events (origins of EIDs) according to pathogen type. Numbers represent the range of values obtained from 10 random draws of the possible grid squares where b represent the regression coefficients and B represents the odds ratio for the independent variables in the model. Higher odds ratios indicate that variable value increases have a higher likelihood of being associated with an EID event and probability value equals the median probability from 10 random draws of the possible grid squares where *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and # $p < 0.1$. (Results from each random draw are given in[13].

Finally, we used the results from these spatial logistic regressions to visualize the true, current risk of EIDs globally (EID 'hotspots'). For zoonotic EIDs from wildlife, these regions are areas with high wildlife biodiversity and high human population pressure (e.g. parts of Latin America, Africa and Asia), as well as regions with exceptionally high anthropogenic change and lower diversity (e.g. North America, Europe, South Asia) – (**Figure 4**).

Figure 4. Global Predicted Risk of EID Outbreak (Risk scores calculated without using reporting bias control variable). Risk scores are categorized by deciles, and mapped on a scale from dark green (lowest decile) to dark red (highest decile).

This predictive map shows the risk of a future EID emerging from any wildlife species, due to a few simple anthropogenic changes. It provides proof-of-concept, and can be refined to focus on specific groups of wildlife that are likely to produce significant new EIDs (e.g. bats) and can be expanded using datasets on other anthropogenic changes (e.g. agricultural production, deforestation, road-building), and with data on future projected changes to these factors. This will likely greatly refine the predictive power of the model. Our paper on this preliminary research is now in press with the journal *Nature* [13].

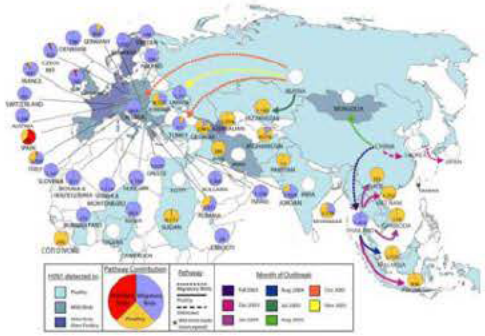


Fig. 5. Spread of H5N1 avian influenza in Asia, Europe, and Africa[29]. Each circle (pie chart) represents a separate spreading event for prior H5N1 spread. Pie charts illustrate the total number of infectious bird-days (# infected birds * days shedding virus) and fraction from each pathway for birds moving between previous H5N1 outbreak countries and the focal country. The orange color denotes poultry trade as the most likely cause of that spreading event, the blue color denotes wild bird migration as the likely cause.

The preliminary data described above provides a strategy to identify regions with the conditions necessary for the initial origins of an EID event. We also developed a strategy to examine what factors cause some EIDs to become pandemic in a case study of H5N1 avian influenza spread. We considered the trade in poultry and pet birds and the movement of migratory birds [29] and found that 44 of 52 country introductions were consistent with trade or migratory bird movement (**Fig. 5, above**).

The success of this approach in explaining H5N1 spread (and demonstrating close congruence with pathogen sequence phylogeny) suggests that this approach can be used for predicting the movement of pathogens linked to trade. We have also modeled the spread of West Nile virus to Galapagos, Hawaii, and Barbados [170-172]. Other workers have published similar analyses predicting the spread of SARS [7], the tiger mosquito (*Aedes albopictus*), an important vector of 22 arboviruses [27], and the future spread of H5N1 should it be viably transmitted among humans [28]. We have demonstrated proof-of-concept that data on trade, combined with virological information and host ecology can be used to predict the pathway and direction of spread of a pathogen globally (**Fig. 6.**). We used data on the global trade of poultry and wild birds, as well as the movements of migratory birds to predict the future spread of H5N1 avian influenza. We found that the risk of spread to the Americas was far greater through poultry trade than by migratory birds.

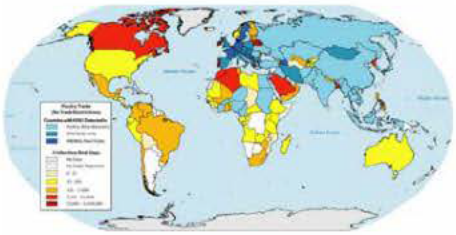


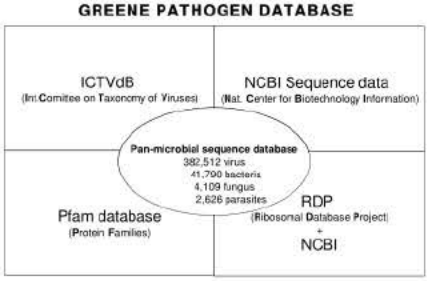
Fig. 6. The predicted risk of H5N1 avian influenza introduction via poultry trade from countries that have previously reported the pathogen (blue) to countries that have not[29]. The risk measures are given in # of infectious bird-days (red = highest, brown = moderate, yellow/white = lowest). This work demonstrates that poultry trade is a far more likely pathway for introduction of H5N1 into the Americas than wild bird migration.

Using this approach, we will build a spatial and temporal database of bat pathogens and human disease emergence events involving bat pathogens, analyse the factors that cause them to emerge, and develop predictive models of 1) the unknown diversity of the bat 'virome'; 2) the future 'hotspots' for emergence of new bat viruses; and 3) the regions within this hotspot map which have the highest connectivity, and which are therefore the most likely to lead to the next emerging pandemic from bats. **Our goal is to expand our sample repository from these hotspot sites, and then use novel virus discovery and pathogenesis techniques to identify new viruses, and understand the likelihood of their future emergence.**

4.2. Microbial surveillance and discovery.

Molecular methods for direct detection of microbes in clinical specimens are rapid, sensitive and may succeed where fastidious requirements for agent replication or the need for high level biocontainment confound cultivation. This program will employ three proven complementary molecular platforms to enable comprehensive surveillance and discovery: MassTag PCR, GreenChip arrays and High Throughput Sequencing (HTS).

4.2.1. Bioinformatics: Establishment of the Greene Microbial Database. A critical early step in the development of the MassTag PCR and microarray tools was the establishment of a viral sequence database. We first implemented a panel that distinguishes 22 different viral and bacterial respiratory pathogens[173]. We later expanded the repertoire to include causative agents of hemorrhagic fever, diarrheal diseases and to subtype influenza viruses. During the period of 10-12/2004, an increased incidence of Influenza-Like Illness (ILI) was recorded by the New York State Department of Health that tested negative for influenza virus by molecular testing, and negative for other respiratory viruses by culture. Concern that a novel agent might be implicated led us to investigate clinical materials [174]. MassTag PCR resolved 26 of 79 previously negative samples, revealing the presence of rhinoviruses in a large proportion of samples, about half of



which belonged to a previously uncharacterized genetic clade. Follow-up studies in Europe, Asia, Africa, and Australia indicated a global distribution of these novel viruses and revealed that they are represent a substantial proportion of unexplained pediatric bronchiolitis and pneumonia. The 2004 New York ILI study confirmed the utility of MassTag PCR for surveillance, outbreak detection and epidemiology by demonstrating its potential to rapidly query with high sensitivity samples for the presence of a wide range of candidate viral and bacterial pathogens that may act alone or in concert.

The development of our viral sequence database was facilitated in 2002 by the move of the ICTVdB (International Committee on Taxonomy of Viruses Database; http://phene.cpmc.columbia.edu), from Biosphere 2 (Earth Institute) in Oracle, Arizona to Columbia; and the establishment of a Northeast Biodefense Center Biomedical Informatics Core (PI Lipkin). To ensure comprehensive coverage, we included every vertebrate virus listed in the ICTVdB, a taxonomic database that describes viruses at the levels of order, family, genus, and species. Construction began by using the Protein Families database of alignments (Pfam, http://pfam.wustl.edu) and Hidden Markov Models (HMM). Sequences for the design of oligonucleotide probes and MassTag PCR primers were selected based on biological parameters, including the degree of conservation of proteins or domains, their expression level during infection, and the amount of data available for the respective region. The majority of viral protein coding sequences in the NCBI database (84%) were represented in the Pfam database; the remainder were mapped using pair-wise BLAST alignments [175]. A pan-microbial database (GreenePmdB) was established by supplementing the GreeneVrdB with ribosomal RNA (rRNA) sequences of fungi, bacteria and parasites obtained from the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu) or the NCBI database. At the time of this writing the GreenePmdB comprises the 382,512 viral sequences of the GreeneVrdB, representing both complete and partial viral genomes; 41,790 bacterial 16S rRNAs; 4,109 fungal 18S rRNAs; and 2,626 18S parasitic rRNAs. **These sequences represent all 2,011 recognized vertebrate virus species** and 135 bacterial, 73 fungal and 63 parasite genera.

4.2.2. GreeneChip Fabrication and Hybridization. DNA arrays have not been widely employed because of limited sensitivity. Using improved methods for sample preparation, amplification, labeling and printing, we have now addressed the challenge of applying this technology to clinical specimens. Together with Agilent Technologies we created a DNA microarray platform suited to analysis of clinical materials without amplification in culture. Investigation by MassTag PCR and viral DNA microarray of blood collected during the 2005 Angola Marburg virus outbreak from an individual who died of hemorrhagic fever failed to yield a pathogen; however, implementation of a *panmicrobial* DNA array, GreeneChipPm, implicated *P. falciparum* infection [176]. Array fabrication studies were initiated by spotting 50, 60, and 70 nt oligonucleotides representing a wide range of viruses with and without amino modifications at the 5' end, onto glass slides. We observed no difference between unmodified 60 or 70 nt oligonucleotide targets. However, hybridization signal improved with the increase in target length from 50 to 60 nt, and amino modification. The enhanced signal with amino modification reflects controlled binding of the oligonucleotide to the slide at one end of the molecule, such that the entire probe is available for hybridization to its intended microbial target. Our intent in implementing the GreeneChip was to identify both known and related agents for which precise sequence information is not available. To assess to what extent a given probe sequence can hybridize to a non-matching but related sequence, we analyzed synthetic mismatch-controls. Whereas up to 15 terminal mismatches had little effect, strings of 5 or more mismatches distributed throughout a sequence, particularly mismatched G/C pairs, resulted in reduced signal; more than 12 mismatches distributed throughout a sequence resulted in no signal. We recently moved from home to commercial printing on the Agilent mask-less printing platform. Advantages include: (1) *Probe orientation*: oligonucleotides are synthesized *in situ* at right angle with respect to the planar surface to allow optimal exposure for hybridization. (2) *Design flexibility*: arrays can be produced in batches of 9 to facilitate modification to include new sequences. (3) *Consistent probe density and morphology*: unlike spotted arrays, Agilent arrays are not confounded by variations in humidity and oligonucleotide concentration. (4) *Higher printing density/higher throughput*: unlike spotted arrays where we have only been able to use 15,000 probes per slide, Agilent slides can accept up to 500,000 probes

per slide, printed in several formats e.g., a 2–well array comprising 220,000 probes/well, a 4-well array comprising 50,000 probes per well, and an 8-well array comprising 15,000 probes per well; gaskets and hybridization chambers have been developed for each of these formats. (5) *Scanning strategy*: implementation of an Agilent scanner allows automatic adjustment of the focal plane for improved resolution; software for extraction of meaningful hybridization data is rapid and user friendly.

Sensitivity is critical to implementation of arrays directly with human, bat or other specimens. Efficiency of individual steps of the protocol was optimized using spiked human samples and real time PCR. First-strand reverse transcription is initiated with a random octamer linked to a specific primer sequence (5'-TCG CGT TAC ATA GTT CGA GNN NNN NN). After RNAse H digestion, cDNA is amplified using a 1:9 mixture of the above primer and a primer targeting the specific primer sequence (5'-CGC TCG CGT TAC ATA GTT CGA). Initial PCR amplification cycles are performed at a low annealing temperature (35^{o}C); subsequent cycles use a stringent annealing temperature (55^{o}C) to favor priming through the specific sequence. Products of this first PCR are then amplified in a second 'labeling' PCR using the specific primer sequence linked to a capture sequence for 3DNA dendrimers containing more than 300 fluorescent reporter molecules (Genisphere Inc.). The PCR product is denatured in hybridization buffer and added to GreeneChips for hybridization. Following washes, a second hybridization step is performed to add Cy3-labeled dendrimers. GreeneChips are incubated with the dendrimers, washed, dried, imaged using an Agilent DNA microarray scanner, and analyzed using Agilent Feature Extractor software. The use of dendrimers provides a 100x gain in sensitivity over microarray labeling methods where reporter molecules are directly incorporated into amplification products.

4.2.3. *GreeneChip analysis.* GreeneLAMP (Log-transformed Analysis of Microarrays using P-values) version 1.0 software was created to assess results of GreeneChip hybridizations. Common analysis software focuses on the differential two-color analysis used in gene expression arrays, which is not applicable to the GreeneChip. GreeneLAMP has a robust and generalized framework for microarray data analysis including: flexible data loading, filtering and control experiment subtraction. Probe intensities are background corrected, $\log_2$-transformed and converted to Z-scores (and their corresponding p-values). Where available, control matched experiments from uninfected samples are used and spots >2 standard deviations (SD) from the mean are subtracted. In instances where matched control samples are not available, the background distribution of signal fluorescence is calculated using fluorescence associated with 1,000 random 60-mers (Null probes). In both scenarios, positive events are selected by applying a false positive rate of 0.01 (the rate at which Null probes are scored as significant) and a minimum p-value per probe of 0.1 (in cases with a matching control) and 0.023 (2 SD) (in cases without a matching control). A map, built from a BLASTN alignment of probes to the GreenePmdB, is used to connect probe sequences to the respective entries in the GreenePmdB. Each of those sequences corresponds to an NCBI Taxonomy ID (TaxID). The individual TaxIDs are mapped to nodes in a taxonomic tree built based on ICTV virus taxonomy or the NCBI taxonomic classification for other organisms. The program output is a ranked list of candidate TaxIDs. Candidate TaxIDs are ranked by combining the p-values for the positive probes for that TaxID using the QFAST method of Bailey and Gribskov [177].

4.2.4. Assessment of GreeneChip Performance. Although our primary goal in developing the GreeneChip platform is to have a tool for clinical applications, there are instances where it may also be useful for characterization of cultured materials. High density GreeneChips allow virus speciation. Additionally, hybridized microbial sequences recovered from GreeneChips, can be cloned and sequenced, eliminating the need for trial and error consensus PCR. However, the most compelling reason to use cultured materials is pragmatic: we have access to large banks of well characterized cultured viruses. Through collaborative relationships with our partners in WHO network laboratories we obtained extracts of cultured cells infected with adeno-, alpha-, arena-, corona-, entero-, filo-, flavi-, herpes-, orthomyxo-, paramyxo-, pox-, reo-, and rhabdoviruses (total of 49 viruses). All were accurately identified. To assess sensitivity, viral RNA extracted from infected cell supernatants was quantitated by real time PCR, serially diluted and subjected to

analysis using template concentrations ranging from 1,000,000 to 10 copies/assay. The threshold detection of adenovirus was 10,000 RNA copies; the threshold for detection of the other viruses tested was 1,000 RNA copies. Array performance was tested using samples obtained from patients with diarrhea, respiratory disease, hemorrhagic fever, tuberculosis and urinary tract infections. In all cases array analysis detected an agent consistent with the diagnosis obtained by culture or PCR. GreeneLAMP analysis revealed the presence of human rotavirus A, human adenovirus F, caliciviruses, astrovirus, human enterovirus A (EV-A), human respiratory syncytial virus A (RSV-A), influenza A virus, Lake Victoria marburg virus (MARV), severe acute respiratory syndrome coronavirus (SARS-CoV), lactobacillus, mycobacteria and gammaproteobacteria. Specific real time PCR analyses indicated viral loads in the clinical specimens of $6.3x10^5$ copies/assay for SARS-CoV [178], $1.1x10^3$ copies/assay for RSV-A [179], and $5.46x10^5$ copies/assay for EV-A [180].

4.2.5. Recovery of Hybridized Sequences from GreeneChips. In pilot experiments using WNV, SARS, and Sindbis isolates, we recovered cDNAs ranging from 200 to 1,000 nt. GreeneChips display 3 or more probes representing different genomic regions for each virus. Sequence recovery, characterization and phylogenetic analysis are straightforward and readily implemented in clinical or field laboratories. A silicon gasket is applied to the slide to define a well over the array. Water is placed in the well at 65°C for 10 min. The water containing the eluted cDNA is used as template for PCR amplification with the specific amplification primer used to generate the hybridized product. Products are cloned, screened by direct colony PCR and sequenced.

4.3. Viral pathogenesis in vitro.

4.3.1. Fusion kinetics. As part of an existing collaboration funded by NIAID (U01 AI056423-01) (Mungall, Co-PI) we have developed methods to quantitate NiV infection in Vero cell culture for the implementation of antiviral assays. To date, we have developed functional and quantitative assays for measuring NiV cell-fusion and virus entry [181-183]. Using recombinant vaccinia viruses expressing henipavirus F and G proteins we can accurately quantitate the kinetics of viral fusion (**Fig. 7**). By producing analogous constructs for each paramyxovirus under investigation, we will evaluate the fusion kinetics of each virus in equivalent cell lines.

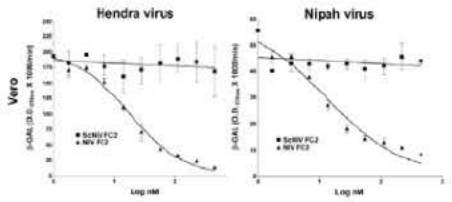


Figure 7. Quantitation of Hendra virus and Nipah virus-mediated cell-cell fusion. HeLa cells were infected with vaccinia recombinants encoding HeV F and HeV G or NiV F and NiV G glycoproteins, along with a vaccinia recombinant encoding T7 RNA polymerase (effector cells). Each designated target cell type was infected with the *E. coli* LacZ-encoding reporter vaccinia virus vCB21R. The cell fusion assay was performed for 2.5 hr at 37°C, followed by lysis in Nonidet P-40 (1%) and β-Gal activity was quantified.

In addition to these kinetic assays, we have also developed assays incorporating fluorescent immunolabelling of viral protein in cell cultures 24hrs after infection providing a reproducible measure of syncytium formation (**Fig. 8**).

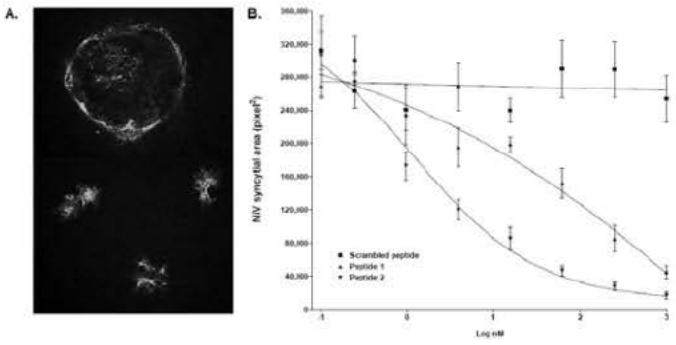


Figure 8. Quantitation of NiV infection of Vero cells. A. Vero cells are infected with NiV in the presence or absence of test antiviral. Cells are incubated for 24 hours, fixed in methanol and immunofluorescently labeled for phosphoprotein prior to digital microscopy. The top image is an untreated control well, the image on the bottom shows a well treated with a test antiviral peptide, resulting in decreased syncytium size. **B.** Image analysis was performed to determine the relative area of each syncytium. Figure shows the relative

syncytial area versus peptide concentration for scrambled peptide control (squares) and two test peptides (triangles).

4.3.2. Viral replication kinetics. In addition to direct virus quantitation assays, we have developed reliable systems for isolation and quantification of NiV from samples and tissues of infected animals [18]. We have established real time PCR assays for NiV, HeV and MenV (Bowden, unpublished data) used to quantify virus in infected tissue culture supernatant and clinical samples (**Fig. 9**). We will establish Taqman PCR assays for detection of each virus under investigation enabling the rapid and accurate quantitation of viral genome *in vitro*.

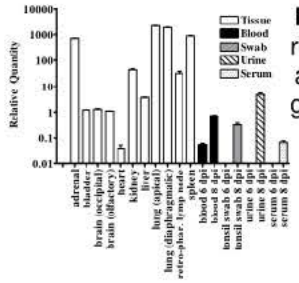


Figure 9. NiV genome in cats detected by Taqman PCR. Normalized, relative NiV genome levels in samples collected during NiV infection in cats and at necropsy. Taqman PCR Ct values were determined in triplicate for NiV genome and normalized by dividing by the 18S rRNA Ct values for each sample. Relative NiV genome was determined by linear regression of NiV cDNA standard curves for each assay. Values are expressed as the average of all replicates and are from a single representative animal.

4.4. Paramyxovirus reverse genetics

As described previously [184] a cDNA representing the full length of the Malaysian NiV genome [51] has been constructed. This was inserted in the plasmid pMDB1 with unique restriction sites included at the end of each gene (pNiV6+ **Fig. 10.**). An additional construct expressing the EGFP gene, inserted downstream of the N gene has also been prepared. Infectious virus was rescued from the full-length clones by infecting CV-1 cells with MVAGKT7, a highly host-restricted strain of vaccinia virus MVA that expresses the T7 polymerase [185]. A mixture of the genome plasmid and N, P, and L supporting plasmids were transfected to the infected CV-1 cells.

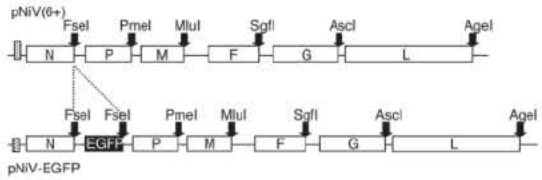


Figure 10. Schematic representation of the pNiV full-length clones constructed. The unique restriction sites used in the cloning procedure are shown. pNiV(6+) has six unique restriction enzyme sites (indicated) and the EGFP construct contains this additional gene inserted between the N and P genes.

The ratio of the plasmids used was that determined previously[186] using a minigenome system for NiV. The transfected CV-1 cells were cultured for 7 days with the addition of new cells. Syncytia were successfully visible 2 days after transfection. Virus was then passaged in Vero cells in which it induced a large number of syncytia within 24 h, characteristic of NiV infection. The rescued NiVs (rNiVs) were further passaged in Vero cells to produce a stock. The recombinant virus expressing EGFP (rNiV-EGFP) was also recovered by using the same procedure. The expression of EGFP in the rNiV-EGFP-infected cells was verified by using fluorescence microscopy (**Fig. 11.**).

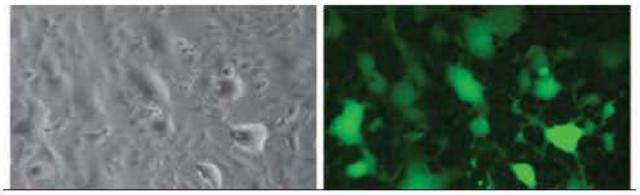


Figure 11. Vero cells were infected with rNiV-EGFP at a multiplicity of infection of 0.01 pfu per cell. The cells were observed after 24 h in a light microscope (left panel) or by fluorescence (right panel). The fluorescence was stably detected after the rNiV-EGFP was grown for three passages in cell culture. The extent of the cytopathic effect (CPE) induced by rNiV or rNiV-EGFP was similar to that of their parental NiV.

While this EGFP expressing virus could theoretically be detected using a fluorimeter we intend to replace the EGFP gene with firefly luciferase, greatly enhancing the sensitivity of virus detection and making use of the luminometer available in our laboratory. Using a commercially available assay system for quantitation of firefly luciferase (Promega), we anticipate being able to rapidly and sensitively quantitate viral load following infection.

4.5. Bat cell lines

Bats including those belonging to the genus *Pteropus*, display an almost uniform asymptomatic response when infected with doses of virus which kill other non-volant mammalian species. A detailed understanding of the immune response of bats at the cellular and molecular level may lead to previously unrecognised mechanisms of disease control. CSIRO-AAHL has initiated a project (Eric French Fellowship to Dr Mungall) to develop tools for viral pathogenesis in bats. At the heart of this program is a concerted effort to develop both primary and immortalized bat cell lines. There are currently no *Pteropus* cell lines commercially available and primary bat cell lines are inherently difficult to establish and maintain. This development program is already in progress and a number of cell lines from a range of Pteropus organs will be available early 2008, prior to the commencement of this NIH proposal. For the collection of bat organs for cell culture AAHL has several veterinarians on staff who are experienced and competent in dealing with bats and AAHL has an approved animal welfare assurance status (**OLAW: A5399-01**). As the bat cell line generation is funded separately, and only immortalized cell cultures will be used for this project, we believe that we should not include a vertebrate animal research section.

4.6. Whole virus genome HTS.

HTS in this aim will be pursued at the Columbia site where there is already considerable expertise in 454 technology. Data will be conveyed to the CSIRO site for detailed bioinformatic and functional analyses.

5. Research Design and Methods

Our Team. This is a unique, multidisciplinary proposal to address the complexity of viral emergence in humans from a key wildlife reservoir group. We have assembled a powerful interdisciplinary team that includes: The director and key staff at the Consortium for Conservation Medicine (CCM) – an institution dedicated to understanding the ecology of emerging diseases from wildlife reservoirs (Daszak, Epstein); a world authority on analysing large databases of reservoir hosts and their pathogens (Jones); the leader and key personnel of the Center for Infection and Immunity (CII) – the premier viral discovery laboratory globally (Lipkin, Briese, Palacios), and a senior staff member at one of few labs dedicated globally to emerging zoonotic pathogens, the Australian Animal Health Lab (Mungall). Each team member has proven expertise in their field, and all have collaborated together under the umbrella of the CCM over the past five years. Indeed, the collaborative approach is central to the way the CCM functions, building multidisciplinary teams to address key issues in disease emergence (www.conservationmedicine.org). Each of the leads at Columbia University (Lipkin), IOZ (Jones), and CSIRO AAHL (Mungall) are long-term adjuncts at CCM, and the CCM faculty are long-term adjuncts at CU and IOZ. This produces a seamless inter-institutional collaboration.

This collaboration has taken place under three projects: **1)** An NIH (Fogarty Intl. Center)-funded project to understand the emergence of Hendra and Nipah virus (R01-TW05869, Daszak PI) which involved Daszak, Epstein and Mungall; **2)** An NSF-funded project to develop a predictive approach to disease emergence (HSD collaborative award #0525216, Daszak PI) which involved Daszak and Jones; **3)** A NIAID-funded training project to understand the emergence of Nipah virus in Bangladesh (K08-AI067549, Epstein PI) which involves Daszak and Lipkin as co-mentors. These collaborative projects have provided some of the preliminary data for this proposal, and demonstrate the cohesion of this group of PIs and their ability to collaborate in this type of multidisciplinary work (see also **"Management Plan"** below).

5.1.Aim 1. Predictive modeling of bat viral diversity and risk of future emergence.

5.1.1. Prediction of global hotspots for bat viral biodiversity, and for the emergence of new zoonoses from bats.

Our approach will follow that used previously for the predictive modeling of EIDs of different types (zoonotic, drug-resistant, vector-borne)[13] and our (Jones) previous development of pathogen databases from carnivores, ungulates and primates [187, 188]. Digital spatial distributions of parasites will be generated from the combined ranges of their hosts using information on the host-parasite combinations from the Bat Disease database and range maps from the Mammal Digital Distribution database[189]. Preliminary data collection has already started on the Bat Disease Database with collaborator Dr. Maarten Vonhof (Western Michigan University) which currently holds data on 5,000 host parasite interactions for over 500 bat species from over 1,000 references. Richness grids (counting the number of parasite taxa per equal area spatial global grid) generated using ArcGIS[166]for all parasites reported from wild bats will be adjusted for measures of sampling effort for each host. GAP analysis will identify areas that have been under-sampled for infectious diseases relative to bat diversity[190]. We will finalize the database, then use GAP analysis to predict the global biodiversity of bat viruses, following the techniques developed by our earlier collaborators for primate pathogens[191]. Using this database, we will also test the hypothesis that bats are reservoirs of a disproportionate number of zoonotic and potentially zoonotic pathogens, which is widely proposed in the recent literature[37, 159, 192], but not supported by previous database analysis[3].

To predict global hotspots of zoonotic disease emergence from bats, we will repeat the analyses in [13] using the database of emerging zoonoses from bats to assess the spatial risk of future zoonotic disease emergence from bats. Specifically, we will regress our gridded datasets of richness of EIDs from bat pathogens onto gridded data sets of bat species richness, rainfall, temperature, human population density and growth and our measure of literature bias (corrected for a more bat-specific measure – e.g. using the authors of articles in the *Journal of Wildlife Diseases* and *Journal of Infectious Diseases*). The richness grid of all mammalian hosts serves as a null model for patterns of parasite richness and including it allows us to determine the influence of the other factors independently of the distribution of mammalian hosts. We will use generalized least squares models (SAS) and likelihood ratio tests for nested models[193, 194] to

quantify the relationship between pathogen species richness in livestock and wildlife (the two response variables) and the core explanatory variables. We will test for spatial autocorrelation of the residuals using Moran's I tests as we have done previously [195, 196], and use autoregressive models to account for spatial autocorrelation between adjacent localities[197, 198]. We will repeat these analyses using the spatial predictions of the unknown diversity of the bat 'virome' to gain a fuller understanding of the predictive risk.

We will then use global, gridded datasets on environmental variables that are shown to more directly impact bat populations and increase contact with people. These will include datasets subnational livestock distribution for sheep, goat, small ruminant, pig, poultry, and bovines from the Food and Agricultural Organization (http://www.fao.org/ag/aga/glipha/index.jsp) and livestock data from other sources. These include data on rearing density, production method, and area of cultivation.

5.1.2. Targeted surveillance in EID 'hotspots' to expand our current knowledgebase of bat viruses.

The CCM is one of the key institutions working on bat viruses globally. The Executive Director, Daszak, heads the Henipavirus Ecology Research Group (HERG) formed five years ago around an NIH-funded program (R01-TW05869) which has investigated the origins of Nipah virus in Malaysia[34, 35], Hendra virus in Australia[199], and SARS CoV in China[158, 160, 200, 201]. This work (Daszak, Epstein) has involved collection and storage of 3,785 biological samples from bats of a range of species from Australia, Indonesia, Malaysia and Bangladesh (**Table 2**).

Bat Species	Sample Type	Number	Country of Origin
Pteropus giganteus	Serum	500	Bangladesh
	saliva	500	Bangladesh
	urine	500	Bangladesh
	colony urine	1250	Bangladesh
P. vampyrus	serum	256	Malaysia
	saliva	256	Malaysia
	urine	256	Malaysia
	dried blood spot	63	Malaysia
	serum	4	Indonesia
P.hypomelanus	serum	789	Malaysia
	saliva	789	Malaysia
	urine	789	Malaysia
	serum	20	Indonesia
Eonycterus spelea	salivary gland	30	Malaysia
	liver	30	Malaysia
	kidney	30	Malaysia
Cynopterus brachyotis	salivary gland	102	Malaysia
	liver	102	Malaysia
	kidney	102	Malaysia

Table 2. List of bat biological samples collected by the Consortium for Conservation Medicine and available for the current project

Samples from these countries are stored at the Center for Infection and Immunity (CII, Lipkin) and the Australian Animal Health Laboratory (Mungall), and are available for our current research. We will expand this collection by targeting bat species within the regions uncovered by our hotspot predictions. Because we don't yet know the location of these hotspots, we cannot yet assess with total accuracy where sampling will need to take place. However, we can refer to our predictive map of the future origins of zoonotic EIDs from all wild mammal species (**Fig. 3**). Bats make up almost a quarter of all mammals[202], and it is likely that the final hotspots will be within the broad regions predicted by the mammalian 'hotspot' map. We therefore anticipate collection of samples in Mexico, Brazil, Cameroon and China in addition to those already collected. To facilitate this, the CCM will use its network of collaborators in the Wildlife Trust Alliance, with which it is formally a partner (www.wildlifetrust.org), and with which CCM scientists collaborate actively (e.g. see[203]). We anticipate no problems in obtaining samples from China, due to our unique formal collaboration with East China Normal University (Dr Shuyi Zhang), which is built around a signed MOU (see www.conservationmedicine.org). Previous

collaboration with Dr Zhang in China involved samples from over 1,500 bats being sent to the Australian Animal Health Lab (Mungall) and the discovery of bats as the likely source of the progenitors of SARS CoV[160, 201].

Potential pitfalls/alternative strategies: It is possible that the predictive modeling will highlight regions which are not covered by the Wildlife Trust Alliance, or other collaborators with CCM. We believe this is unlikely, because the risk map is essentially likely to be a product of regions of high anthropogenic pressure and high bat biodiversity. Regions of bat biodiversity closely correlate with mammalian biodiversity [202, 204], and therefore these areas are likely to be correlated with the hotspot map in Figure 3. Furthermore, if the regions are different, we believe that collaborative arrangements could easily be set up to collect samples. Our groups are very well connected in the conservation and ecology community, and we have been able to conduct such sampling without much difficulty, even in politically sensitive countries (e.g. China and India) in the past.

5.1.3. Risk assessment of future viral emergence from bats. In years 04 and 05, we will expand our hotspot modeling to incorporate global, gridded datasets on projected future changes in EID drivers, and on international travel and trade. This will provide a refined, more accurate risk assessment for future viral emergence from bats. We will use the regression models from **5.1.1** with only significant terms to generate likelihood for disease emergence for each global grid square. Next, we will produce an integrated, spatial database of drivers projected through the year 2050. Third, we will apply the parameters derived from the Aim 1 statistical findings to generate first-order estimates of future disease emergence risks, for four different scenarios of the future. Finally, we will compare the results across scenarios and conduct sensitivity analyses determine the degree to which the results are influenced by different drivers and projected patterns of change.

Central to this activity is the spatial database of projected drivers. To help achieve a high degree of internal consistency, we will seek to utilize model outputs from an integrated collection of linked models utilized widely within the global change research community, the IMAGE model[205, 206]. The IMAGE model, and its linked modules, provide the following useful parameters on a 0.5 degree grid, to the year 2100: Human population; Urbanization; GDP; Land cover; Livestock density; Temperature; and Precipitation. A benefit of utilizing these data sets and models is that it facilitates consideration of our results by the broader global change modeling community. We will supplement this collection of spatial drivers with select additional data, including projections of international air travel obtained from Boeing [207, 208].

We will utilize the same alternative scenarios used in the IPCC[209] to calculate different ranges of future drivers and impacts. The scenarios contain alternative packages of assumptions regarding a number of factors that influence global change, most important of which for our purposes are population and per-capita income growth rates. We will apply the results from the statistical analyses to the projected values of the socioeconomic and environmental drivers to estimate possible disease emergence risk surfaces, for each of the disease categories. We will then forecast and map changes in the probability of the panzootic spread of bat-origin EIDs using projections of climate change, human population growth, livestock production increases (**Figure 12**), relative GDP growth, and global trade and travel (**Figure 13**).

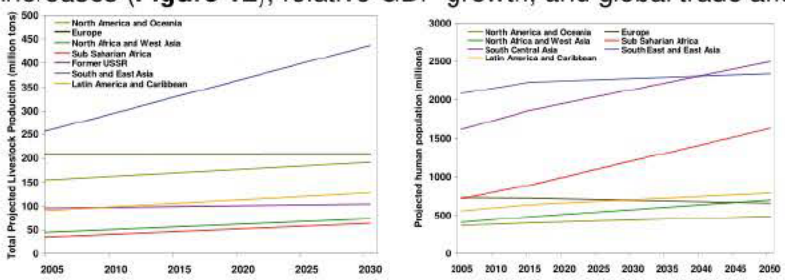


Figure 12 (above). Projected changes in livestock production (left, in 10^6 tons) and human population, by region (right, in thousands)[148, 205].

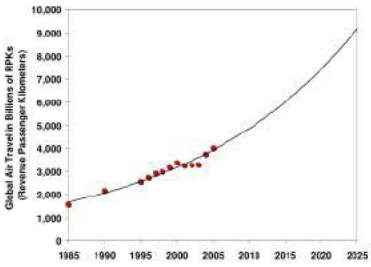


Figure 13 (left). Past and projected change in global air traffic volume[210]. RPK is the number of kilometers traveled annually multiplied by the number of people traveling. In our dataset, air traffic volume data are available on an airport-by-airport basis.

For both analyses, we will test alternative functional forms (allowing for non-linearities, variable interactions, multilevel models), to explore the degree to which such projections are sensitive to such variation. We will compare the results of the alternative scenarios to see which differences are most significant in terms of EID risk. This analysis will enable us to set priorities for future research, by illuminating areas of current uncertainty that have the highest potential to resolve uncertainties about the future.

5.2. Aim 2. Bat viral pathogen identification using a staged strategy.

5.2.1. MassTag PCR analysis.

We will implement an efficient, staged strategy for microbial surveillance and discovery. In year -01 MassTag PCR panels will be used to rapidly screen bat samples for the presence of known and closely related paramyxoviruses, lyssaviruses, and coronaviruses. In years -02 and -03 GreeneChips will be used to survey all vertebrate virus taxa. In years -03 and -04 a selected subset of samples from the CCM collection will be subjected to HTS analysis to identify microbes not captured by MassTag PCR or GreeneChip and to profile microflora. (Co-PIs Lipkin & Briese).

Our first strategy will be to establish bat housekeeping gene controls. Housekeeping gene controls are critical for assay calibration and monitoring RNA integrity (MassTag PCR, GreeneChips, HTS). CSIRO-AAHL is in the process of sequencing an Australian *Rhinolophus* and an Australian *Pteropus* transcriptome (funded by the Australian Biosecurity CRC). Sequence will be available late 2007. Employing these as a guide, the coding sequences for commonly used expression control genes like beta-actin and GAPDH, will be cloned from bat mRNA by cPCR with primers designed using GreeneSCPrimer.

MassTag PCR assays for detection of paramyxoviruses, lyssaviruses and coronaviruses. We will inventory viruses known and closely related to taxa already identified in bats using an inexpensive, high throughput screen. Assay development will be completed within 6 months with implementation immediately thereafter. We will rapidly identify candidate viruses for further characterization through analysis of sequence, phylogenetic, and prevalence data. These analyses will focus investment in culture and pathogenesis in years 1-2. We will establish bat housekeeping gene controls for MassTag PCR assays and optimize MassTag PCR assays for bat feces, saliva, urine and serum. We will then implement MassTag PCR assays for paramyxoviruses, lyssaviruses and coronaviruses, confirm identity of viruses identified by MassTag PCR analysis and establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance. Utilyzing a large panel of uncharacterized virus isolates obtained from bats and stored at the Australian Animal Health Laboratory (AAHL – Consortium), extracted RNA will be provided to the Columbia University lab for MassTag PCR analysis.

Optimize MassTag PCR assays for bat feces, saliva, urine and serum. The effects of sample type on assay specificity and sensitivity will be tested using bat feces, saliva, urine and serum spiked with known concentrations of paramyxovirus, lyssavirus and coronavirus. Routine sample preparation will use Tri-Reagent. In the event that PCR inhibition is detected with housekeeping gene controls, alternative extraction procedures will be evaluated; including Qiagen silica columns (Qiagen); NucliSens soluble silica extraction (Biomerieux) or a combined Ultraspec II extraction system (Biotecx).

Implement MassTag PCR assays for paramyxoviruses, lyssaviruses and coronaviruses. Primer sets will be selected in conserved regions of the viral genomes to screen for the presence of coronaviruses, rhabdo-/lyssaviruses, paramyxoviruses. For coronaviruses the available SARS and bat coronavirus sequences will be used and primers will be selected in the same conserved polymerase regions that were used to initially identify this virus. For other viruses multiple

sequence alignments based on sequences listed in **Table 3** will be used to select appropriate primer sets using SCPrimer software. MassTag PCR panels will be built as 20-plex reactions; in the event that more than 20 primer sets are needed to cover the targeted sequences 2 MassTag panels will be assembled. We will include in addition screening primers targeting flavi-, bunya-, filo- and reoviruses that have been described or are suspected to occur in bats (Table 3).

Table 3. Significant viruses isolated from, or identified in, bats.

Lyssaviruses	**Paramyxoviruses**	**Flaviviruses**	**Bunyaviruses**
rabies	Hendra	Bukalasa bat	Kaeng Khoi
Duvenhage	Nipah	Carey Island	Nepuyo
Lagos bat	Mapuera	Dakar bat	Hantaan
Australian bat	Menangle	Entebbe bat	Rift Valley fever
European bat 1 and 2	Tioman	Japanese encephalitis	Toscana
West Caucasian bat	Beilong	Jugra	
Aravan		Kyasanur Forest disease	**Togaviruses**
Khujand	**Filoviruses**	Montana myotis	Chikungunya
Irkut	Ebola Reston	Phom-Penh bat	
		Rio Bravo	
Other Rhabdoviruses	**Reoviruses**	St. Louis	
Kern Canyon	Melaka	Saboya	
Mount Elgon bat	Nelson bay	Sokuluk	
Oita 296	Pulau	Tamana bat	
		Uganda S	
		Yokose	

Confirm identity of viruses identified by MassTag PCR analysis. Either conserved genetic regions amplified in MassTag assays (typically 100-200nt) will be cloned and sequenced or, where possible, adjacent genome regions with greater phylogenetic information will be amplified using published primer sets, or sets selected from multiple sequence alignments by SCPrimer. We will then establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance. Control templates will be cloned and sequenced; primers will be tested for sensitivity and specificity and modified as required to achieve threshold of 50 RNA copies. We have extensive experience in the development of Taqman PCR assays such that we don't envisage technical difficulties in this aim.

Anticipated results: Given our recent success in establishing and utilyzing MassTag PCR assays [118, 211], we do not anticipate major impediments to the swift completion of this aim. After establishing suitable bat internal controls, we will rapidly accumulate sequence data and characterize bat paramyxoviruses, coronaviruses and lyssaviruses, forming a substantial base with which to compare and contrast novel viruses discovered through the targeted sampling and surveillance of bats in EID hotspots identified in Aim 1.

Potential pitfalls/alternative strategies: Given the largely unknown nature of bat sequence information, it is possible that sequence variability between bat species may confound cloning of housekeeping gene controls. Bat mitochondrial genes are well represented in GenBank and are typically employed for phylogenetic studies. We have established primer sets that detect NADH dehydrogenase subunit 2. This primer set will be used in the unlikely event we fail to identify suitable pol II transcript controls. NADH dehydrogenase subunit 2 primers address a wide range of bat species including: *Pteropus giganteus*,*P. rodricensis*, *Pteropus hypomelanus, Pteropus scapulatus, Pteropus pumilus, Pteropus vampyrus, Cynopterus brachyotis, Paranyctimene raptor, Boneia bidens, Macroglossus minimus, Nyctimene albiventer, Nyctimene aello, Cynopterus sphinx, Thoopterus nigrescens, Syconycteris australis, Rousettus aegyptiacus* and *Eidolon helvum.* We acknowledge that the limit of detection of MassTag PCR assays may prevent the identification of viral targets that are either present in samples at a concentrations <100 RNA copies or are sufficiently different in nt sequence to abrogate primer binding. We cannot address the issue of sensitivity; however, detection of viruses that elude detection due to sequence variation may be captured by GreeneChip or HTS.

5.2.2. GreeneChip assays for detection of vertebrate viruses.

Samples negative in MassTag, and a random sample of 10% of positive samples (detection of co-infections) will be analysed by GreeneChip. Samples positive in GreeneChip assays will be further characterized by release and sequence analysis of hybridized nucleic acid as described section 4.2.5 of Preliminary Studies. Specific real time PCR assays will be established and implemented for quantitation of pathogen burden and surveillance
This aim requires optimization GreeneChip assays for bat feces, saliva, urine and serum. We will then implement GreeneChip assays for bat feces, saliva, urine and serum using the same strategy employed in Aim 5.2.1.

Potential pitfalls/alternative strategies: Our consistent success in implementing GreeneChips for pathogen identification in cultured as well as clinical specimens indicate that we will not have difficulties establishing assays [16, 17, 212, 213]. Viral targets may escape detection that are either present in samples at concentrations below the sensitivity threshold (<5,000 RNA copies) or differ in nt sequence by more than 10% from any printed viral probe. Novel viruses missed in this aim may be identified through HTS.

5.2.3. Metagenomic sequence analysis of bat feces, saliva, urine and serum

Samples negative in GreeneChip, and a random sample of 10% of positive samples (detection of co-infections) will be analysed by HTS. We will design and implement software for subtraction of bat sequences. The method will be analogous to the one that we developed for the subtraction of human host sequences to identify a novel arenavirus in human transplant tissues (manuscript in press, NEJM) or to subtract bee sequences in our study of Colony Collapse Disorder of the honey bee [157]. Bat sequences for this purpose will be obtained through a bat transcriptome project that is currently conducted at CSIRO-AAHL This project is funded by the Australian Biosecurity CRC (Daszak and Lipkin are members of the International Standing Advisory Committee), and is expected to be completed in late 2007. Co-PI Mungall is a member of the bat transcriptome project, which is based at AAHL. We will then implement metagenomic assays of bat feces, saliva, urine and serum subjected to HTS. Sample preparation for metagenomic assays of bat feces, saliva, urine and serum will be optimized by spiking bat feces, saliva, urine and serum with a known target nucleic acid. Extraction efficiency and amplification performance will be traced using the established housekeeping gene controls as well as real time PCR quantitation of the introduced target nucleic acid.

To confirm identity of microbes identified in metagenomic assays, we will use data obtained through HTS to design specific and cPCR primers for amplification of products that can be used for phylogenetic analyses. Finally, we will establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance. Data obtained through HTS will be used to design real time primers and probes and to clone control templates. This will proceed as previously described in sections **5.2.1 and 5.2.2.** HTS is rapidly establishing itself as a viable strategy for the mass sequencing of viral (and other) genomes.

Potential pitfalls/alternative strategies: Microbial targets may escape detection that represent either <1% of total sequence present after subtraction of chromosomal DNA and host rRNA or have less than <30% aa similarity to microbial sequences in the database. As for the previous assays, sensitivity will always be a limiting factor in any pathogen discovery strategy but we believe the staged assay implementation strategy we have chosen has a high chance of identifying novel pathogens, if present in samples. If we suspect that nt sequence variation is responsible for assay failure, novel sequences will be used to design primers for cloning larger fragments by 3' and 5' RACE, and to establish and implement specific real time PCR assays for quantitation of pathogen burden and surveillance.

It is possible that we will not be able to identify new viruses in the wildlife samples we have collected, and will collect. We believe this is unlikely. A simple analysis (Daszak, unpublished data) shows that for the known 50,000 vertebrate species present, if we estimate that each species harbours 20 endemic viruses (likely a significant underestimate, given that there are 217 known viruses found in humans [4]), it follows that there are around 1,000,000 vertebrate viruses, yet we have only discovered 2,000 viruses in total, an underestimate of pathogen diversity by 99.8%. If this degree of underestimation is equivalent across the vertebrate classes, then bats are likely to have over 20,000 viruses as yet undiscovered, given

an estimated total for bat host species diversity of 1116 species[214]. It is likely that prevalence of most viruses (which tend to be acute) will be low. However, we will pool samples to maximize the likelihood of viral discovery. Finally, even if no new viruses are discovered, we are likely to find significant new information on the incidence of known emerging viruses, which has great relevance to the study of bat virus ecology and biology, as well as public health.

5.3. Aim 3. Bat Viral Pathogenesis

3.1. In vitro evaluation of bat derived paramyxoviruses, coronaviruses and lyssaviruses in Vero and bat cell lines to determine correlates of infection.

We will initially characterise the infection of a number of prototype viruses from each of the three main bat virus families in cell lines that will not only readily propagate virus (Vero and BHK cells), but in primary or continuous bat cell lines (developed during a parallel CSIRO funded project) to evaluate potential tissue tropisms and cellular responses to infection in the bat. In addition to comparing the cellular (host) responses, we will also evaluate the viral quasispecies changes (using whole genome sequencing) apparent following single or multiple passages to examine adaptation during the establishment phase of viral infections.

Host response to infection. Routine cell culture systems (Vero cells or BHK cells) or primary and/or continuous bat cell lines (unrelated CSIRO funded project) will be utilyzed to evaluate host responses to a number of recently emerged, non-biodefence related paramyxoviruses (MenV, TPMV, SalV, Tioman, Mapuera and PoRV Virus), coronaviruses (bat SARS CoV, bat CoV, and a number of bat coronavirus isolates from Hong Kong) in addition to several lyssaviruses (and related rhabdoviruses). Further, we will harness the power of whole genome, rapid pyrosequencing for the determination of molecular correlates of infection and pathogenesis. A suite of routine assays for evaluating the state of cellular physiology and function will be incorporated to compare uninfected versus infected cells. These include markers for apoptosis and necrosis, fusion kinetics, viral replication kinetics and local immune function (including interferon inhibition utilized by each virus).

Assessment of apoptosis and necrosis. There are a number of simple add, mix and measure assays available for rapid quantitation of cell viability and apoptotic events, enabling the rapid characterisation of viral infection in vitro. The MultiTox-Fluor (MT-F) Multiplex Cytotoxicity Assay is a single-reagent-addition fluorescent assay that simultaneously measures the relative number of live and dead cells in cell populations. The MT-F Assay gives ratiometric, inversely correlated measures of cell viability and cytotoxicity. In addition to information about relative cell viability, assays that directly measure caspase activity can provide valuable information about the mechanism of death in infected or dying cells. The Caspase-Glo® Assays use the luminogenic caspase-8 tetrapeptide substrate (Z-LETD-aminoluciferin), the caspase-9 tetrapeptide substrate (Z-LEHD-aminoluciferin) or the caspase-3/7 substrate (Z-DEVD-aminoluciferin) and a stable luciferase in proprietary buffers. The buffers are optimized for the specific caspase activity, cell lysis and luciferase activity. In the absence of active caspase, the caspase substrates do not act as substrates for luciferase and thus produce no light. Upon cleavage of the substrates by the respective caspase, aminoluciferin is liberated and can contribute to the generation of light in a luminescence reaction. The resulting luminescent signal is directly proportional to the amount of caspase activity present in the sample.

Fusion kinetics. As part of an existing collaboration funded by NIAID (U01 AI056423-01) (Mungall, Co-PI), we have developed methods to quantitate NiV infection in Vero cell culture for the implementation of antiviral assays. To date, we have developed functional and quantitative assays for measuring NiV cell-fusion and virus entry [181-183]. Using recombinant vaccinia viruses expressing henipavirus F and G proteins we can accurately quantitate the kinetics of viral fusion (see preliminary data). By producing analogous constructs for each paramyxovirus under investigation, we will evaluate the fusion kinetics of each virus in equivalent cell lines.

Viral replication kinetics. In addition to direct virus quantitation assays, we have developed reliable systems for isolation and quantification of NiV from samples and tissues of infected animals [18]. We have established real time PCR assays for NiV, HeV and MenV (Bowden, unpublished data) used to quantify virus in infected tissue culture supernatant and clinical samples (see preliminary data). We will establish Taqman PCR assays for detection of each virus under investigation enabling rapid and accurate quantitation of viral genome *in vitro*.

Immune function. We will comprehensively evaluate a panel of cytokines likely to be released during the initial phases of viral infection. Using an established real-time PCR assay, modified for each cell type (species), which allows the precise quantification of changes in the expression level of six relevant porcine cytokines, and three housekeeping genes [215] we can simultaneously detect nine sequences by measuring 3x3 targets in a triplex-format. The mRNA of the lymphokines IL-2, IL-4, IL-10, and IFN-γ, of the proinflammatory cytokines IL-1α and IL-6, and of the housekeeping genes are quantified using TaqMan-probes by means of standard dilution series on the ABI 7500. The standard consists of equal aliquots of the experimental cDNAs under investigation. Simultaneously the most suitable combination of 3 out of the four housekeeping genes h-actin, HPRT, GAPDH, and cyclophilin can be selected, and their averaged expression values constitute a normalisation factor. The raw data of all targets of interest is then calculated relative to this normalisation factor, making eventual changes of the relative expression level of the single housekeeping genes controllable and quantifiable.

These cytokine assays will additionally enable us to evaluate the mechanisms employed by each virus for the inhibition of host-cell transcription and translation and the consequent failure to synthesize IFN, inhibition of dsRNA-signalling and IFN-signalling pathways, and antagonizing the IFN-induced antiviral effecter proteins. For example, the anti-IFN activities of many paramyxoviruses are encoded by the viral P gene. Products of the P gene inhibit both dsRNA signaling [134-137] and IFN signaling [138-140], but often by slightly different approaches. We are still teasing out the intricacies of henipavirus P/V/W/C interactions with STAT molecules and the recent addition of a NiV reverse genetics system to our arsenal, combined with monospecific antisera for NiV P, V, W and C proteins (supplied by Dr Rota, CDC, Atlanta) should rapidly enable evaluation of the relative role of each of these proteins during experimental infection. We anticipate generating an equivalent set of reagents for each paramyxovirus under investigation.

Viral adaptation using rapid whole genome virus sequencing. Using established techniques for multiple passaging of viruses *in vitro*, we will perform whole genome sequencing before during and after variable numbers of replication cycles. By comparison with established rates of mutation for paramyxoviruses and other RNA viruses [39] we may be able to assess the relative roles of genetic drift versus specific adaptation within relevant cell types. Further, comparison of multiple passaged viruses in a range of cell types may provide clues to selective adaptation pressures present in different tissues.

Anticipated results. These analyses will provide a quantitative assessment of the relative infectivity and pathogenicity of a subset of bat-derived viruses from three important viral families. We anticipate that this will enable the identification of molecular patterns among isolates which can then be correlated directly with pathogenicity. For the first time, we will develop a quantitative understanding of the differences in host animal/cell events in response to viral infection by closely (and distantly) related viruses. Additionally, harnessing the power of HTS technology, we will perform quasispecies analysis of viral evolution and host adaptation, as it happens.

Quasispecies analysis of experimental viral populations before (inoculum), during and after (shedding) infection in animals/cells will provide a measure of the population of potential emergent viruses and may indicate the relative role of genetic drift (random fluctuations in allele frequencies) versus the natural selection of advantageous mutations for host adaptation. Longitudinal samples collected during serial passaging in cell lines are ideal for this type of comparison. Quite simply, changes in the dominant viral species at specific time points may provide identifiable indicators of the relative viral fitness and how this adapts over time within the host. Repeated passaging of virus in cell lines may also provide an insight into whether successful host infection results in adaptation of the virus or rather, successful infection only occurs if the virus already possesses the necessary mutations. For the latter, pre-adaptation of the virus in a secondary cell line may be required to establish infection.

Potential pitfalls/alternative strategies: As already mentioned, we already have unique access to this set of related bat-derived paramxyoviruses that display either mild clinical signs during infection, asymptomatic infections, or are as yet uncharacterised with respect to their pathogenicity in terrestrial mammals. We have incorporated a range of established, routine assays for evaluating virus infection and pathogenicity so we do not anticipate technical limitations to this approach. While access to pyrosequencing technology can be expensive,

CSIRO has made an AUD$800,000 commitment to ensuring access to this technology as part of studies unrelated to this proposal.

Aim 3.2. Evaluation of correlates of paramyxovirus infection using reverse genetics.
Rationale

Once wild type virus pathogenicity has been established (**Aim 3.1**), the entire virus genome has been sequenced and we have identified possible molecular determinants of infection/pathogenicity, we will clone out the gene of interest and reverse engineer specific genetic mutations (associated with specific correlates of infection identified through comparative whole virus sequencing) and evaluate these effects on virulence independently using viral chimera's (NiV parent systems with pseudotyped viral proteins incorporated. If cell lines are largely refractory to a particular virus we will reverse engineer genetic correlates expected to confer pathogenicity and evaluate their effect *in vitro*.

Using a NiV reverse genetics system similar to that described in the preliminary data section above (provided by Dr. Paul Rota, CDC – see attached letter of support), we can manipulate specific genes (or portions of genes) to make viral chimera's facilitating the evaluation of specific genetic correlates of infectivity. We have unique access to a set of related bat-derived paramxyoviruses that display either mild clinical signs during infection, asymptomatic infections, or are as yet uncharacterised with respect to their pathogenicity in terrestrial mammals. In this project we will conduct comparative analysis of these related viruses via whole genome sequencing, manipulation of viral genomes using established reverse genetics technologies, combined with assessment of their relative pathogenicity and the nature of induced host immune responses using a range of cell lines, either as a surrogate for humans (normal human bronchial epithelial - NHBE cells) or using bat cells to evaluate the characteristics of infection in the natural or reservoir host.

Anticipated results: Detailed analysis of the physiological and molecular markers of viral infection, using controlled systems only possible via reverse genetics, we expect to identify specific genetic correlates that make particular viruses more (or less) infectious to specific cells types. By teasing apart these molecular correlates of pathogenicity, we can then begin to understand what adaptation steps may be required to make a non-infectious, or non-pathogenic virus infectious and pathogenic to human cell lines. This information will then enable a more targeted approach to mitigation of these possibilities, through the identification of targeted antiviral strategies.

Potential pitfalls/alternative strategies: Previous studies with minigenome systems have indicated that the support proteins of HeV and NiV are interchangeable [186] but that Measles Virus proteins are not, suggesting that although the replication strategies are similar, there are also genus specific differences between related paramyxoviruses. While this may indicate that viral chimera's may not be functionally transcribed, the approach suggested here is likely to be successful as only the gene coding regions will be altered, leaving the highly conserved intergenic sequences [58, 59, 216-219] and genomic termini unaltered [220]. Unfortunately, we do not have reverse genetics systems for coronaviruses or lyssaviruses so our evaluations will need to be confined only to paramyxovirus pathogenicity.

3.3. Identification of viral or host correlates of infection.

Using a range of mammalian (including bat derived) cell lines and the NHBE cell as a surrogate model for human respiratory infection, we will evaluate the cellular pathogenicity of novel viruses discovered through Aims 1 and 2 using the same suite of assays for *in vitro* characterisation of paramyxoviruses, coronaviruses and lyssaviruses (Aim 3.1.). Incorporating rapid, whole virus genome sequencing technologies, we will rapidly evaluate quasispecies changes relevant to host adapatation. By comparison of data for these newly discovered viruses to well characterised, but closely related viruses, we expect to determine potential viral or host correlates of infectivity and pathogenicity, or both.

Comprehensive sequence analysis of viruses at various times before and after the host transfer event are required to tease out the evolutionary changes of biological significance in host switching. For the most rewarding interpretation of this data, dissection of the molecular controls of host range and of the host barriers restricting infection are also required. In parallel with an

unrelated project (funded by CSIRO), we will be in a position to characterize the cellular physiology and immunology of cell lines derived from the natural host for these viruses, namely the bat. There are currently no continuous bat cell lines available, significantly hampering efforts to understand these important reservoir hosts. This CSIRO funded project will not only establish bat primary cell cultures, we have recently developed the expertise to transform primary bat cells, into continuous cell lines amenable for multi-passage research

Anticipated results: As for the previous studies in paramyxoviruses using reverse genetics, detailed analysis of the physiological and molecular markers of viral infection is likely to identify specific genetic correlates that make particular viruses more (or less) infectious to specific cells types. The knowledge gained using control reverse genetics systems for paramyxoviruses (Aim 3.2.) should provide essentially a road map, with which to evaluate and characterise novel virus pathogenicity.

Potential pitfalls/alternative strategies: As with any pathogen discovery program, there is an inherent risk that no novel viruses will be identified. We have elected to perform controlled characterization studies on a range of paramyxoviruses, coronaviruses and lyssaviruses in parallel with the pathogen discovery aims such that irrespective of the results of the targeted sampling and screening process, we will still be able to identify specific virus and/or host correlates of infection in vitro. We have access to a large number of uncharacterised pathogens, so a number of these could also be incorporated into the analyses as the results of the screening process become clear.

Management Plan

This brings together different disciplines to unravel the complexity of the process of zoonotic disease emergence. All members of this team have worked together for the past 5 years, and are able to seamlessly collaborate on this program. The potential risks in this proposal have been outlined above, and here we lay out our management strategy which builds cohesiveness, and increases the likelihood that this proposal will yield significant advances in this critical field. This is a collaborative proposal among four institutions: The CCM at Wildlife Trust, New York (Daszak, Epstein); The Greene Lab, Columbia Univ., New York (Lipkin, Briese, Palacios); The Institute of Zoology at Cambridge University, UK (Jones); and the Australian Animal Health Lab (Mungall). All PIs are connected via adjunct status in other's labs (Daszak, Epstein at Columbia;

Year	CCM/IOZ	Greene Lab	AAHL
	Build bat database, conduct modeling, train field teams (Wildlife Trust Alliance) in sample collection.	Establish housekeeping gene controls, optimize and implement Mass Tag PCR	*In vitro* evaluation of paramyxo-, corona- and lyssaviruses.
2	Expand sample collection following hotspot modeling	Mass Tag PCR; Optimize and implement GreeneChip analyses	Continue *in vitro* evaluations. Quasispecies analysis.
3	Continue sample collection. Begin refining datasets	Metagenomic high throughput sequencing (HTS)/ 454; Analyses, publication	Paramyxovirus reverse genetics studies. Begin novel virus characterization.
4	Sample collection in newly targeted hotspots. Begin refined modeling	Metagenomic high throughput sequencing (HTS)/ 454; Analyses, publication	Continue paramyxovirus reverse genetics studies. Continue novel virus characterization.
5	Final sample collections. Finalize risk assessment.	Analyses of sequence data and publications	Finalize molecular correlates of infection and/or pathogenicity.

Daszak at Inst. Zool., Jones, Mungall, Lipkin at CCM).

Research will be co-ordinated via monthly conference calls involving all PIs and led by Daszak. Mungall will visit the USA each year as part of this research (funded by AAHL) and PIs Lipkin and Daszak will visit Australia each year to meet with Mungall and other team members as part of (and funded by) their membership of the AB-CRC (see bios Lipkin, Daszak). The CCM postdoctoral assistant will conduct all sample collection and inventory, and all modeling tasks

under the supervision of Daszak, Jones and Epstein. Postdocs at CU and at AAHL will conduct work on aims 2 and 3 under the direction of Lipkin and Mungall respectively.

12. Vertebrate Animals

Vertebrate animal use is confined to collection of blood from wild animals (bats) in foreign countries that are listed as "other performance sites". The primary institution for this project (Wildlife Trust) has it's own IACUC Committee, which follows the structure of those set up under OLAW guidelines, and this project is pending approval by that committee. However, Wildlife Trust does not have an Animal Welfare Assurance Number and is unable to acquire one, because (as stated by the AAALAC) the organization works only on wildlife and does not have a laboratory animal facility.
If this application is funded, the correct procedure is for us to apply to NIH's OLAW for approval on a project basis. We have done this previously for work on wildlife, and will be able to progress rapidly should the project be approved for funding, because we will have our own organization's IACUC committee's approval at that time. Below are the answers to the 5 questions on vertebrate animal use:

1. Detailed description of animal use.
All work with vertebrate animals will be conducted in the field, in the countries listed as 'other performance sites'.
Bat capture. Capture and bleeding techniques are in accordance with those used by other workers[1, 2] and by ourselves in previous published studies[3-5] and have previously been approved by our IACUC committee. Bats will be captured using a mist net manned continuously by two people during the entire capture period, and bats are removed from the net as soon as they become entangled to minimize stress and prevent injury. In my experience, a maximum of 15-20 pteropodid fruit bats or 30 insectivorous or small fruit bats can be safely held and processed by a team of three people per trapping period using gas anesthesia. Duration of trapping will depend on the capture rate. Bats are placed individually into small bags and hung from a branch or post until samples are collected. Bats are held for a maximum of six hours.

Chemical restraint. Chemical restraint will be used only for large pteropodid bats. Bleeding and swabbing of small bats (insectivorous bats or small fruit bats) can be safely undertaken without anesthesia. We will use isoflurane and a portable vaporizer to restrain bats, as described in the literature[6] and used by us previously. Isoflurane has been shown to be safe and effective for short-term chemical restraint of bats. The CCM has two portable isoflurane vaporizers (Harvard Apparatus, MA, USA). Bats will be under anesthesia for 10-12 minutes, and recovery is determined by presence of palpebral and withdrawl reflexes, as well as biting reflex. Bats are kept in a quiet, cool place while waiting to be processed and while recovering from anesthesia. Bats are given mango juice orally by syringe prior to release. Bats are released at their site of capture and are allowed to climb into a tree where they can either rest or fly.

Sample Collection. Bats will be anesthetized prior to sampling. Two sets of three swabs will be taken from each bat: throat, urogenital tract, and rectal. Blood (3.0 ml) will be collected from the radial artery or vein using a 23 gauge needle and 3cc syringe.

Animal Identification Bats will be banded on the first phalanx of digit I, using stainless steel thumb bands (Gey Band & Tag Co, PA, USA) stamped with a unique serial number (Kunz, pers. com). A veterinary microchip (AVID Identification Systems, LA) carrying a unique ID number will be implanted subcutaneously between the scapulae according to manufacturer's instruction. These ID numbers can be retrieved using a microchip reader (AVID). This allows for two means of animal identification: the thumb bands can be

viewed from a distance, allowing for crude censusing of marked bats using binoculars; and the microchip insures animal ID for collecting accurate recapture data in the event that the thumb band is lost.

Species and number used in study:
Fruit bats (*Pteropus spp.*, *Eidelon*) 1,000 each
Small fruit bats (*Cyanopterus* spp., *Eonycterus* spp., *Rousettus* spp.) 1,000 each
Insectivorous bats (*Rhinolophus* spp., *Pippistrellus* spp., other members of the family Verspetillionidae): 1,000 each

2. Justify use of animals, choice of species, numbers to be used.
These bats are reservoirs of a number of emerging zoonoses. The sample size (1,000 bats per species) was chosen to provide enough samples given approximately 5 sampling sites globally (=200 samples per bat species per site) and the 5 year grant period (=40 samples per bat species per site per year). This should provide an opportunity to detect viruses that are often found at low viral prevalence of 1%-3% (e.g. the SARS-like Coronaviruses[5].

3. Provide information on veterinary care. Animals will receive emergency veterinary care if necessary. There is no specific veterinary care that is appropriate for this project, nor are clinical veterinary facilities included as a performance site, as animals will be released within hours of capture.

4. Procedures for ensuring animal comfort, lack of distress, pain, or injury.
Bats will not be held longer than 6 hours. In my experience, bats tolerate this period well and there have been no clinical adverse effects seen in any of the bats captured and sampled in Malaysia and Bangladesh. Mist nets will be attended during capture periods, and bats will be extracted from the net as soon as they become entangled. This will minimize stress and prevent injury from entanglement. Bats will be placed in pillowcases and hung from tree branches while awaiting processing and during recovery. The pillowcases are sufficiently porous as to allow for ventilation. The enclosed environment seems to calm the bats, as they do not struggle once inside, but they hang quietly. Bats are protected from extreme heat or cold while under anesthesia, and lubrication is used on their eyes to protect them from injury. Bats are monitored by a veterinarian during all stages of capture, processing, and release. Bats are kept in a cool place while in the pillowcases. Prior to release, bats will be syringe-fed fruit juice to accommodate any hypoglycemia from capture.
We have placed collars on captive Australian flying foxes and observed them for two months. These bats were free to forage at night and tolerated the collars well (C. Smith, pers. comm..). In Malaysia we have had a flying fox carrying a transmitter for seven months. Tidemann and Nelson report Grey-headed flying foxes carrying transmitters for up to a year [7].

5. Euthanasia: To date, there has been no mortality of fruit bats in CCM's or collaborator's work related to Nipah virus. More than 1,000 bats representing seven species of *Pteropus* have been captured for projects in Malaysia, India, and Australia. In the event of injury to an animal that results in pain and suffering, and reasonable veterinary care is unavailable, the animal will be euthanized by Dr Epstein or a trained veterinary officer using ketamine injected intramuscularly 37.5mg/kg (81) and sodium pentobarbital injected intravenously at a dose of 1.0ml per 5kg injected intravenously. This protocol is in accordance with the AVMA euthanasia report (2001).

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Consortium/Contractual Arrangements

This project is a multi-institutional collaboration led by the Consortium for Conservation Medicine at Wildlife Trust, New York (Daszak, PI), which will subcontract funds to two institutions: Columbia University (Lipkin) and the Australian Animal Health Laboratory (AAHL – Mungall), which is a foreign institution (see attachment to Research and Related, other project information Form for justification of work at foreign site). Dr Daszak has over 10 years previous experience managing collaborative projects, including an R01 on Nipah virus that involved 5 separate foreign institutions (including AAHL where Co-PI Mungall is based) and a 5-year NSF/NIH Ecology of Infectious Disease award on West Nile virus which involves 3 domestic subcontractees.

The applicant organization (Wildlife Trust) is justified in taking the lead on this project because this group (specifically it's Consortium for Conservation Medicine), led by Dr Daszak, specializes in understanding the ecological, and virological processes underlying zoonotic disease emergence. Dr Daszak has conducted significant preliminary work on this issue (see preliminary data), including collection of over 3,000 samples which will be used in the study, and the building of a large database of emerging infectious disease ecological information which will be analyzed in this study. Wildlife Trust also acts as the headquarters of the Wildlife Trust Alliance – an international group of organizations which will supply new samples to the group as this project progresses. The subcontractees will work on specific issues that they have proven expertise in. These areas are: viral discovery (Columbia University, Dr Lipkin) from the samples that Dr Daszak's group collects, and viral pathogenesis (AAHL, Dr Mungall) on the viruses that are discovered in these samples.

Department of Health and Human Services
Public Health Service

Measles, Mumps, Rubella,
and Herpesviruses Virus
Branch
MS-C-22
Centers for Disease
Control and Prevention
1600 Clifton Rd.
Atlanta, GA, USA 30333
Tel: 404-639-4181
Fax: 404-639-4187

27 September, 2007

Dr. Bruce Mungall
Project Leader
Henipavirus Therapeutics
Australian Animal Health Laboratory
5 Portarlington Rd East Geelong Vic. 3220

Dear Bruce:

I am of course quite eager and extremely pleased to continue to work with you in your initiation of an exciting viral pathogen discovery and characterization proposal. The combined expertise and resources provided by the Consortium for Conservation Medicine, Columbia University and AHHL make this an ideal research plan and one that very few investigators have the opportunity to execute. I am very excited about the possibility that certain novel pathogens could be discovered, but I agree that there is some risk that no new pathogens will be identified. Your back-up plan of characterization of the six most recently emerged paramyxovirus pathogens will proved valuable data towards understanding the viral and host correlates of infection. As you know, we have already developed a full length infectious clone for Nipah virus as testimony toward our existing collaborations. One of my graduate students will be spending three months in your laboratory in order to establish this reverse genetics system and rescue a number of recombinant viruses. These will be important tools for elucidating the molecular correlates of infection and pathogenicity in among important pathogens

As in our past collaborations I and my associates will always be available for help and consultations should the need arise, as well as for joint experiments aimed at characterization of novel viruses that your team may discover. Your laboratory's progress these past few years in both the *in vitro* and *in vivo* Nipah virus infection assays, including the recently published animal model, has been impressive, and the research plan that you have outlined may provide critical new data and important advances toward understanding the host-pathogen interaction for these important emerging viruses.

I look forward to a continued, and now expanded, collaboration on these exciting projects!

Yours Sincerely,

(b) (6)

Paul Rota, Ph.D.
Supervisory Microbiologist

ZSL
LIVING CONSERVATION

PATRON: H M THE QUEEN
The Zoological Society of London (ZSL), founded in 1826, is devoted to achieving and promoting the worldwide conservation of animals and their habitats.

INSTITUTE OF ZOOLOGY
REGENT'S PARK LONDON NW1 4RY
T 020 7449 6600 F 020 7586 2870
www.zsl.org

Tel: +44 (0)207449 6627
Email: kate.jones@ioz.ac.uk

3rd October 2007

Peter Daszak
Consortium for Conservation Medicine
460 West 34th Street, 17th Floor
New York, NY 10001

Dear Peter,

I am very pleased to be involved on this grant 'Quantifying the risk of viral emergence from bats' and it has my full support.

You and I have been collaborating predicting modelling of emerging diseases for over 3 years and have seen it develop to a stage where further funding is now crucial for it to continue. We have been extremely productive with our previous funding, developing a global dataset of human emerging diseases (Human Emerging Infectious Disease Event Database) and analysing these patterns, with one book chapter and paper in review in Nature.

I have been investigating bat evolution, biodiversity and disease macroecology for over 15 years and bring a significant level of expertise to this research project. My particular involvement in your project is to support the further development of the bat disease database and more detailed analyses of the patterns to develop a predictive model of bat zoonotic disease emergence.

I anticipate that this research will produce results that will have a profound impact on the way we manage the impacts of global change.

Best wishes

Kate Jones

INSTITUTE OF ZOOLOGY
LONDON ZOO WHIPSNADE WILD ANIMAL PARK
The Zoological Society of London is a registered charity. No: 208728

PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

● New ○ Resubmission ○ Renewal ○ Continuation ○ Revision

Federal Identifier: GRANT00349359

2. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

❑ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ○ No ○

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ○ No ○

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

○Yes ●No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance, where applicable, provide an explanation and attach below.

Explanation:

Attachments

CertificationExplanation_attDataGroup0

File Name	Mime Type

Grant Number: 5R01AI079231-02

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Risk of Viral Emergence from Bats

Aleksei A. Chmura
Wildlife Trust
61 Route 9W
Palisades, NY 109648000

Award e-mailed to: (b) (6)

Budget Period: 09/01/2009 – 08/31/2010
Project Period: 09/18/2008 – 06/30/2013

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $535,156 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to WILDLIFE TRUST in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI079231 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Ann W. Devine
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI079231-02

Award Calculation (U.S. Dollars)

Federal Direct Costs	$493,333
Federal F&A Costs	$41,823
Approved Budget	$535,156
Federal Share	$535,156
TOTAL FEDERAL AWARD AMOUNT	$535,156

AMOUNT OF THIS ACTION (FEDERAL SHARE) $535,156

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$535,156	$535,156
3	$534,739	$534,739
4	$534,968	$534,968
5	$547,542	$547,542

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI079231A
Fiscal Year:	2009

IC	*CAN*	*2009*	*2010*	*2011*	*2012*
AI	8472302	$535,156	$534,739	$534,968	$547,542

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M34A / **OC**: 414E / **Processed**: (b) (6) 09/15/2009

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI079231-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI079231-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI079231-02

NIAID records show that the non-competing grant progress report was submitted late. It is of utmost importance that we receive accurately completed non-competing grant progress reports in a timely manner, so that we can ensure timely awards without interrupting your research.

The next non-competing grant progress report is due on 07/15/10. Future late submission of your non-competing grant progress report and any subsequently requested documentation will result in a reduction of time and/or funds for this grant.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Shadetra Robinson
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Eun-chung Park
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI079231-02

INSTITUTION: WILDLIFE TRUST

Facilities and Administrative Costs	***Year 2***	***Year 3***	***Year 4***	***Year 5***
F&A Cost Rate 1	26.1%	26.1%	26.1%	26.1%
F&A Cost Base 1	$160,242	$154,822	$164,924	$182,152
F&A Costs 1	$41,823	$40,409	$43,045	$47,542

OMB No. 0925-0001

Grant Number 5R01AI79231-2	**Total Project Period** From: 09/18/2008 To: 06/30/2013
EIN: **Review Group:** 1311726494A1 IRAP	**Requested Budget Period:** From: 09/01/2009 To: 08/31/2010
Title of Project: Risk of Viral Emergence from Bats	**Due Date:** 07/16/2009 **Submitted Date:** 08/16/2009

Program Director/Principal Investigator: PETER DASZAK 460 West 34th Street New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** **Email Address:** (b) (6)	**Applicant Organization:** WILDLIFE TRUST WILDLIFE TRUST 460 West 34th Street New York , NY 10001 **Department:** **Major Subdivision:**
Administrative Official: Aleksei Avery Chmura WILDLIFE TRUST 61 ROUTE 9W PALISADES , NY 109648000 **Phone Number:** (b) (6) **Fax Number:** **Email Address:** (b) (6)	**Signing Official:** Aleksei Avery Chmura WILDLIFE TRUST 61 ROUTE 9W PALISADES , NY 109648000 **Phone Number:** 845 365-8466 **Fax Number:** **Email Address:** (b) (6)
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☐ No ☒ Yes **Animal Assurance Number:** A3415-01 **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: WILDLIFE TRUST		
DUNS: 077090066		
Street 1: WILDLIFE TRUST	Street 2: 460 West 34th Street	
City: New York	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 10001
Congressional Districts: 08		

Additional Project/Performance Site Location		
Organizational Name: Wildlife Trust		
DUNS: 077090066		
Street 1: 460 West 34th Street	Street 2: 17th Floor	
City: New York	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 10001
Project/Performance Site Congressional Districts: 08		

Additional Project/Performance Site Location		
Organizational Name: The Trustees of Columbia University		
DUNS: 621889815		
Street 1: 630 West 168th Street	Street 2: Box 49	
City: New York	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 10032
Project/Performance Site Congressional Districts: 15		

Additional Project/Performance Site Location		
Organizational Name: The Institute of Zoology		
DUNS: 227012275		
Street 1: Regent's Park	Street 2:	
City: London	County:	State:
Province:	Country: UNITED KINGDOM	Zip/Postal Code: NW14RY
Project/Performance Site Congressional Districts: 0		

Additional Project/Performance Site Location		
Organizational Name: Australian Animal Health Laboratory (AAHL)		
DUNS: 754307957		
Street 1: 5 Portarlington Road	Street 2:	
City: East Geelong	County:	State:
Province:	Country: AUSTRALIA	Zip/Postal Code: VIC 3219
Project/Performance Site Congressional Districts: 0		

Program Director/Principal Investigator:	**Grant Number**
PETER DASZAK	5R01AI79231-2
Applicant Organization:	**Period Covered by this Report:**
WILDLIFE TRUST	09/18/2008 - 08/31/2009

Title of Project:

Risk of Viral Emergence from Bats

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Citation ID:	**Citation Source:**	**Citation Text:**

Research Accomplishments:

File is not uploaded

Other Document File:

File is not uploaded

Other Support File:

File is not uploaded

Senior/Key Personnel Report						
Program Director/Principal Investigator: PETER DASZAK			**Grant Number** 5R01AI79231-2			
Name:	**Degree(s) Name:**	**SSN:**	**Role on Project:**	**Months Devoted to Project**		
				Cal	**Acad**	**Sum**
PETER DASZAK	PHD, BS	(b) (6)	PD/PI	(b) (4), (b) (6)		
Thomas Briese	PHD		Co-PD/PI			
Peter Daszak	PHD, BS		PD/PI			
Jonathan H. Epstein	DVM		Co-PD/PI			
Kate Elizabeth Jones	PHD		Co-PD/PI			
W. Ian Lipkin	PHD		Co-PD/PI			
Bruce Andrew Mungall	PHD		Co-PD/PI			
Gustavo F. Palacios	PHD		Co-PD/PI			

PROGRESS REPORT (5R01AI079231 - Risk of Viral Emergence from Bats) YEAR 1

We have completed 8 months of this 5-year R01 and have already made significant advances. We have expanded our bat pathogen database from 6,400 host-parasite interactions for over 584 bat species and over 1,200 references to 25,000 datapoints. We have collected 528 new bat samples (from *Pteropus* spp. fruit bats in South Asia), including sera, urine, saliva and urogenital swabs. We have used high throughput pyrosequencing on pregnant bats in Bangladesh **to discover and characterize a previously undescribed virus** with 54% homology to GBV C virus (family *Flaviviridae,* genus *Hepacivirus*) in chimpanzees (*Pan troglodytes*). We have also expanded our field surveillance to five countries China, Mexico, Bangladesh, Brazil, and the USA – the first four of which are emerging disease hotspots. We now have standardized field protocols for sampling microchiroptera, in addition to our existing protocols for megachiroptera (e.g. old world fruit bats). These small insectivorous bats comprise the majority of the bat species in the world and thus the majority of bats we plan to screen for novel viruses. Below is a detailed report of our progress in the first year of this grant.

1) Database analysis of the diversity of unknown bat viruses

We have begun to update our initial database for bat parasites which we will use to analyze the risk for emerging diseases from bats globally. We have now entered ~25,000 records, including literature searches from Web of Science, BIOSIS, and Google, and the 'Literature Cited' sections of every paper published on bat parasites. We've identified 2600 ectoparasite and 1025 endoparasitea and virus citations, restricting the literature to post-1950. We've already mined records from ~90% of the ectoparasite literature and 25% of the current citations for endoparasites. In year 2 we will complete the database and begin analyses of the temporal and geographic patterns and trends in bat parasites. We aim to use the database to identify regions and species most likely to have 1) a high diversity of unknown bat viruses and 2) a propensity for these viruses to emerge into people. In year 3 we will focus our targeting for sample collection based on these analyses.

2) Protocols for microchrioptera sampling established

We have developed a standardized sampling protocol for michrochiroptera to be used in conjunction withour existing protoocls for megchripotera (e.g. pteropid bats). Specific challenges exist with small bats – namely the minute blood volumes that we are safely able to obtain with our non-destructive sampling approach. The protocol is based on the desire for enough serum to run several serological assays for antibodies to known and yet-to-be discovered viruses. We use buffered PBS to dilute blood samples and increase the volume of serum obtained after centrifugation. The complete protocol is listed below:

1. Date & Location are recorded using GPS along with temperature and weather conditions including time (or duration) of trapping and any other variables. All notes are written in a notebook and later transferred to an excel file. After sampling, photographs are taken of each note page to ensure against loss of hard-copy.
2. Pre-printed bar-scan labels from the Green Lab at affixed to all vials. Bat samples are coded with the following method:

04.12.09 – XRD
BZ – *P. hypomelanus*
Oral XXX

3. Bats are caught using mistnet, handnet, or harptrap and placed into cloth bags (with draw-string mouths). These cloth bags are then suspended from pole permitting air to circulate between each bat-with-bag. In warm months, someone must monitor captured bats' local environment to ensure adequate ventilation and to prevent excessive humidity.
4. Bats are weighed (in grams) while still in bags. Scale with or without container or cup is tared and both bat & bag are weighed. Once bat is removed for biometry, bag is re-weighed and subtracted from previous total.
5. Bat is removed from bag, identified (genus, species, age class, and sex) and photographed.
6. The following biometrics (in centimetres) are taken (Menzel *et al.*, 2002):
    a. Forearm length ('elbow to wrist')
    b. Ear Length (most distal tip of ear to base)
    c. Tragus length (top of tragus to base of ear)
    d. Body Length (measured ventrally from top of nose to base of tail)
    e. Hind Foot Length ('ankle to toe')
    f. Tail Length (from base to tip)
    g. Tibia ('knee to ankle')

7. 2 2mm wing punches are taken from each bat and placed in ethanol in a 0.5ml vial.
8. Oral and rectal swabs are taken with fine aluminium or wooden-shaft sterile swab and placed into nuclisense lysis buffer. Sealed, labelled vials with samples are put into dry-shipper filled with liquid N2 or otherwise stored at -80˚C.
9. The venipuncture site is prepared with a pre-injection swab of ethanol and a 25 gauge ¾ needle is used to only pierce the saphenous, brachial, or propatagial veins. Blood beads on the skin surface and may be collected using a 100 µl pipette with sterile tip and added directly to PBS in a Eppendorf vial and mixed briefly with the pipette tip. NB, quantity of blood extracted from bat should not exceed a ratio of 1g of bat weight to 10µl of blood.
10. Once all trapped bats are processed and returned to their respective bags, the collected blood in Eppendorf diluted with PBS is then spun in a micro-centrifuge briefly (1-3 mins) and allowed to settle overnight or for a minimum of one hour.
11. Once the blood-plus-PBS in the Eppendorf tubes has settled, the serum is removed with a sterile pipette tip and put into duplicate, labelled 700µl vials without buffer for storage and later analysis. Serum vials may be stored on ice for up to 24 hours during transit and then at -80˚C or put directly into N_2 for return to laboratory.
12. Remaining blood cells are pipetted into two vials and Nuclisens lysis buffer (approximately equivalent to the serum fraction removed) is added to the remaining blood cells to maintain – at most - a 1:10 dilution and provide a haemostatic buffer. To maintain the viability of cellular DNA for further genetic analysis the diluted blood cell fraction is stored with oral/anal samples (above) in a dry-shipper filled with liquid N2 (in collar) until they may be deposited in a -80˚C freezer.
13. Bats are released as close to their site of capture as possible.
14. For euthanized bats, liver, kidney, brain, heart, spleen, reproductive tissue (testes & uterus), and lung will be sampled and frozen directly into vials with Nuclisens. Each sample should be mashed briefly in the buffer solution. Salivary gland (not vital, if already swabbing for saliva).

3) Expanded coverage of bat surveillance

We have begun sampling bats in five countries: Bangladesh, Brazil, China, Mexico and the United States. Preliminary field data are as follows:

Brazil

The Brazil bat Project is beginning the capture and sampling of the bats in São Paulo state, in a rural area, near of small farms. The total number of bats sampled are 44, and the species are *Sturnira lilium, Carollia perspicillata, Artibeus lituratus, Anoura caudifer.*

The number of species sampled is described in the figure below.

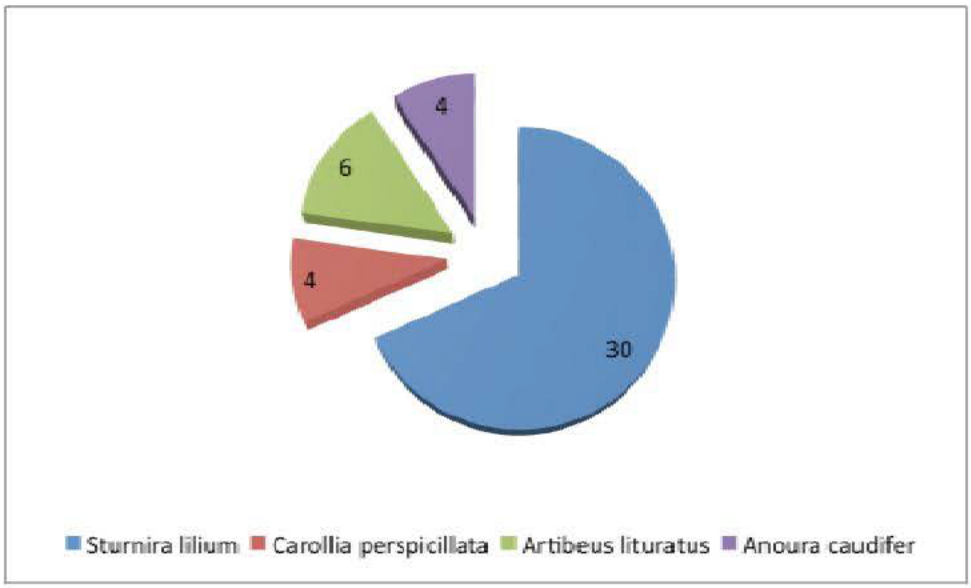

Figure 1. Number of bats species sampled.

The abundance of *Sturnira lilium* have been reported in others ecological studies in Brazil (MARINHO-FILHO 1991, PEDRO & TADDEI 1997). In other localities *Carollia perspicillata* is the dominant species (BERNARD 2002, PEDRO & PASSOS 1995, PEDRO & TADDEI 2002, STONER 2001). *Anoura caudifer* (long-tongued bat) is a nectar feeder bat. *Sturnira lilium*, *Carollia perspicillata* and *Artibeus literatus* are fruit eating bats. The rabies virus was isolated for the first time from a *Artibeus literatus* in 1997 in São Paulo state (PASSOS et.al, 1999). **Figure 2** below shows the mean weight for each species sampled. Our next field trip is scheduled for spring, when the warmer weather will yield more species and samples.

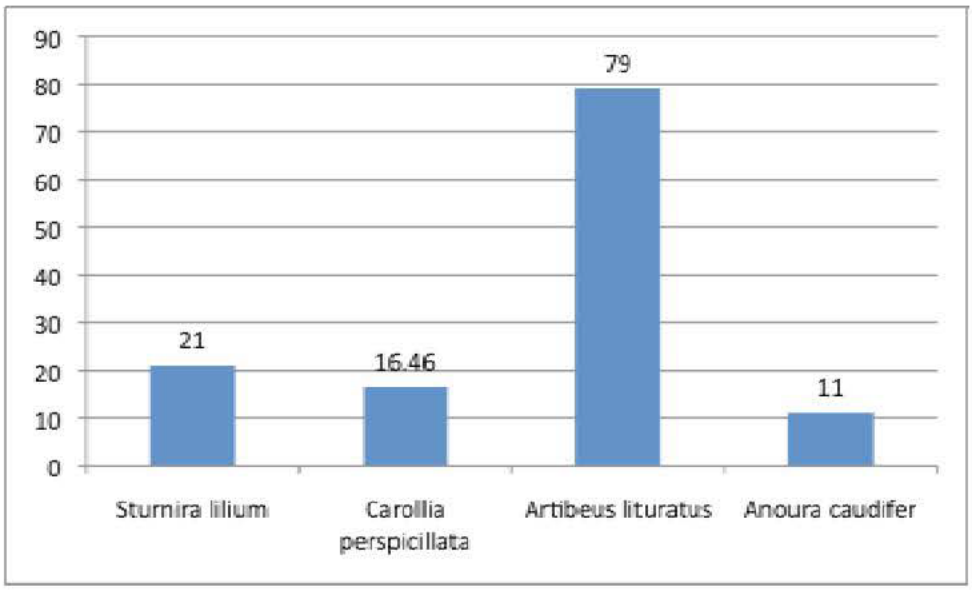

Figure 2. Mean weight of bats sampled

Mexico

We are working with collaborators at Departamento de Etología y Fauna Silvestre, Universidad Nacional Autónoma de México and UNAM in Mexico. Bats were collected between Jun 17th and June 25th, 2009. We collected bat specimens from 18 species from four locations in two different ecological landscapes: disturbed and undisturbed (see Table 1). Serum samples from each bat will be screened for anti-rabies virus antibodies in Mexico, and aliquots of serum, urine, feces, and saliva, preserved in Nuclisense lysis buffer (BioMerieux, USA) will be exported to the Center for Infection and Immunity at Columbia University to be screened for known and unknown viruses using a combination of MassTag PCR (see Bat panel development) and high throughput pyrosequencing. From June 15th to July 15th, we had a second-year veterinary student, Ms. Ashley Case, from Tufts Cummings School of Veterinary Medicine participate in our field research under a conservation medicine applied learning experience with Wildlife Trust, funded partially by this grant. Ms. Case was testing the hypothesis that there was a greater seroprevalence of rabies in a disturbed habitat compared to an undisturbed one. She was also conducting surveillance for white nose syndrome. The results of our field sampling are listed in Table 1.

Table 1. List of species and numbers sampled in each location in Mexico, June –July 2009.

	Undisturbed			Disturbed		
	Lacandona	Laguna Belgica	Total	Azufres	Santa Ana	Total
Artibeus jamaicensis	7		7	7	5	12
Artibeus lituratus	4		4	10	16	26
Artibeus phaeotis				3	1	4
Bauerus dubiaquercus	11		11			
Carollia perspicillata		1	1		3	3
Carollia sowelli	4	2	6			
Desmodus rotundus	2		2		1	1
Glossophaga comissarisi	2		2			
Glossophaga soricina	7	1	8	3	3	6
Micronycteris schmidtorum	2		2			
Myotis californicus					1	1
Myotis keaysi		2	2			
Platyrrhinus helleri	1		1			
Pteronotus davyi	1		1			
Pteronotus parnelli	2		2			
Sturnira illium					11	11
Sturnira ludovici	1	1	2		8	8
Trachops cirrhosus		1	1			
			52			72

China

In July, 2008 we began fieldwork in China. From June to August 2008, 392 bats representing the genera *Hipposideros, Myotis, Pipistrellus, Rhinolophus,* and *Scotophilus* were trapped and sampled throughout China's Hainan province, an island in the South China Sea (Table 2). Field sampling of species was coordinated with the support of the Guangdong Entomological Institue, Guangzhou China, and the South China Institute of Endangered Animals, Chinese Academy of Sciences. In the field, bats were captured upon exit from roost sites using hand nets or mist nets and were held individually in cloth bags until sample collection. For each individual the relative age, sex, body mass, body measurements, and reproductive status were recorded. Serum and whole blood were taken along with oral and anal swabs. All samples were taken in duplicate.

In China, we also had a veterinary student from TCSVM, Kristie Taylor, who was also working on a Conservation Medicine Applied Learning Experience under the mentorship of WT scientist Aleksei Chmura. Samples will be screened for SARS-like coronaviruses in China. Aliquots from these bat samples will be sent to the Center for Infection and Immunity for testing using MassTag PCR and HTPS.

TABLE 2. Species of bats sampled in China

Genus, species	No. of individuals
Hipposideros	99
pomona	75
larvatus	24
Rhinolophus	62
luctus	7
affinis	23
sinicus	29
pusillus	3
Scotophilus	125
kuhlii	125
Pipistrellus	85
abramus	27
pipistrellus	58
Myotis	21
daubentoni	19
ricketti	2
Total	392

USA

Through our collaboration with Western Michigan University (M. Vonhoff, Co-PI) we are developing a bat database. We began collecting bat samples at various sites within the US in June, 2009. Bat species sampled include *Eptesicus fuscus*, *Myotis lucifugus* and *Lasiurus borealis* in Southern Michigan and southern Indiana.

4) Laboratory diagnostics: MassTag PCR "bat panel"

In collaboration with Ian Lipkin, Director of the Center for Infection and Immunity, we are developing a "Bat Panel" for MassTag PCR. The panel will allow us to efficiently screen bat samples for 10-12 groups of viral pathogens of high interest. The panel is in the process of being validated, and will test for the the following viral groups: Henipaviruses; ebolaviruses (including Reston); Marburg viruses; Australian Bat Lyssavirus; SARS-like coronaviruses; bat coronaviruses; GB viruses.

5) Discovery of a novel Flavivirus from *Pteropus giganteus* in Bangladesh using high throughput pyrosequencing

Preliminary Data:

RNA was extracted from serum samples collected from 12 *Pteropus giganteus* captured in Faridpur as part of a Nipah virus surveillance study (NIAID K08AI067549-01A2, Epstein PI). The samples were pooled in groups of four based on sex and reproductive status: Adult male; Adult Female (nonpregnant); and adult female (pregnant); in order to test the hypothesis that pregnant females were more likely to be infected with pathogens due to the physiologic stress of pregnancy when compared to males and non-pregnant females. A metagenomic study was performed on the pooled samples (Epstein et al, in prep). The resulting sequences mainly showed genomic DNA and commensal or environmental bacteria; however, the pooled sample containing RNA from pregnant females did have two sequences that did not match anything in Genbank at the nucleotide level. During year one of this grant, we focused on characterizing this novel virus, and have now nearly sequenced the complete genome. The results are described below.

Results from Unbiased High-throughput Sequencing.

A total of 1,837 sequences were generated from UHTS from pooled serum. Two of the sequences, 53 and 146 nucleotides respectively, were matched to Dourocouli virus (GBV-A) using a BLASTX amino acid-level search of Genbank. None of the three sequences matched anything in Genbank at the nucleotide level. None of the other three pooled samples contained sequences that matched any of the GB viruses. Reverse transcription PCR using primers designed from these initial sequences was used to detect additional sequence, and 8,667 nucleotides of the genome were sequenced using a combination of RT-PCR, touchdown PCR, Two-Step walking PCR, and 5'and 3'RACE, as described [1, 2]

Genomic characterization

Initially, nucleotide level BLAST searches of GenBank using oligonucleotides generated by HTPS did not detect any relationship to known viruses. An amino-acid level search (BLASTX), showed that the sequences most resembled flaviviruses, particularly GB virus A [3]. Like other flaviviruses, this virus has a positive sense single-stranded RNA genome with a single, large open reading frame. There is a 5' UTR, envelope proteins E1 and E2 followed by non-structural genes NS3, NS4(A and B), and NS5 (A and B). Two genomes were characterized to near full-length from two bats. We have sequenced 8,571 nucleotides from "Bat 93" and 8,667 from "Bat 68." Genomic sequences were 99% similar at the nucleotide level across 8,571 nucleotides. Pair-wise amino acid comparisons to the full length genome of GBV-A showed 49.4% homology and 48.8% homology to GBV A over. Phylogenetic trees were constructed from the E2, NS3 and NS4 gene regions of all the known GB viruses, HCV, and bat GB-like virus, and the same basic relationship was upheld for each gene (**fig 3**). Bat GB-like virus appears to be more closely related to GBV-A and GBV-C than HCV or GBV-B. A BlastX search of Genbank showed a 38% match to GB virus A [4]. We found residues that make up the catalytic triad of the serine protease within the NS3 region (Hist, Aspartate, Serine) that are highly conserved among GB viruses and HCV [5]. Genewise comparisons between the bat GB-like virus, GB viruses A, B, and C, and HCV showed maximal amino acid-level homology (50.3%) in the putative helicase NS3 gene region. Phylogenetic relationships are conserved among NS3, NS4 and NS5 genes, and it appears that this virus may be in a new genus separate from *Hepacivirus* and the GB viruses, however most closely related to GBV-A and GBV-C.

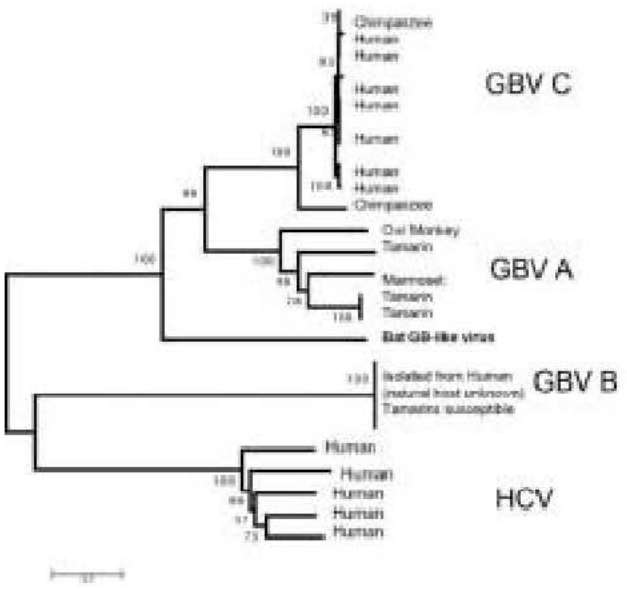

Figure 3. Neighbor-joining tree of the non-structural protein 3 (NS3) gene for GB viruses, Hepatitis C, and **our novel bat Flavivirus isolated from *P. giganteus* in Bangladesh (Epstein *et al.*, in prep.).** Preliminary data show a lack of host-specificity within the GBV-A and GBV-C clades suggesting that these viruses have an evolutionary history of host-switching.

We have found this new virus in five bat samples originating from the same colony, suggesting it is not a spillover from primates. This is the **first finding of a GB virus from bats** and our analyses suggest that **1)** the low homology to its nearest relative suggest this is likely a new genus of the family *Flaviviridae*, **2)** the long branch length and lack of distinct, but related viruses suggest there are other bat GB viruses to be discovered, and **3)** the lack of viral clade structure by host species, and presence of other viruses in non-human primates, suggests there is potential for zoonotic spill-over. We have now identified some divergence among individual bat GBVs, suggesting potential for analysis of evolution within the clade. We are currently preparing a manuscript for publication based on these findings.

Literature Cited

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2. Pilhofer, M., et al., Characterization of bacterial operons consisting of two tubulins and a kinesin-like gene by the novel Two-Step Gene Walking method. Nucleic Acids Research, 2007. 35(20): p. 8.
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4. Leary, T.P., et al., The sequence and genomic organization of a GB virus A variant isolated from captive tamarins. Journal of General Virology, 1997. 78: p. 2307-2313.
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11. STONER, K.E. 2001. Differential habitat use and reproductive patterns of frugivorous bats in tropical dry forest of northwestern Costa Rica. Canadian Journal of Zoology, Ottawa, 79: 1626-1633.

Grant Number: 5R01AI079231-03 REVISED

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Risk of Viral Emergence from Bats

Aleksei A. Chmura
Wildlife Trust
406 W 34th Street
17th Floor
New York, NY 10001

Award e-mailed to: (b) (6)

Budget Period: 09/01/2010 – 08/31/2011
Project Period: 09/18/2008 – 08/31/2013

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to WILDLIFE TRUST in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release or other document that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Award Number R01AI079231 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health."

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Artisha Y. Wright
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI079231-03 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$444,119
Federal F&A Costs	$36,304
Approved Budget	$480,423
Federal Share	$480,423
TOTAL FEDERAL AWARD AMOUNT	$480,423

AMOUNT OF THIS ACTION (FEDERAL SHARE) $0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$480,423	$480,423
4	$524,321	$524,321
5	$536,647	$536,647

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI079231A
Fiscal Year:	2010

IC	*CAN*	*2010*	*2011*	*2012*
AI	8472302	$480,423	$524,321	$536,647

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M34A / **OC**: 414E / **Processed**: (b) (6) 01/07/2011

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI079231-03 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI079231-03 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI079231-03 REVISED

Revision #1: This award removes all restrictions on foreign and domestic work based upon acceptable documentation that was received, meeting administrative requirements.

Supersedes Notice of Award (NoA) issued 9/15/2010.

*****

This award has been reduced by one month of funding due to consistently late submission of progress reports. The next non-competing grant progress report is due on 07/16/2011. Future late submission of your non-competing grant progress report and any subsequently requested documentation will result in a reduction of time and/or funds for this grant.

This non-competing award reflects an adjustment of the amount recommended on the previous Notice of Award, per the NIAID implementation of the NIH FY 2010 fiscal policy (See NIH Guide Notice NOT-OD-10-039). Future year recommended levels also have been adjusted consistent with the referenced Notice.

This award includes funds awarded for consortium activity with the Trustees of Columbia University. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

This award includes funds awarded for consortium activity with Australian Animal Health Laboratory/AUSTRALIA. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Michael A. Wright
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Eun-chung Park
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI079231-03 REVISED

INSTITUTION: WILDLIFE TRUST

Facilities and	*Year 3*	*Year 4*	*Year 5*

Administrative Costs			
F&A Cost Rate 1	26.1%	26.1%	26.1%
F&A Cost Base 1	$139,096	$161,641	$178,528
F&A Costs 1	$36,304	$42,188	$46,596

OMB No. 0925-0001

Grant Number 5R01AI79231-3	**Total Project Period** From: 09/18/2008 To: 06/30/2013
EIN: **Review Group:** 1311726494A1 IRAP	**Requested Budget Period:** From: 09/01/2010 To: 08/31/2011
Title of Project: Risk of Viral Emergence from Bats	**Due Date:** 07/16/2010 **Submitted Date:** 08/19/2010
Program Director/Principal Investigator: PETER DASZAK 460 West 34th Street New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** **Email Address:** (b) (6)	**Applicant Organization:** WILDLIFE TRUST WILDLIFE TRUST 460 West 34th Street New York , NY 10001 **Department:** **Major Subdivision:**
Administrative Official: Aleksei Avery Chmura 460 W 34th St., 17th Floor New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** 1.212.380.4465 **Email Address:** (b) (6)	**Signing Official:** Aleksei Avery Chmura 460 W 34th St., 17th Floor New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** 1.212.380.4465 **Email Address:** (b) (6)
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☐ No ☒ Yes **Animal Assurance Number:** A3415-01 **Inventions and Patents:** ☒ No ☐ Yes ☐ Previously Reported ☒ Not Previously Reported
Program Income: ☒ No ☐ Yes	

Budget Period	**Anticipated Amount**	**Source**
F&A Changes:		

Primary Project/Performance Site Location

Organizational Name: Wildlife Trust		
DUNS: 077090066		
Street 1: 460 West 34th Street	Street 2: 17th Floor	
City: New York	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 10001
Congressional Districts: 08		

Additional Project/Performance Site Location			
Organizational Name: The Trustees of Columbia University			
DUNS: 621889815			
Street 1: 630 West 168th Street		Street 2: Box 49	
City: New York	County:		State: NY
Province:	Country: UNITED STATES		Zip/Postal Code: 10032
Project/Performance Site Congressional Districts: 15			

Additional Project/Performance Site Location			
Organizational Name: The Institute of Zoology			
DUNS: 227012275			
Street 1: Regent's Park		Street 2:	
City: London	County:		State:
Province:	Country: UNITED KINGDOM		Zip/Postal Code: NW14RY
Project/Performance Site Congressional Districts: 0			

Additional Project/Performance Site Location			
Organizational Name: Australian Animal Health Laboratory (AAHL)			
DUNS: 754307957			
Street 1: 5 Portarlington Road		Street 2:	
City: East Geelong	County:		State:
Province:	Country: AUSTRALIA		Zip/Postal Code: VIC 3219
Project/Performance Site Congressional Districts: 0			

Program Director/Principal Investigator:	Grant Number
PETER DASZAK	5R01AI79231-3
Applicant Organization:	**Period Covered by this Report:**
WILDLIFE TRUST	09/01/2009 - 08/31/2010

Title of Project:

Risk of Viral Emergence from Bats

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ No ☐ Yes

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ No ☐ Yes

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ No ☐ Yes

Justification:

Changes in Select Agent Research? ☒ No ☐ Yes

Changes in Multiple PD/PI Leadership plan? ☒ No ☐ Yes

Change in human embryonic stem cell (hESC) line(s) used? ☒ No ☐ Yes

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ No ☐ Yes

Has the Involvement of Animal Subjects changed since previous submission? ☒ No ☐ Yes

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

Cover Letter: File is not uploaded
Research Accomplishments: File is not uploaded
Other Document File: File is not uploaded
Other Support File: File is not uploaded

All Personnel Report

Program Director/Principal Investigator:	Grant Number
PETER DASZAK	5R01AI79231-3

Name:	Commons ID:	Degree(s) Name:	SSN:			
PETER DASZAK	(b) (6)	PHD, BS	(b) (6)	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			(b) (6)	(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Thomas Briese		PHD	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Jonathan H. Epstein		DVM	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Kate Elizabeth Jones		PHD	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
W. Ian Lipkin		PHD	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Bruce Andrew Mungall		PHD	XXX-XX-	Months Devoted to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**

Co-PD/PI				(b) (4), (b) (6)		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Gustavo F. Palacios		PHD	XXX-XX-	**Months Devoted to Project**		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

Progress report year 2

We have completed 2 years of this 5-year R01 and have already made significant advances in global sample collection, mathematical modeling, and detection of novel pathogens in bats. We have expanded our bat pathogen database from 41,608 host-parasite interactions for over 584 bat species and over 5,418 references to more than 25,000 datapoints. We have now collected approximately 7,900 bat samples, including sera, urine, saliva, urogenital swabs, white blood cells, wing punch biopsies, and blood smears, and expanded our geographic surveillance to include the Phillippines under a program designed to determine whether bats are the reservoir for Ebola Reston virus (REBOV). We now have implemented standardized field protocols for sampling microchiroptera, in addition to our existing protocols for megachiroptera (e.g. old world fruit bats). These small insectivorous bats comprise the majority of the bat species in the world and thus the majority of bats we plan to screen for novel viruses. Below is a detailed report of our progress in the first year of this grant.

1) Database analysis of the diversity of unknown bat viruses

We have continued work on our comprehensive, spatially- and temporally-explicit database of bat-pathogen records, which we will use to analyze the risk for emerging diseases from bats globally. Based on literature searches using Web of Science, BIOSIS, Google Scholar, and the 'Literature Cited' sections of every paper on bat parasites we have identified, we have collected 2600 ectoparasite citations (restricting the literature to post-1950) and 3300 endoparasite citations (with no restrictions on date of publication). Based on this literature, we have now entered ~32,000 records, representing ~95% of the ectoparasite literature and 80-90% of the current citations for endoparasites. The next stage is to update the parasite records to a standard, modern taxonomy for each parasite group, and georeference all localities. Figure X shows the number of parasite records, from the literature, for each bat family. We aim to use the database to identify regions and species most likely to have 1) a high diversity of unknown bat viruses and 2) a propensity for these viruses to emerge into people. In year 3 we will focus our targeting for sample collection based on these analyses.

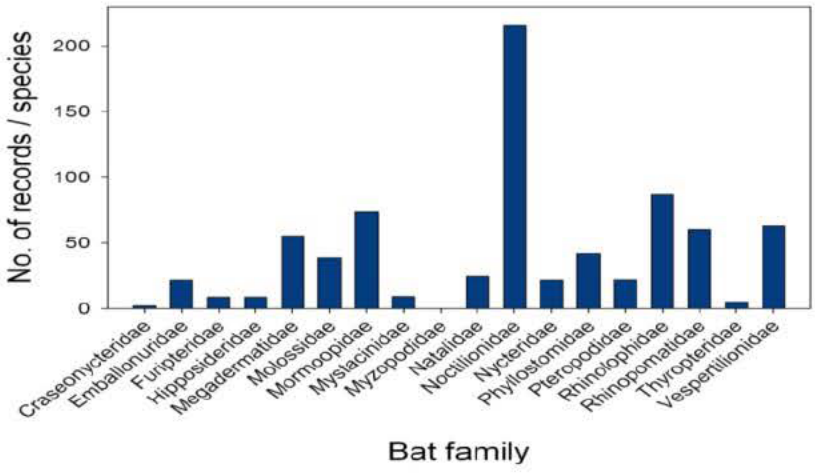


Fig X. Number of parasite records for each bat family, corrected for number of bat species in the family

2) Protocols for insectivorous and small fruit bat sampling implemented

We have successfully implemented a standardized sampling protocol for michrochiroptera and small fruit bats, where low blood sample volumes are expected to be obtained based on body mass. These protocols have been used in conjunction with our existing protocols for megachripotera (e.g. large pteropodid bats) (1). Specific challenges exist with small bats – namely the minute blood volumes that we are safely able to obtain with our non-destructive sampling approach. The protocol is based on the need for enough serum to run several serological assays for antibodies to known and yet-to-be discovered viruses. We use buffered PBS to dilute blood samples and increase the volume of serum obtained after centrifugation. Insectivorous bats may be less than 10g, which only allows for up to 60uL of blood to be safely drawn. In such instances, blood is diluted 1:10 using PBS (buffered saline) to increase serum volume. We have implemented this protocol in each of our field sites, and have modified it for intermediate sized fruit bats where safely obtainable blood volumes are still relatively low (<300uL). In these instances we have used a 1:5 dilution with PBS to increase the overall blood volume. This has dramatically expanded our ability to obtain quality blood samples from very small bats.

3) Expanded coverage of bat surveillance

To date, we have sampled bats in seven countries: Bangladesh, Brazil, China, Mexico, India, Philippines, and the United States. Preliminary field data are summarized below:

Bangladesh

This past year we have made significant progress to expand our bat sampling in Bangladesh to include a diversity of other bat species, in addition to *Pteropus giganteus*. In March/April 2010 we sampled fruit bat species never sampled before for viruses in the country. We conducted extensive field surveys and identified a colony of approximately two thousand *Rousettus leschenaulti* from which we sampled 70 individuals, collecting urine, oropharyngeal and fecal swabs, whole blood, and sera (350 samples). These samples have been exported to our collaborative labs at The Australian Animal Health Laboratory (AAHL, Geelong) and The Center for Infection and Immunity, Columbia University (Lipkin lab, New York) to conduct assays for Nipah virus (AAHL, CII), Ebola virus (AAHL) and viral discovery (CII). Nucleic acid has been extracted from these samples, and we are in the process of screening them for known and novel pathogens under this grant. Preliminary results include detection of Nipah virus nucleic acid from a single *Roussettus* bat using a real-time PCR assay. We are in the process of sequencing the amplicon to confirm the identity. Additionally we have trained 3 new veterinarians and 2 additional field technicians in Bangladesh to capture and collect specimens from bats; this increased capacity for field surveillance will accelerate our program in Bangladesh to assess bat viral diversity over the next year.

Brazil

For this year, Brazil NIAID project had been capture bats in four different states in Brazil : São Paulo, Minas Gerais, Rio de Janeiro and Goiás. A total of n= 605 (3025 samples) bats were captured in pristine areas (protected areas) and urban areas with different levels of disturbance.

The families / species sampled are *Lichonycteris obscura, Glossophaga sorisina, Family Vespertilionidae, Family Molossidae, Desmodus rotundus, Carollia perspicillata, Artibeus lituratus* and *imbriatus, Anoura caudifer, Platyhirrinus lineatus* and*, Sturnira lilium.*

For all this animals, were collected saliva (oral swab), feces (rectal swab), urine, wing punch and blood. The samples are waiting for an import permit before being sent to the Center for Infection and Immunity (Columbia University) for viral discovery work.

Since in Brazil has a great diversity of bat species, involving different habitats and widespread distribution, it is essential to collect information regarding their behavior, population dynamics, use of rural and urban man-made constructs for shelter and the circumstances by which potentially infectious bats may interact with people. Rabies virus has been isolated from 36 species of bats present in the country. Moreover, 29 different bat species have been observed using houses to roost, which may increase the chance of contact with humans and domestic animals. Alterations in the environment due to urban and suburban development for the expanding human populations may have reduced both roosting and foraging habitats. In addition, urban ecosystems offer a large amount of food and shelter associated with a lack of predators, consequently some bat species have adapted to roosting in buildings. We have focused our sampling across a pristine to urban habitat gradient in order to compare the viral diversity within bat species that occur in these habitat types, which represent different interfaces with people or livestock and thus potentially different risk profiles for viral emergence from bats.

Mexico

We are working with collaborators at Departamento de Etología y Fauna Silvestre, Universidad Nacional Autónoma de México and UNAM in Mexico. Bats were collected between Jun 17th and June 25th, 2009. We collected bat specimens from 18 species from four locations in two different ecological landscapes: disturbed and undisturbed (**see Table X**). Serum samples from each bat will be screened for anti-rabies virus antibodies in Mexico, and aliquots of serum, urine, feces, and saliva, preserved in Nuclisense lysis buffer (BioMerieux, USA) (492 samples) will be exported to the Center for Infection and Immunity at Columbia University to be screened for known and unknown viruses using a combination of MassTag PCR (see Bat panel development) and high throughput pyrosequencing. We are in the process of finalizing importation and export permits so that these samples can be send to the CII, and we anticipate shipment and testing in the first half of year 3 of this award. The results of our field sampling are listed in Table 1.

Table 1. List of species and numbers sampled in each location in Mexico, June –July 2009.

Undisturbed			Disturbed		
Lacandona	Laguna Belgica	Total	Azufres	Santa Ana	Total

Artibeus jamaicensis	7		7	7	5	12
Artibeus lituratus	4		4	10	16	26
Artibeus phaeotis				3	1	4
Bauerus dubiaquercus	11		11			
Carollia perspicillata		1	1		3	3
Carollia sowelli	4	2	6			
Desmodus rotundus	2		2		1	1
Glossophaga comissarisi	2		2			
Glossophaga soricina	7	1	8	3	3	6
Micronycteris schmidtorum	2		2			
Myotis californicus					1	1
Myotis keaysi		2	2			
Platyrrhinus helleri	1		1			
Pteronotus davyi	1		1			
Pteronotus parnelli	2		2			
Sturnira illium					11	11
Sturnira ludovici	1	1	2		8	8
Trachops cirrhosus		1	1			
			52			72

China

In July, 2008 we began fieldwork in China. From June to August 2008, 392 bats (1600 samples) representing the genera *Hipposideros, Myotis, Pipistrellus, Rhinolophus,* and *Scotophilus* were trapped and sampled throughout China's Hainan province, an island in the South China Sea (Table 2). Field sampling of species was coordinated with the support of the Guangdong Entomological Institue, Guangzhou China, and the South China Institute of Endangered Animals, Chinese Academy of Sciences. In the field, bats were captured upon exit from roost sites using hand nets or mist nets and were held individually in cloth bags until sample collection. For each individual the relative age, sex, body mass, body measurements, and reproductive status were recorded. Serum and whole blood were taken along with oral and anal swabs. All samples were taken in duplicate.

Samples are being screened for SARS-like coronaviruses, henipaviruses, and filoviruses at the Wuhan Institute of Virology in China.

TABLE 2. Species of bats sampled in China

Genus, species	No. of individuals
Hipposideros	99
pomona	75
larvatus	24
Rhinolophus	62
luctus	7
affinis	23
sinicus	29
pusillus	3
Scotophilus	125
kuhlii	125
Pipistrellus	85
abramus	27
pipistrellus	58
Myotis	21
daubentoni	19
ricketti	2
Total	392

India

Through collaboration with the Indian Institute of Science and the Asian Nature Conservation Foundation, we have completed a four-day training course focused on the safe capture and sampling of mega (fruit) and micro (insectivorous) bats. Participants included two post-doctoral bat ecologists who will be responsible for sampling bats according to our viral discovery protocols in key regions of biodiversity in India including the Western Ghaats; West Bengal, and Assam. Field sampling will begin in year 3 of the grant. West Bengal presents an ecological landscape similar to Bangladesh, and we expect results from bat viral screening here to be comparable to those from Bangladesh, and this represents and excellent opportunity to test the hypothesis that Nipah virus exists in bats with similar prevalence and distribution in India (as Bangladesh), and risk of spillover is similar, but lack of surveillance and reporting is the main reason for the apparent difference in human Nipah virus cases.

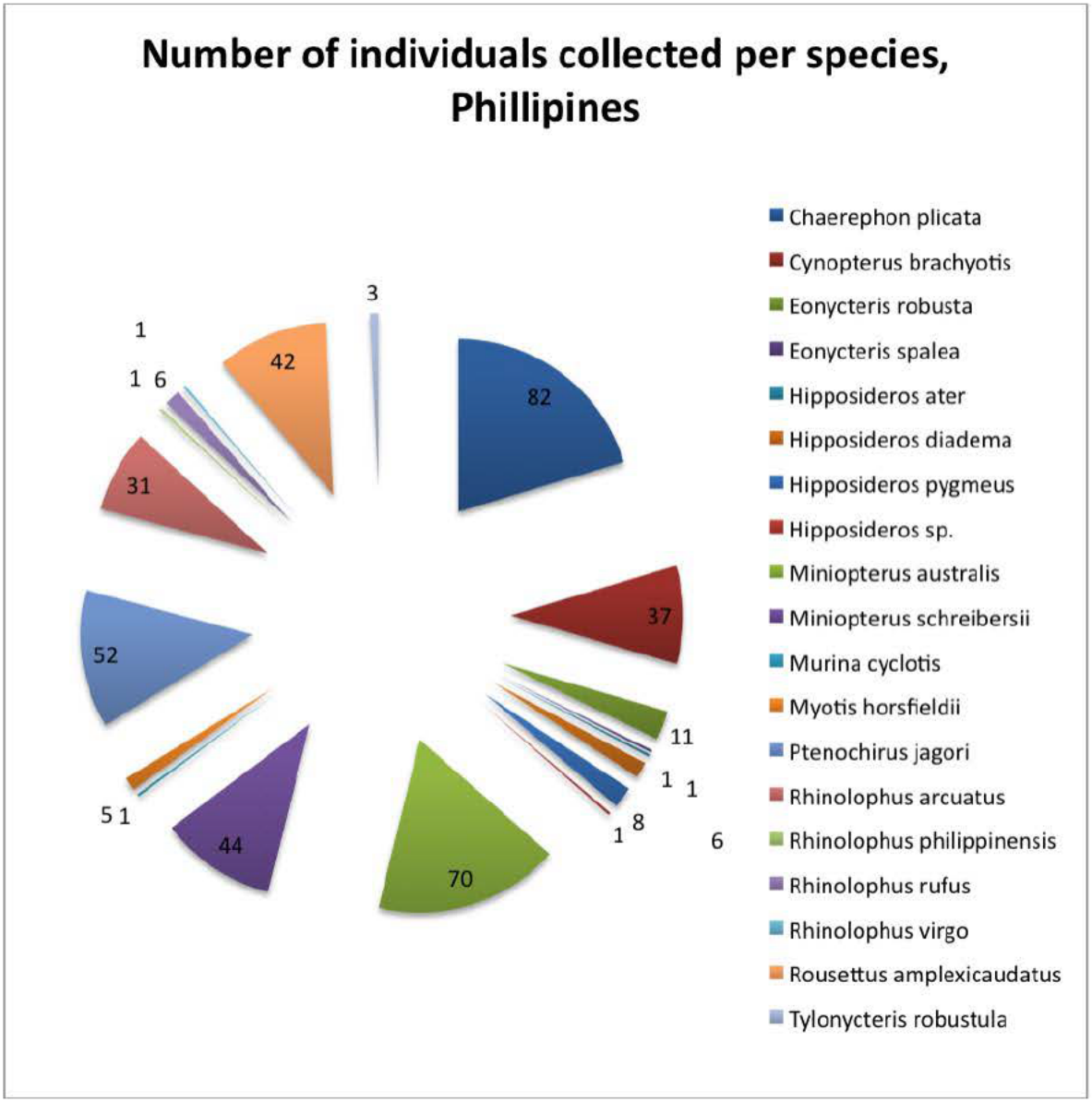
Philippines

We led a 3-week sampling trip of bat species at multiple sites in Luzon, Philippines in April 2010. Thorough a collaboration with FAO, and in-country partners at the Philippine Ministry of Health, Protected Areas and Wildlife Bureau, and Department of Livestock, we conducted training in the capture and sampling of a diverse range of bats and collected a large number of samples (n=403, ~2442 samples, details below) for our viral discovery work. One of the main goals of the sampling was to investigate range of natural reservoirs for Reston Ebola virus (REBOV), which has recently emerged in pigs in the Philippines. Tests for REBOV are currently underway in our partner labs.

Species	Count
Chaerephon plicata	82
Cynopterus brachyotis	37
Eonycteris robusta	11
Eonycteris spalea	1
Hipposideros ater	1
Hipposideros diadema	6
Hipposideros pygmeus	8
Hipposideros sp.	1
Miniopterus australis	70
Miniopterus schreibersii	44
Murina cyclotis	1
Myotis horsfieldii	5
Ptenochirus jagori	52
Rhinolophus arcuatus	31
Rhinolophus philippinensis	1
Rhinolophus rufus	6
Rhinolophus virgo	1
Rousettus amplexicaudatus	42

Tylonycteris robustula	3
Grand Total	**403**

Samples	Aliquot 1	Aliquot 2
Throat	355	355
Urine	189	189
Rectal	245	245
Blood	350	-
Serum	341	341
Blood Smear	283	-
Filter Paper	315	-
Wing punch	364	-
Total	**2442**	


Number of individuals collected per species, Phillipines
3
82
42
1
1 6
31
37
52
11
1 1
5 1
1 8
6
44
70
Chaerephon plicata
Cynopterus brachyotis
Eonycteris robusta
Eonycteris spalea
Hipposideros ater
Hipposideros diadema
Hipposideros pygmeus
Hipposideros sp.
Miniopterus australis
Miniopterus schreibersii
Murina cyclotis
Myotis horsfieldii
Ptenochirus jagori
Rhinolophus arcuatus
Rhinolophus philippinensis
Rhinolophus rufus
Rhinolophus virgo
Rousettus amplexicaudatus
Tylonycteris robustula

USA

Through our collaboration with Western Michigan University (M. Vonhoff, Co-PI) we are developing a bat database. We began collecting bat samples at various sites within the US in June, 2009. Bat species sampled include *Eptesicus fuscus*, *Myotis lucifugus* and *Lasiurus borealis* in Southern Michigan and southern Indiana. The following progress has been made with the database:

- Identified 5,418 articles, from which we have mined 41,608 bat-parasite associations to date
- The database is essentially complete for Acari, Bacteria, Fungi, Insecta, and Protozoa.
- ~70% of Virus and Helminth articles have been examined to date.

In year three of this award we will complete the following activities with respect to the database:

- Completion of data entry
- Updating of parasite records based on current taxonomy
  - Database is currently uncorrected for scientific name changes and taxonomic revisions.
- Georeferencing
  - Conversion of location descriptions to geographical coordinates (latitude and longitude).

We will address the following questions:

- How do patterns of host specificity vary across different parasite groups with different life histories and transmission strategies?
- Has high parasite species richness led to a change in host diversification rates across their evolutionary history?
- Do spatial patterns of parasite diversity overlap patterns of host diversity?
- Do bat parasites follow a species-area relationship?
- Is high parasite species richness associated with threat status in bats?
- How will parasite distributions change under various global warming scenarios?

4) Laboratory diagnostics: MassTag PCR "bat panel"

In collaboration with Ian Lipkin, Director of the Center for Infection and Immunity, we are developing a "Bat Panel" for MassTag PCR. The panel will allow us to efficiently screen bat samples for 10-12 groups of viral pathogens of high interest. The panel is in the process of being validated, and will test for the the following viral groups: Henipaviruses; ebolaviruses (including Reston); Marburg viruses; Australian Bat Lyssavirus; SARS-like coronaviruses; bat coronaviruses; GB viruses.

Discovery of novel coronaviruses from Africa using Next Generation Sequencing

A coronavirus was detected in Hipposideros ssp bats in Nigeria from GIT specimens using a consensus coronavirus PCR. A nearly complete genome sequence was obtained using next-

generation 454 sequencing and consensus PCRs. Comparative amino acid sequence showed that this coronavirus is most similar to the group 2b coronaviruses which comprises SARS and SARS-like coronaviruses. Phylogenetic analysis further shows that this coronavirus forms a branch distinct from group 2b coronaviruses. Based on sequence and phylogeny analyses we propose that this coronavirus is the first member of a novel subgroup among group 2 coronaviruses. This manuscript is currently in preparation.

Discovery of novel Filovirus from Europe using Next Generation Sequencing

A novel Filovirus was detected in insectivorous bats (Miniopterus schreibersii) following a bat die-off in Lloviu cave, Spain. The virus was detected in the liver of a diseased bat using a consensus PCR approach, and the full genome of the virus then sequence using next-generation 454 sequencing. Phylogenetic analysis of the genome suggests that this virus could represent a new genus within the Filovirus family, which also contains the genera Ebolavirus and Marburgvirus. This manuscript is currently in preparation.

5) Discovery of a novel Flavivirus related to Hepatitis C virus from Pteropus giganteus in Bangladesh using high throughput pyrosequencing

We have now published this study in PLoS Pathogens (2). The major findings of this study were:

- Discovery and full characterization of GBV-D, a flavivirus related to HCV and ancestral to previously described GB viruses which have only been described in humans and non-human primates.
- Epidemiological profile showing a prevalence of 5% in a single bat colony
- Clinical profile showing no apparent impact on hepatic function in the host species
- Detection of nucleic acid in saliva from one bat, indicating that zoonotic transmission, if possible, could plausibly occur via the same pathway as Nipah virus – via food-borne routes (e.g. date palm sap)

The next steps for following up the discovery of this virus are to screen sera from people with a high likelihood of exposure to Pteropus giganteus, specifically, Nipah virus IgG positive patients and hepatitis patients from villages where date palm sap is commonly consumed. We will also work towards the development of a serological assay for this virus. We submitted a proposal to provide additional funds for this work.

Literature Cited

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Grant Number: 5R01AI079231-04

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Risk of Viral Emergence from Bats

Aleksei A. Chmura
Wildlife Trust
406 W 34th Street
17th Floor
New York, NY 10001

Award e-mailed to: (b) (6)

Budget Period: 09/01/2011 – 08/31/2012
Project Period: 09/18/2008 – 08/31/2013

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $510,005 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, that cites results from NIH grant-supported research must include an acknowledgment of NIH grant support and disclaimer such as "The project described was supported by Grant Number R01AI079231 from the National Institute Of Allergy And Infectious Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute Of Allergy And Infectious Diseases or the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients are required to comply with the NIH Public Access Policy. This includes submission to PubMed Central (PMC), upon acceptance for publication, an electronic version of a final peer-reviewed, manuscript resulting from research supported in whole or in part, with direct costs from National Institutes of Health. The author's final peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process. For additional information, please visit http://publicaccess.nih.gov/.

Award recipients must promote objectivity in research by establishing standards to ensure that the design, conduct and reporting of research funded under NIH-funded awards are not biased by a conflicting financial interest of an Investigator. Investigator is defined as the Principal Investigator and any other person who is responsible for the design, conduct, or reporting of NIH-funded research or proposed research, including the Investigator's spouse and dependent children. Awardees must have a written administrative process to identify and manage financial conflict of interest and must inform Investigators of the conflict of interest policy and of the Investigators' responsibilities. Prior to expenditure of these awarded funds, the Awardee must report to the NIH Awarding Component the existence of a conflicting interest and within 60 days of any new conflicting interests identified after the initial report. Awardees must comply with these and all other aspects of 42 CFR Part 50, Subpart F. These requirements also apply to subgrantees, contractors, or collaborators engaged by the Awardee under this award. The NIH website http://grants.nih.gov/grants/policy/coi/index.htm provides additional information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael A. Wright
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI079231-04

Award Calculation (U.S. Dollars)

Salaries and Wages	$109,831
Fringe Benefits	$27,458
Equipment	$11,761
Supplies	$3,902
Travel Costs	$13,280
Other Costs	$2,940
Consortium/Contractual Cost	$299,749
Federal Direct Costs	$468,921
Federal F&A Costs	$41,084
Approved Budget	$510,005
Federal Share	$510,005
TOTAL FEDERAL AWARD AMOUNT	$510,005
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$510,005

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
4	$510,005	$510,005
5	$529,571	$529,571

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI079231A
Fiscal Year:	2011

IC	*CAN*	*2011*	*2012*
AI	8472302	$510,005	$529,571

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M34A / **OC**: 414E / **Processed**: (b) (6), 08/04/2011

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI079231-04

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI079231-04

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award may be subject to the Transparency Act subaward and executive compensation reporting requirements of 2 CFR Part 170. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI079231-04

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

This non-competing award reflects an adjustment of the amount recommended on the previous Notice of Award, per the NIAID implementation of the NIH FY 2011 fiscal policy (See NIH Guide Notice NOT-OD-11-068). Future year recommended levels also have been adjusted consistent with the referenced Notice.

No studies, work or any Federal funds can be expended in the countries of NIgeria, Republic of Congo and Israel until approved.
****

This award includes funds awarded for consortium activity with the Trustees of Columbia University. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

This award includes funds awarded for consortium activity with Australian Animal Health Laboratory/AUSTRALIA. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Michael A. Wright
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-493-0597

Program Official: Eun-chung Park
Email: (b) (6) **Phone**: (b) (6) **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI079231-04

INSTITUTION: ECOHEALTH ALLIANCE, INC.

Budget	*Year 4*	*Year 5*
Salaries and Wages	$109,831	$112,028
Fringe Benefits	$27,458	$28,007
Equipment	$11,761	
Supplies	$3,902	$16,662
Travel Costs	$13,280	$13,280
Other Costs	$2,940	$2,940
Consortium/Contractual Cost	$299,749	$311,523
TOTAL FEDERAL DC	$468,921	$484,440
TOTAL FEDERAL F&A	$41,084	$45,131
TOTAL COST	$510,005	$529,571

Facilities and Administrative Costs	*Year 4*	*Year 5*
F&A Cost Rate 1	26.1%	26.1%
F&A Cost Base 1	$157,411	$172,917
F&A Costs 1	$41,084	$45,131

EcoHealth Alliance

01 July 2011

Dr. Eun-Chung Park
Virology Branch, DMID, NIAID, NIH
6610 Rockledge Dr., Rm 4103
Bethesda MD 20892-7630

Dear Dr Park,

Thank you for giving us guidance on our reporting. Please find our Year 3 progress report for grant #5R01AI079231 attached. Please note that since our last report our Institutional Name has been updated within eRA Commons – from "**Wildlife Trust**" to "**EcoHealth Alliance**".

We would like to add Dr. Kevin J. Olival as a staff scientist (doctoral level). The aim of Dr. Olival's work will be to manage field activities under this grant, and help develop predictive models of zoonotic bat virus emergence. His CV is attached in our report.

At the end of our progress report we also request permission to work in three new countries in years 4-5 of this project: Nigeria, Republic of Congo, and Israel. We provide you with contact details for our local coordinator for each country as well as more details on the scientific justification and target species for each country. Please let us know if there is any other information you require for State Department approval.

As per your request, we provide additional details in this report regarding safe animal handling as per our approved IACUC protocol, and also issues of biosafety that we adhere to carefully with all of our projects and partners.

Please let me know, via our SRO Mr. Chmura, if there is any other information you need, or further clarification of any issues. We will rapidly respond when you contact us.

Thanks again for your advice and guidance,

Yours sincerely,

Dr Peter Daszak
President, EcoHealth Alliance
460 West 34th St.,17th Floor
New York, NY. 10001
(b) (6)
(b) (6)

OMB No. 0925-0001

Grant Number 5R01AI79231-4	**Total Project Period** From: 09/18/2008 To: 08/31/2013
EIN: **Review Group:** 1311726494A1 IRAP	**Requested Budget Period:** From: 09/01/2011 To: 08/31/2012
Title of Project: Risk of Viral Emergence from Bats	**Due Date:** 07/15/2011 **Submitted Date:** 07/14/2011
Program Director/Principal Investigator: PETER DASZAK 460 West 34th Street New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** **Email Address:** (b) (6)	**Applicant Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK , NY 100012317 **Department:** **Major Subdivision:**
Administrative Official: Aleksei Chmura 460 W 34th St., 17th Floor New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** 1.212.380.4465 **Email Address:** (b) (6)	**Signing Official:** Aleksei Chmura 460 W 34th St., 17th Floor New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** 1.212.380.4465 **Email Address:** (b) (6)
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☐ No ☒ Yes **Animal Assurance Number:** A3415-01 **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☐ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: ECOHEALTH ALLIANCE		
DUNS: 077090066		
Street 1: ECOHEALTH ALLIANCE, INC.	Street 2: 460 W 34TH ST	
City: NEW YORK	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 100012317
Congressional Districts: 08		

Additional Project/Performance Site Location		
Organizational Name: The Trustees of Columbia University		
DUNS: 621889815		
Street 1: 630 West 168th Street	Street 2: Box 49	
City: New York	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 10032
Project/Performance Site Congressional Districts: 15		

Additional Project/Performance Site Location		
Organizational Name: The Institute of Zoology		
DUNS: 227012275		
Street 1: Regent's Park	Street 2:	
City: London	County:	State:
Province:	Country: UNITED KINGDOM	Zip/Postal Code: NW14RY
Project/Performance Site Congressional Districts: 0		

Additional Project/Performance Site Location		
Organizational Name: Australian Animal Health Laboratory (AAHL)		
DUNS: 754307957		
Street 1: 5 Portarlington Road	Street 2:	
City: East Geelong	County:	State:
Province:	Country: AUSTRALIA	Zip/Postal Code: VIC 3219
Project/Performance Site Congressional Districts: 0		

Additional Project/Performance Site Location		
Organizational Name: International Centre for Diarrhoeal Disease Research, Bangladesh		
DUNS: 000000000		
Street 1: GPO 128	Street 2:	
City: Dhaka	County:	State:
Province:	Country: BANGLADESH	Zip/Postal Code: 1212
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: East China Normal University		
DUNS: 420945495		
Street 1: 3663 Zhongshan Beilu	Street 2:	
City: Shanghai	County:	State:
Province:	Country: CHINA	Zip/Postal Code: 200062
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Universidad Mayor de San Simon		
DUNS: 950009451		
Street 1: Centro de Biodiversidad y Genetica	Street 2: PO Box 538	
City: Cochabamba	County:	State:
Province:	Country: BOLIVIA	Zip/Postal Code:
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Fundacion Universitaria San Martin		
DUNS: 880303115		
Street 1: Kra 10 No 128-70 Torre 1	Street 2: apto 504	
City: Bogota	County:	State:
Province:	Country: COLOMBIA	Zip/Postal Code:
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Veterinary Research Institute		
DUNS: 000000000		
Street 1: D203, Block D, Paradesa Tropika	Street 2: Persiaran Meranti, Bandar Sri Damansara	
City: Kuala Lumpur	County:	State:
Province:	Country: MALAYSIA	Zip/Postal Code: 52200
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Universidad Nacional Mayor de San Marcos		
DUNS: 934842774		
Street 1: Museo de Historia Nacional	Street 2: Peruvian Wildlife Department, Apto 140434	
City: Lima	County:	State:
Province:	Country: PERU	Zip/Postal Code: 14
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Department of Veterinary Medicine		
DUNS: 000000000		
Street 1: Bureau of Animal Industry & FAO	Street 2: Eliptical Road, Quezon City	
City: Manila	County:	State:
Province:	Country: PHILIPPINES	Zip/Postal Code:
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location			
Organizational Name: Western Michigan University			
DUNS: 062230560			
Street 1: 3431 Wood Hall		Street 2:	
City: Kalamazoo	County:		State: MI
Province:	Country: UNITED STATES	Zip/Postal Code: 49008	
Project/Performance Site Congressional Districts: 06			

Program Director/Principal Investigator:	**Grant Number**
PETER DASZAK	5R01AI79231-4
Applicant Organization:	**Period Covered by this Report:**
ECOHEALTH ALLIANCE, INC.	09/01/2010 - 08/31/2011

Title of Project:

Risk of Viral Emergence from Bats

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification: n/a

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:
	18288193	PUBMED	NIH Manuscript Submission System was not available at the time of submission
	81508600	MYNCBI	NIH Manuscript Submission System was not available at the time of submission
	81508759	MYNCBI	NIH Manuscript Submission System was not available at the time of submission
	81509248	MYNCBI	NIH Manuscript Submission System was not available at the time of

submission

Research Accomplishments:

File is not uploaded

Other Support File:

File is not uploaded

All Personnel Report

Program Director/Principal Investigator:	Grant Number
PETER DASZAK	5R01AI79231-4

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
PETER DASZAK	(b) (6)	PHD, BS	(b) (6)			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			(b) (6)	(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Thomas Briese		PHD	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
JONATHAN H EPSTEIN	(b) (6)	DVM	XXX-XX-0358			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator			09/74	(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
Kate Elizabeth Jones	(b) (6)	PHD	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project		
W. Ian Lipkin		PHD	XXX-XX-			
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	Months Devoted to Project
Bruce Andrew Mungall		PHD	XXX-XX-	

Role on Project:	Supplement Support:		DoB: (MM/YY)	Cal	Acad	Sum
Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	
Kevin J. Olival	(b) (6)	PhD	(b) (6)	Months Devoted to Project

Role on Project:	Supplement Support:	DoB: (MM/YY)	Cal	Acad	Sum
Staff scientist (Doctoral level)		(b) (6)	(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:	
Gustavo F. Palacios		PHD	XXX-XX-	Months Devoted to Project

Role on Project:	Supplement Support:	DoB: (MM/YY)	Cal	Acad	Sum
Co-PD/PI			(b) (4), (b) (6)		

NIAID Bat Virus Grant 5R01AI079231, Progress report year 3

10 July 2011

Overview:

We have completed 3 years of this 5-year R01 and have made significant advances in: novel viral discovery from samples collected in Years 1-2, optimization of laboratory diagnostic protocols and primers for bat viral assays, expanding field surveillance and sample collection, and mathematical and geographic "bat virus hotspot" modeling. We have collected over 22,000 clinical samples from >50 species of free-ranging bats captured in the past 12 months from Bangladesh, Bolivia, China, Malaysia, Mexico, and the Philippines. This includes over 2,500 animals captured, sampled, and released by our field teams (Table 1). We have discovered 35 completely new viruses from 5 viral families (Corona-, Herpes-, Astro-, Parvo-, and Adenoviridae) from samples tested from Bangladesh, Mexico, and China. Our viral detection was improved using our refined diagnostic testing protocols and consensus PCR primers that were developed this year. Additionally we have completed unbiased sequencing (454 sequencing) on two clinical bat samples from Thailand, which yielded leads on several additional new pathogens including Herpes- and Adenoviruses and possibly novel retroviruses. We also have leads from serological evidence for infection of Bangladesh fruit bats by what may be a novel Filovirus. We have nearly completed our spatially- and temporally-explicit database of bat-pathogens that has over 42,000 records. Using this georeferenced database on bat pathogens, we have generated the first "hotspot" risk models for bat diseases – from these initial modeling runs, we have mapped zoonotic risk from bat-borne emerging infectious diseases (EIDs) and also developed predictive maps of global bat viral diversity based on all known bat viruses. This past year we also assembled an entirely new database of all known mammal host-virus associations from 60 years of literature to answer the question, "are bats more important viral reservoirs than other mammal groups"? We present details of all of these Year 3 activities in the report below.

Table 1. Number of bats captured and number of samples obtained from the field in last 12 months.

Country	Bats	Samples
Bangladesh	932	8,388
Bolivia	306	2,448
China	231	1,848
Malaysia	119	1,071
Mexico	941	8,469
Philippines	61	517
Total	2,590	22,741

Pathogen Discovery in Bat Samples:

We have made significant progress in our diagnostic capacity and novel viral discovery in Year 3 from samples collected in approved countries under our grant. In the past year, we have

sequenced and confirmed 35 completely new viruses from 5 viral families (Corona-, Herpes-, Astro-, Parvo-, and Adenoviridae). These new viruses were discovered in a diversity of bat species from the most species-rich families of bats globally (Vespertilionidae, Pteropodidae, Phyllostomidae, and Rhinolophidae). Additionally, we have important leads on several other new viruses using viral family-level serological assays and nucleic acid fragments retrieved in unbiased sequencing runs (454 sequencing). We have nucleic acid reads from 454 sequencing that likely represent novel Herpes- and Adenoviruses from bats from Thailand, although these have not yet been confirmed with PCR or additional sequencing. While running a panel of tests we also have serological results that suggest infection of a unique Filovirus in Bangladeshi fruit bats (*Rousettus spp.*). All of these results are presented below in greater detail organized by viral family.

Optimized diagnostic algorithm and conserved primers for broad screening of viral families in bats

We have developed and adopted a two-stage algorithm for pathogen discovery in bats (Figure 1). The first is an extensive molecular screen of all bat samples collected using consensus and standard PCR for non-select agents at a viral family level. For this stage, we have recently developed and optimized a suite of consensus primers to broadly screen for DNA and RNA viral families/genera, shown in Figure 2. Our second approach is the targeted use of next generation deep sequencing (454 and/or Illumina) for the unbiased detection of novel agents. Samples are selected for deep sequencing if a) they are well characterized and collected along a gradient of anthropogenic change, b) they have been collected from bats following an outbreak in humans in the same region, or c) they are considered to come from a species of high profile and/or concern (discussed in more detail in the modeling section). Over the past six months we have worked to optimize protocols for both consensus PCR assays and deep sequencing – validating their use on different clinical samples from bats (including blood, urine, feces and saliva). In particular, we are completing the development of the 454 protocols before their widespread use increases later this year as part of a detailed metagenomic viral discovery study in bats (Malaysia),

Figure 1

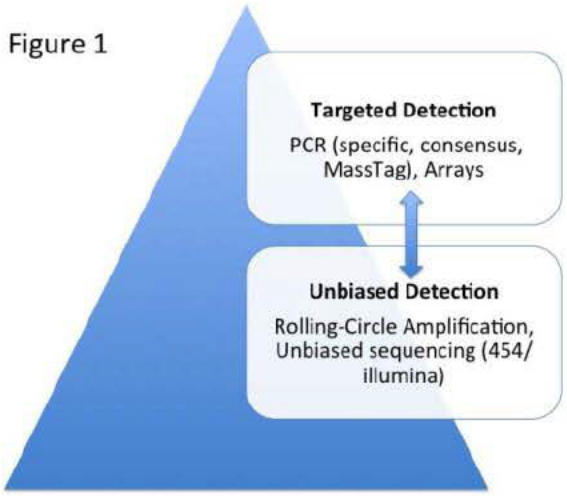
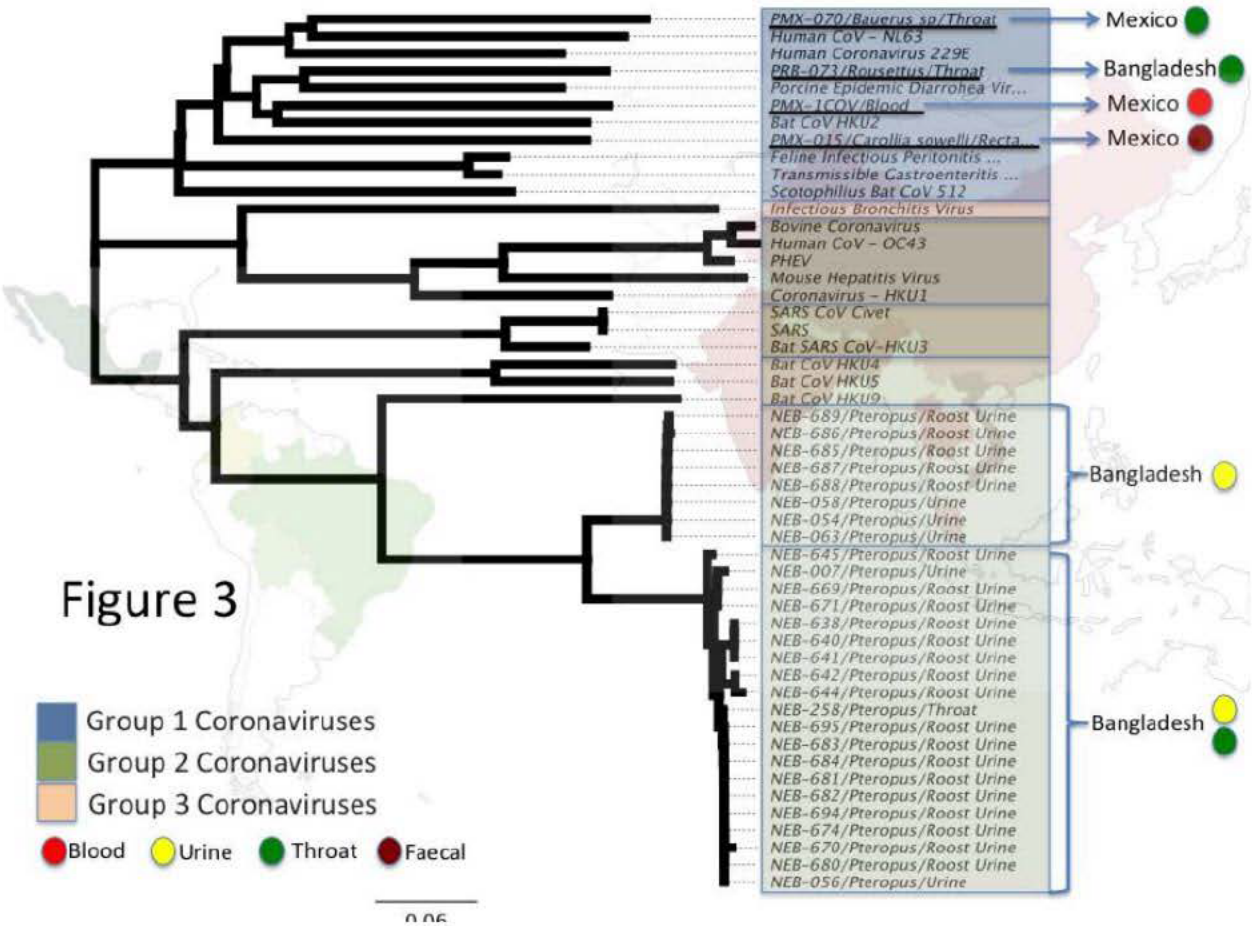


Fig 2. Consensus PCRs for bats

and for other high value samples collected at our field sites globally.

Novel Coronaviruses (Bangladesh, Mexico, and China)

Bat samples from Bangladesh, Mexico, and China have been screened for the presence of known and novel coronaviruses. We have identified 7 completely new coronaviruses, in phylogenetic clades not previously described. A total of 882 samples from 18 species from Mexico and Bangladesh were processed and screened for coronaviruses – including blood, urine, feces and/or throat swabs. Of these, 63 samples were confirmed positive for coronavirus. Phylogenetic analysis showed that the 63 positives from Bangladesh and Mexico represent six novel coronaviruses – four of which cluster with group 1 coronaviruses, and two of which cluster with group 2 (Figure 3). Each of these novel Coronaviruses represents unique clades described for the first time and this result significantly expands our understanding of the viral diversity within this viral family known to have zoonotic potential. We are currently screening additional samples from both Mexico and Bangladesh in an attempt to exhaust the discovery curve of novel coronaviruses in these regions/species – i.e the point at which we no longer identify novel viruses, but only those we have seen before. Once completed, this dataset will also be used for spatial and temporal analysis of the evolution of coronaviruses in bats. Additionally we discovered a novel Group 1 coronavirus from China, from the same geographic region where SARS-coronavirus first emerged, and are now working with our colleagues at the Wuhan Institute of Virology in China to obtain a more complete genome sequence from this virus and better characterize the coronavirus diversity from Chinese bats.



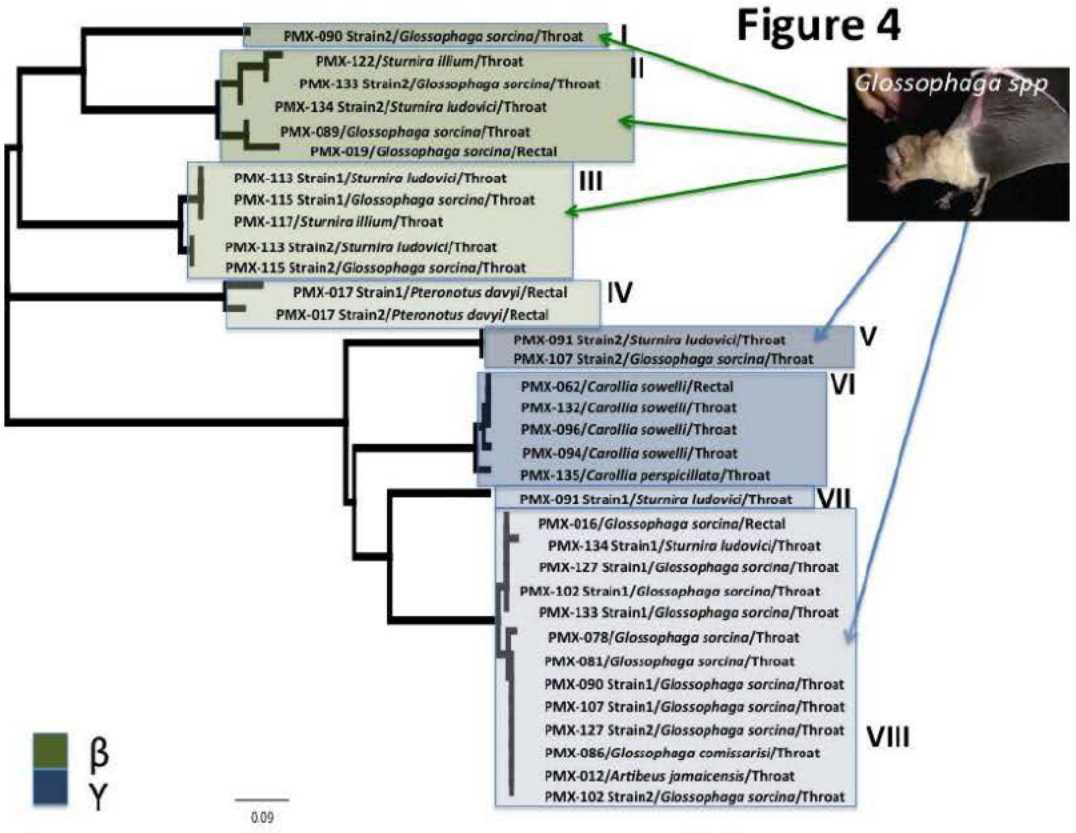
Novel Herpesviruses (Bangladesh and Mexico)

Bat samples have also been screened for the presence of known and novel herpesviruses (HVs). From Mexico, eight new HVs were identified from five different bat genera – with four of the HVs belonging to the betaherpesvirus subfamily, and four belonging to the gammaherpesvirus subfamily. Strain variation was also detected for each of the eight novel viruses – with a maximum of 9.8% nucleotide sequence variation/species.

HVs are often considered to be very host specific. We therefore investigated the variation of host species associated with each of the eight HVs from Mexico. While some viruses seemed to show specificity for one species of bat, the results do appear to suggest that bat HVs will co-infect multiple species sharing similar ecological niches. We demonstrated 1) that certain bats were only infected with one of the eight novel viruses, 2) that others were infected with >1 of either the betaherpesviruses or gammaherpesviruses, and 3) that some bats that were infected with >1 virus from each subfamily (Figure 4). It is unknown whether bat HVs follow the hypotheses of host-viral speciation that have been proposed for other HVs. Our data suggest they may not. We are currently investigating this by sequencing host genes (Cytb) for use in models of virus-host coevolution.

In addition to Mexico, we have screened *Pteropus giganteus* from Bangladesh for the presence of HVs. To date, four novel viruses have been identified, although their complete genome classification and characterization is still underway. These data, together with additional new viruses identified from yet unscreened species, will contribute to our global analysis of herpesviruses phylogenetic diversity and improve our understanding of the mechanisms that drive viral evolution among bat species.

Figure 4. Phylogenetic analysis of 8 novel Herpesvirus clades discovered from seven bat species (5 genera) from Mexico. Some bat species were only infected with one Herpesvirus (*Carollia sowelli*), but others were found infected with multiple HV stains/clades and even from both beta- and gammaherpesvirus subfamilies (e.g. *Glossophaga sorcina*, shown).



Novel Astroviruses (Mexico, Bangladesh, and China)

Bat samples from Mexico (n=254) and Bangladesh (n=247) were screened for the presence of known and novel astroviruses, including blood, feces, throat swabs and blood representing from a total of 18 different species. In Mexico, four novel astroviruses have been detected in six different bat species from feces and throat swabs (Figure 5). Novel astroviruses have also been detected in *Pteropus* bats from Bangladesh, although their full classification and characterization has yet to be completed. As for CoV and HV, the completion of a global dataset for astroviruses in bats will allow for spatial and temporal analysis of viral evolution, and

modeling of viral-host speciation. Taken together, comparative analyses of how each viral family evolves in bats through time and space will provide great insight into viral emergence in new hosts.

In collaboration with the Wuhan Institute of Virology in China, we have also discovered 9 new bat astroviruses in China from 4 bat species. These viruses are diverse and span the phylogenetic tree of astroviruses when analyzed with other known viruses. The position of these new viruses is based on PCR detection, sequencing, and phylogenetic analysis of the RNA-dependent RNA- polymerase (RdRp) gene (Figure 6).

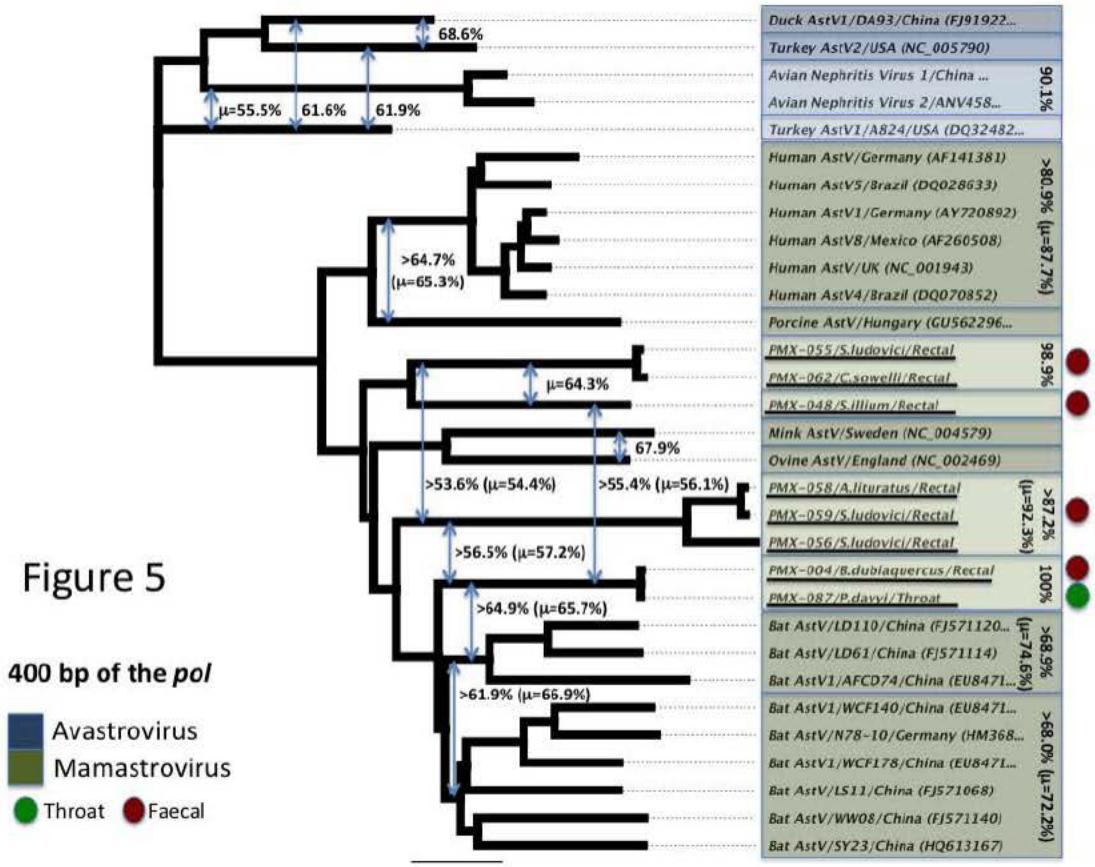


Figure 5

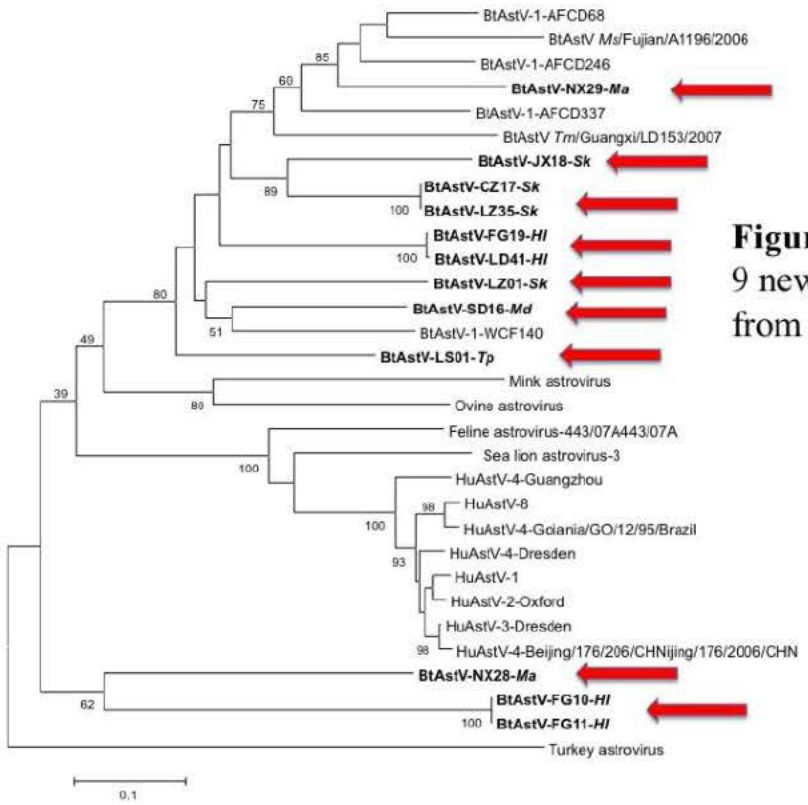
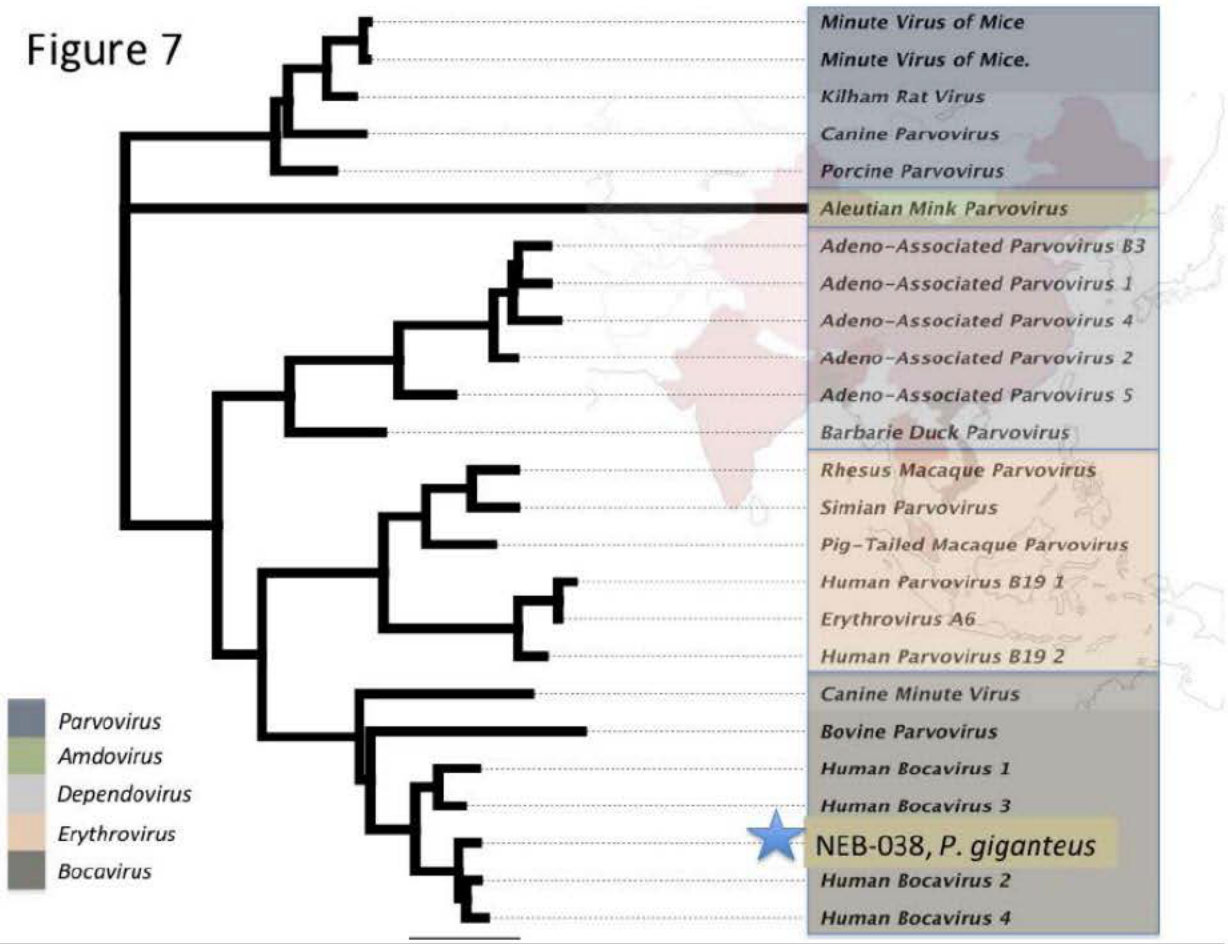


Figure 6
9 new Astroviruses from Chinese bats

Novel Bocavirus (Bangladesh)

In Bangladesh we recently discovered a novel bocavirus (Parvoviridae). We sequenced 277 bp of the polymerase gene and showed that this new virus groups phylogenetically between three known human strains of bocavirus (Figure 7). The virus was identified from a urine sample from a *Pteropus giganteus* fruit bat, and only 1/255 samples were positive. Further characterization of this virus is necessary and ongoing. Little is known about the diversity of Bocaviruses, but they are thought to be important (emerging) human respiratory and/or enteric pathogens. This is the first and only non-human bocavirus known and its phylogenetic position may suggest zoonotic potential for this virus.

Figure 7

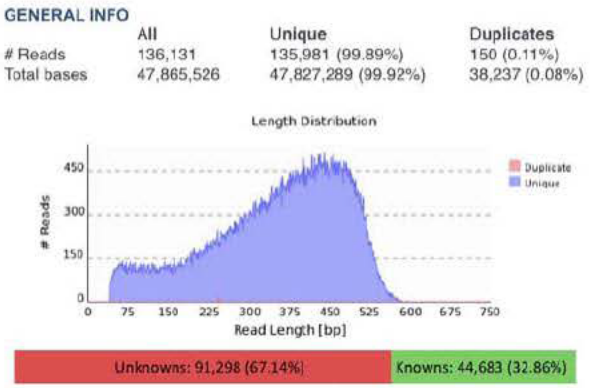


Next-generation sequencing results and viral discovery in Thailand: leads on novel Herpes-, Adeno-, and Retroviruses

In a partnership with Drs. Thiravat Hemachudha and Supaporn Wacharapluesadee from Chulalongkorn University, Thailand, we have been testing the 454 'Junior' next-generation sequencing machines (Roche) as an effective tool for viral discovery in clinical samples from bats. We have completed two 454 Jr. sequencing runs on two samples, one pooled saliva sample from *Rousettus leschenaulti* and one pooled urine sample from *Pteropus lylei*, both sampled from bats in central Thailand. Our preliminary findings from this study provide critical leads on the presence of several viral families not previously known from these bat species. A majority of the reads were around 400 base pairs and were unique, as they did not match to known nucleic acid sequences in the BLAST database (Figures 8a and 9a). A small subset of hits matched to known viruses (0.05% of all raw reads). We performed additional analyses on this subset using TblastX specifically against a database of all known viruses with search criteria set at 80% coverage and 60% identity (Figures 8b and 9b). Excluding a large number of bacteriophage viruses, we had several important leads. This includes a large number of hits ~22% of viral reads in both the saliva and urine samples for several novel herpesviruses, most close match BLAST was with cercopithecine herpesvirus 5, a primate virus. We had additional

convincing evidence (7 hits with good coverage) for a novel Adenovirus in the *Pteropus* urine sample (Figure 9b) with the closest relative a Tree Shrew Adenovirus (Mastadenovirus). Additionally there were =several hits for retroviruses in both samples, although we are confirming whether or not these are the result of endogenous retroviruses vs. an active infection. We are next planning to follow up with these viral families using consensus PCR assays on more samples collected from these same species to get larger nucleic acid fragments and confirm the initial results from RNA and DNA sequence reads from the 454 runs.

Figure 8a. *Rousettus* Saliva – unassembled reads



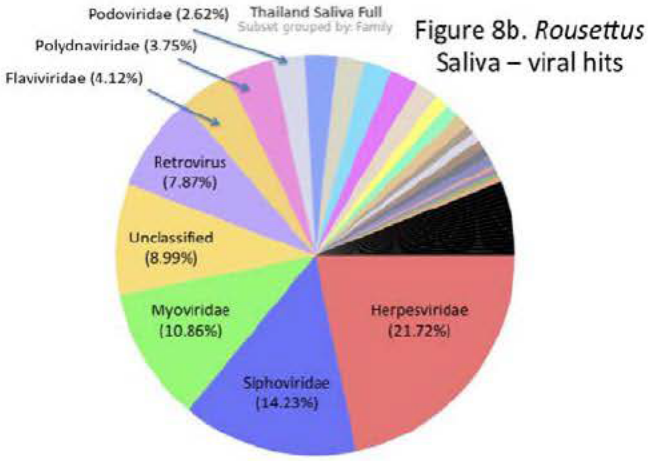
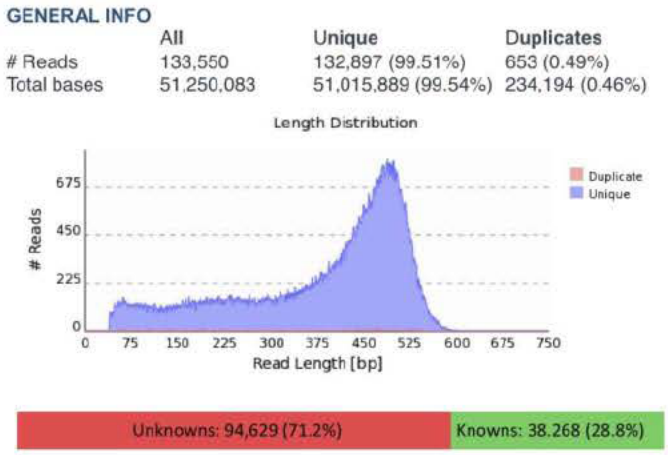


Figure 8b. *Rousettus* Saliva – viral hits

Figure 9a. *Pteropus* Urine – unassembled reads



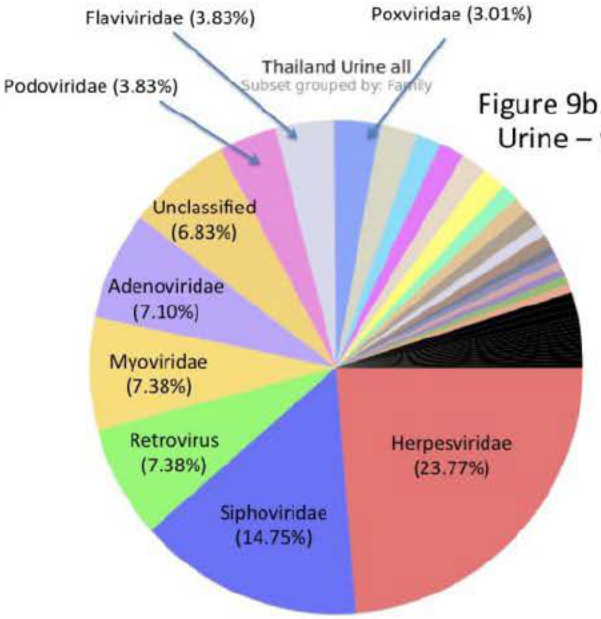


Figure 9b. *Pteropus* Urine – viral hits

Filovirus Seropositive bats in Bangladesh

We collected a total of 2583 samples from 141 *Rousettus leschenaulti,* 75 *Cynopterus* spp, and 107 *Megaderma lyra* bats in Bangladesh this past year. We have screened all sera from these bats using a series of tests including Filovirus assays, including recombinant expressed EboV-N antigens by ELISA. Six of the 141 *Rousettus* sera showed weak positive antibody levels on the Filovrius ELISA. These results were confirmed for all samples using Western Blot. We have run conserved primer PCR assays on urine, feces, and saliva samples from half (n=70) of the

Rousettus samples but all were negative for Filoviruses. Without nucleic acid from these samples at this stage, we are unable to characterize the strain of Filovirus circulating in these bats. Our suspicion, because of the relatively "weak positive" results from the serological assay, that this may represent a past infection in these bats from a Filovirus perhaps distantly related to Ebola Reston, Ebola Zaire, or Marburg viruses.

Photo: *Rousettus leschenaulti* sampled and released in Faridpur, Bangladesh.

Biosafety

Despite the fact that the bats being studied under this award predominantly appear healthy, and the aims of this study are to identify non-biodefense agents in bats, bats are known to carry agents that cause severe disease and mortality in people. Because of this, and because of the fact that bats infected with zoonotic viruses including Ebola virus, Marburg virus, Rabies virus, Nipah and Hendra viruses, and SARS coronavirus may appear clinically "normal," all field and laboratory personnel follow strict safety and biohazard protocols during all phases of the study: capture, sampling, sample handling, sample shipment, and testing. Field work involves the risk of exposure to bat-borne pathogens as well as physical injury. One of the Co-PIs (Dr. Epstein) is a veterinarian with extensive experience working with wildlife species and high-biosecurity pathogens (Nipah virus, ebolavirus, SARS), and several of the key personnel are veterinarians and wildlife biologists who are also experienced in handling wildlife under disease surveillance activities. Thus, great care has been taken in the field to limit the risk of accidental exposure to known or unknown bat pathogens. Field team members handling animals and/or working in caverns or enclosed environments with large bat populations utilize personal protective equipment and practice proper environmental disinfection techniques. This includes wearing coveralls or dedicated clothing, nitrile gloves, eye protection (safety glasses or a face shield, and a P95 or P100 respirator. All field clothing and equipment is disinfected using virkon disinfectant following capture and sampling activity. All biological waste from field surveys is disposed of in the appropriate container (sharps box or a biohazard autoclave bag) and brought to local hospitals or university labs to be autoclaved. All field personnel have been immunized against rabies and have demonstrated a neutralizing antibody titer, in accordance with WHO and CDC recommendations. Field teams carry rabies boosters in the field (or have identified a regional clinic where rabies vaccine is available) and will receive a booster in the event of a potential exposure.

Field and lab safety protocol

Our procedures to deal with bites, needle-sticks etc. are as follows: the wound is washed thoroughly with soap and water to clean away dirt and debris, then vigorously scrubbed with a sterile gauze bandage and benzalkonium chloride for 5 minutes. If bleeding, pressure is applied with a sterile bandage until bleeding has stopped. If the wound continues to bleed, medical

attention will be sought at the nearest hospital. The bat from which the bite or exposure originated is identified, and the samples collected from it labeled on the data sheet that these were involved in an exposure. Our procedures require that the person potentially exposed reports to a major hospital within 24 hours, if the wound is severe, to have wound examined and receive a rabies booster (as per WHO/CDC protocols). Minor injuries are treated in the field and a rabies booster is provided either in the field or at a local clinic.

Samples placed in lysis buffer are non-infectious and may be handled under BSL 2 conditions. Serum or plasma is heat inactivated (at 56^{o} C for 30 min) prior to shipping or testing. Samples placed in viral transport medium or frozen without medium will be handled as clinical diagnostic samples. Samples will be stored at ultra-low temperatures (-80^{o} C) until viral isolation is required, and then they will be packaged and shipped as diagnostic specimens according to IATA standards for clinical specimens. Samples designated for viral isolation from a bat that had a potentially zoonotic virus identified via molecular techniques (e.g. PCR) will be treated as potentially infectious for the purposes of shipping and handling, and will be tested under the appropriate biosafety lab standards. Potentially infectious samples (PCR positive) will be received at partner laboratories and placed in BSL-3 (or BSL 4 if there is suspicion of a virus related to a select agent) laboratories to be tested.

Mathematical Modeling of Bat Viruses: Viral Diversity, Distribution, Host Range, and Risk Maps

Database analysis of the diversity of unknown bat viruses

Our work on the comprehensive, spatially- and temporally-explicit database of bat-pathogen records is near completion. Based on literature searches using Web of Science, BIOSIS, Google Scholar, and the 'Literature Cited' sections of every paper on bat parasites we have identified, we have collected 2617 ectoparasite citations (restricting the literature to post-1950) and 3764 endoparasite citations (with no restrictions on date of publication). We have now entered ~42,000 records, representing >95% of the bat parasite literature. These records include 8,751 viral, 1,668 protozoan, 449 fungal, and 291 bacterial records. All other records (~30,000) are of helminths (Platyhelminths and Nematodes) and external mite and insect parasites. So far our focus has been on completion of data mining. However, we have now also started on the process of updating the parasite records to a standard, modern taxonomy for each parasite group.

We are now confident in the extensive coverage of our database, and have begun to use the database to model and identify regions and species most likely to have 1) a high diversity of unknown bat viruses and 2) a propensity for these viruses to emerge into people. We have completed initial runs of our spatial regression -- bat virus "hotspot" analysis -- and produced a first round of risk maps for bat-borne emerging infectious diseases. Similarly, this year we have used this database along with a comparable database we put together for all known mammal viruses (Olival, Bogich, et al. unpublished data) to address whether or not bats are host to a

disproportionate number of viruses relative to other mammalian groups, as has been repeatedly suggested in the literature.

Are bats exceptional viral reservoirs as compared to other mammals?

As part of our R01 funded activities this past year we completed an extensive literature review and opinion paper posing the question above (Olival et al. In Press). Although researchers have recently suggested that bats are somehow "exceptional" viral reservoirs as compared to other mammalian Orders, no one had tested this hypothesis using in any rigorous or data-driven approach.

To further expand on our recent paper and investigate whether or not bats carry a disproportionate number of viruses per species compared to other mammals, after correcting for bias from study effort, we conducted a preliminary statistical investigation using our new database on all mammal viruses and their known mammalian hosts, as outlined below.

Methods: We used our new database on mammalian virus host range (Olival, Bogich et al., unpublished data) to obtain all viruses for each mammalian Order. Host species without a standardized synonym (Wilson and Reeder 2005) were excluded. For each host species, viral richness was counted, and taxonomic order was noted. Two orders (*Scandentia* and *Diprotodontia*) were excluded as they only had a single species, and therefore could not be well-modeled.

The dataset also contained two measure of literature bias for each host species: 'Bias', was the number of papers published over all years in the ISI Zoological Abstracts database for each host species; and 'Disbias', which included the nmber of disease-related papers from the same source for each species. Both of these measures give a good proxy for research effort and were used to correct for systematic biases in detection at a host level. After cleaning, the dataset covered 290 host species across 13 orders.

Results:

From the raw data, Chiroptera have the highest median viruses per species than any other order (Fig. 10) and the second highest mean viruses per species, after *Perissodactyla* (Table 2). Basic scatter plots suggest a weak positive association between extent of literature bias and number of viruses discovered for species in most orders (Fig. 11).

Figure 10. Box plots showing viruses per species split by order. Outliers are denoted by circles.

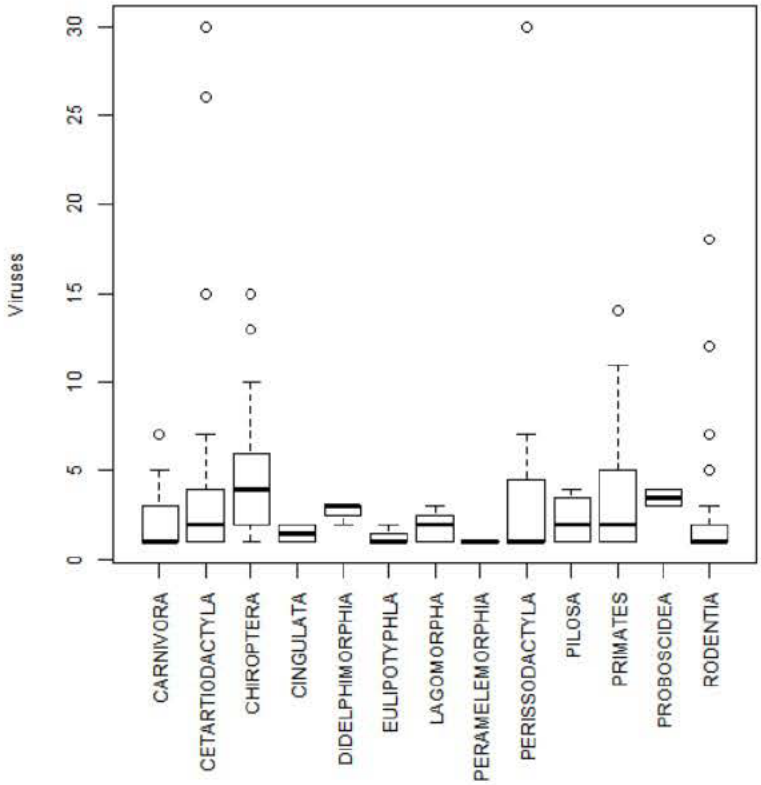


Figure 11. Scatter plots of number of viruses per species against number of total papers published for that species in the 7 taxonomic orders with n>5.

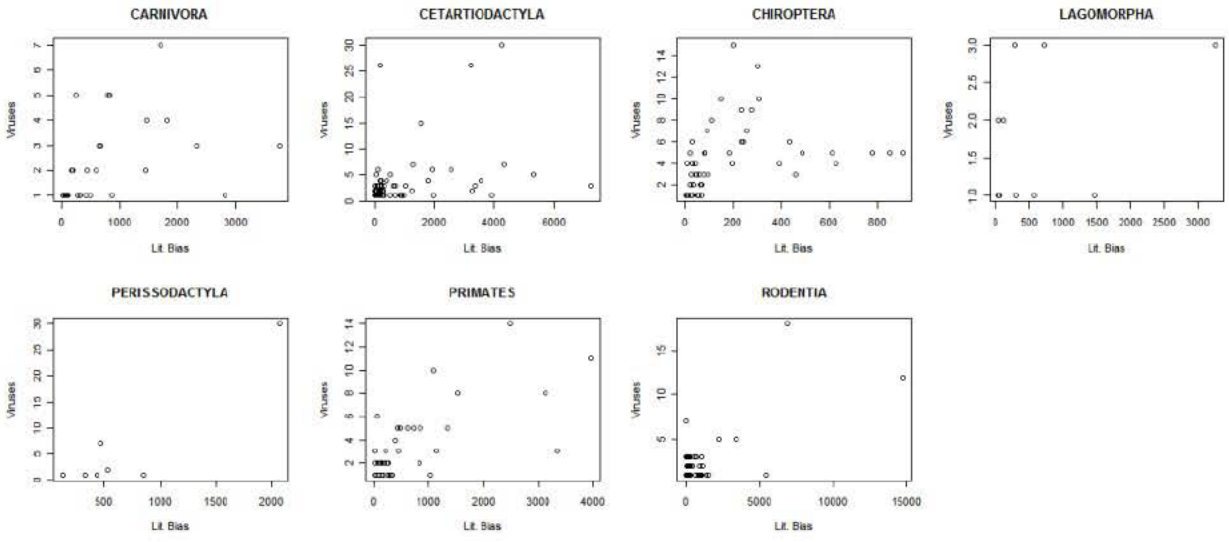


Table 2. Sample sizes, mean viruses and mean bias measures for non-human species in 13 taxonomic orders after data correction/cleaning. SCANDENTIA and DIPROTODONTIA are excluded (see main text).

Order	n	Mean viruses per species	Mean 'Bias' measure per species
CARNIVORA	36	2.03	654.7
CETARTIODACTYLA	62	3.97	1023.2
CHIROPTERA	50	4.42	188.5
CINGULATA	2	1.50	296.0
DIDELPHIMORPHIA	3	2.67	672.0
EULIPOTYPHLA	4	1.25	479.5
LAGOMORPHA	11	1.82	630.2
PERAMELEMORPHIA	2	1.00	166.5
PERISSODACTYLA	7	6.14	685.9
PILOSA	4	2.25	110.3
PRIMATES	39	3.49	693.7
PROBOSCIDEA	2	3.50	2261.5
RODENTIA	68	2.10	729.8

In order to fulfill the assumptions of ANOVA framework models, the response variable needed to a) be normally distributed/parametric, and b) have homogeneity of variance across groups (Orders). Quantile-quantile plots suggested virus richness was not normally distributed, and none of the transformations applied (including log and nth root) changed this (data not shown). Fligner tests showed virus richness did not have homogeneity of variance ($p<0.001$), though log(richness) did ($p=0.235$). The response variable was therefore chosen as log(richness).

Modeling: GLMs were created starting with a saturated model and using a stepwise reduction function. Order was considered to be main effect predictor, and Bias to be covariate. The best model based on AIC score was Model 1, the starting saturated model, which contained an interaction (Table 3).

Table 3. R outputs for ANOVA comparison of models. Model 1 is the saturated model, while Model 2 is the model with a first-step reduction (removal of the Order-Bias interaction).

Model	Resid. df	Resid. Dev	AIC

Model	Resid. df	Resid. Dev	AIC
1. log(Vir)~Order:Bias + Order + Bias	264	116.73	613.07
2. log(Vir)~Order + Bias	276	131.28	623.14

All model components were highly significant (Table 4), giving evidence that known viral richness within a species is dependent upon its taxonomic order, and study effort. The significant interaction suggests the effect of study effort upon discovered virus richness varies between orders.

These results were robust, and held true when using different model families (including Poisson), and when using Disbias (disease-related literature only) instead of Bias (data not shown). Similarly, a Quantile-Quantile plot of the model showed a good fit, indicating robustness against violation of the normality assumption.

Table 4. R output from ANOVA testing of components of the best fitting model (Model 1) using Chi squared tests.

Model Component	Df	Deviance	Resid. df	Resid. Dev	P value
Intercept	-	-	289	184.01	-
Order	12	26.06	277	157.95	3.51E-008
Bias	1	26.67	276	131.28	8.09E-015
Order:Bias	12	14.55	264	116.73	<0.001

Randomization/Bootstrapping: The model was then bootstrapped order to further test robustness of the model. Manly's method of unrestricted permutations for ANOVA (Manly 2007) was selected, as it does not require normally distributed data, and can bootstrap models featuring interactions. 5000 bootstrap resamples were carried out which randomly reassigned each virus richness value observation to a different Order-Bias pair. F statistics were calculated and stored, and compared to F statistics from the true data.

Output probability values of $F_{true,data} < F_{random.data}$ (i.e the chance of randomized data producing stronger associations between virus richness and covariates than the actual data) were as follows-

Order: prob<0.001, Bias: prob<0.001, Order:Bias interaction: prob=0.0026.

Bootstrapping gives much less support for the interaction than previously suggested, though still significant. Again, results show strong robustness.

Posthoc testing: There was strong evidence for an association between taxonomic Order and virus richness per species. To test our hypothesis that Chiroptera have a disproportionate virus richness, posthoc testing was carried out to see which Order-Order comparisons were significant, using Tukey's Honest Significant Difference methods. This also implicitly controls for multiple testing.

Table 5. Tukey's HSD outputs for pairwise comparison of viral richness between taxonomic Orders. For clarity, only those significant or close to significance are displayed, (n=3) excluding most other comparisons (n=75). Estimates are positive if the first Order listed in comparison is > the second Order, and negative if vice-versa.

Comparison	Estimate	Std. Error	z value	P value
CHIROPTERA - CARNIVORA	0.690	0.185	3.736	<0.01
PERISSODACTYLA - CHIROPTERA	-1.214	0.401	-3.026	7.30E-002
RODENTIA - CHIROPTERA	-0.609	0.149	-4.097	<0.01

Chiroptera appeared to significantly carry more viruses than *Carnivora* or *Rodentia* (Fig. 10), and there is weak evidence they also carry more viruses than *Perissodactyla*. However, this must be interpreted very cautiously since Tukey's method does not fully account for the significant Order:Bias interaction. Comparisons between *Chiroptera* and many other Orders with vastly smaller mean virus richness were unexpectedly non-significant, however this is likely due to high uncertainty as these Orders had low sample sizes.

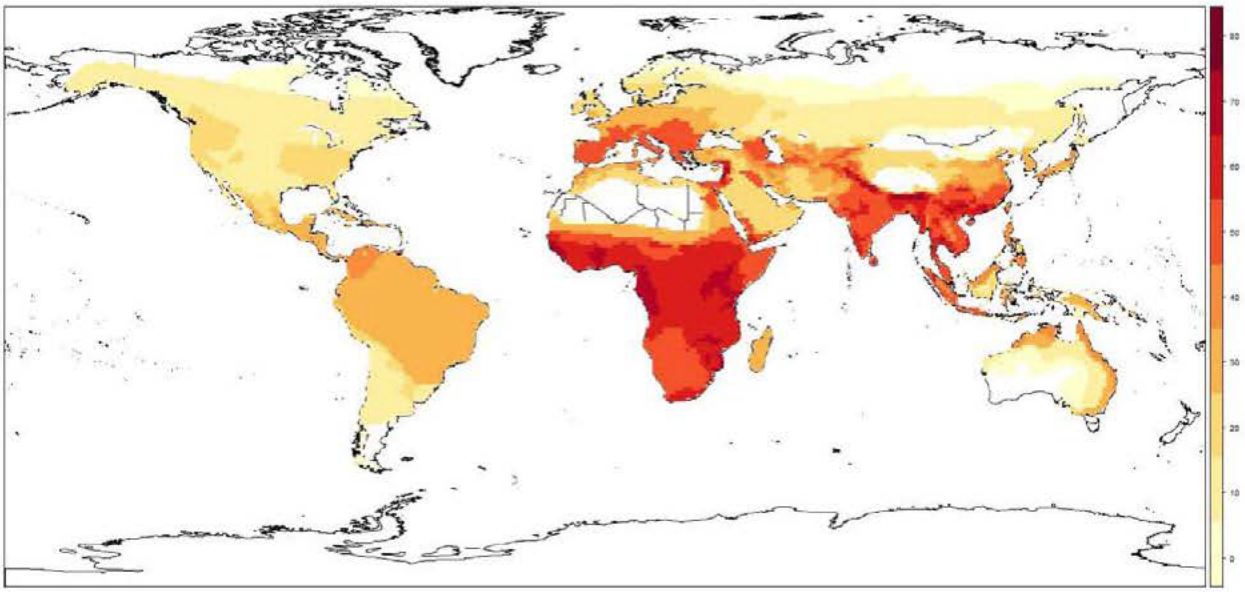
Conclusion: Virus richness per species significantly varied by Order, even after correcting for sampling bias. The influence of literature bias upon discovered virus richness was also significant, but varies between Orders (Order:Bias interaction), the positive relationships suggests intuitively suggested that more we look, the more viruses we find, and in many cases these appear to not be approaching an asymptote. Our results were robust to different bias measures, different model methods and bootstrapping/randomization, and thus provides preemptive evidence that bats have more viruses per species than other Orders.

Mapping global "hotspots" of predicted bat virus diversity

Data on all known unique bat-virus species pairs was be sourced from our bat parasite database (co-PI Vonhof). Entries were corrected to remove redundancy in synonyms by referencing Mammal Species of the World 2005 for host species and ICTVdb Virus Taxonomy, 8th ed for virus species. After correction, the data spanned 179 unique viruses (including substrains/subspecies) across 211 unique host species.

We created proxy maps for the distribution of each virus by assuming it to be present in the entire range of each of its confirmed host species, sourced from IUCN Red List data. Host species ranges were then fused to give a flat single-layered map for each virus. All virus maps (n=179) were overlaid and a species richness algorithm was used to calculate the richness in each 1-degree grid cell on the map (Fig. 12).

Fig. 12. Potential global virus richness within bat hosts.



Within the original dataset, there were a large number of unique *Coronavirus* names, as species/strains were named using different methods, such as references to genetics, place and host species. As these could not be reconciled, an additional map was created that excluded these entries (n=144, Fig. 13). This appeared to comparatively reduce richness in parts of East Asia. A separate map was also created for only those species shared with humans (n=47, Fig. 14), which shows a slightly different pattern: Richness is comparatively lower in Africa and most other areas, whilst richness is comparatively higher in the Americas and South Asia. This could indicate important global differences in emergence of zoonoses from bat reservoirs.

Fig. 13. Potential global virus richness within bat hosts excluding excess *Coronavirus* richness

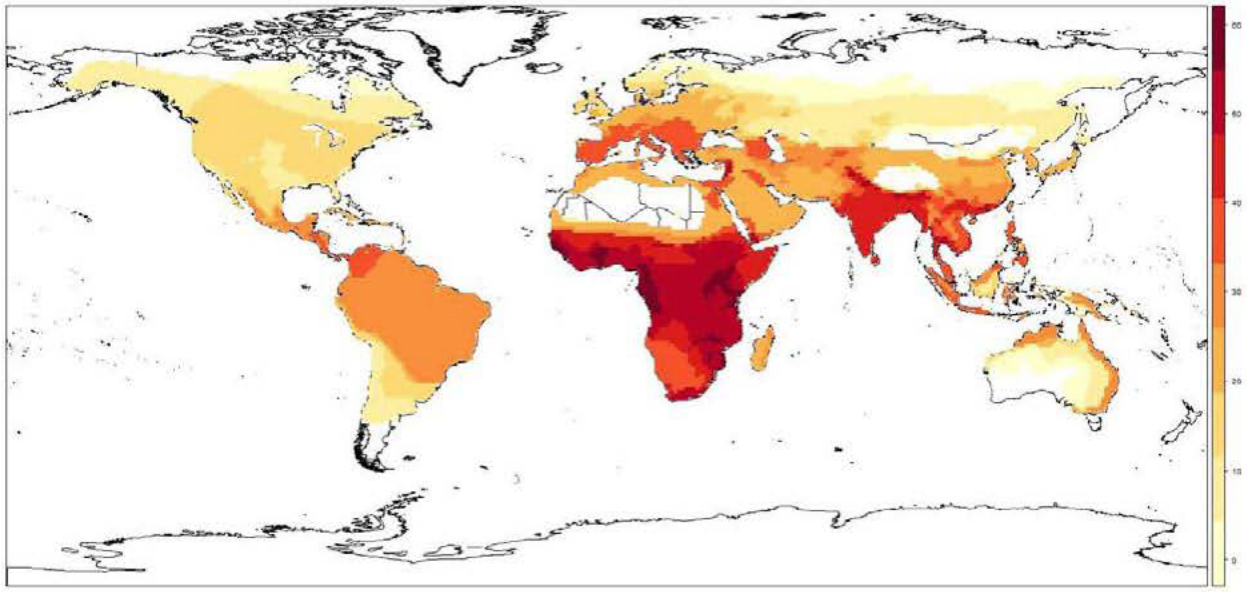
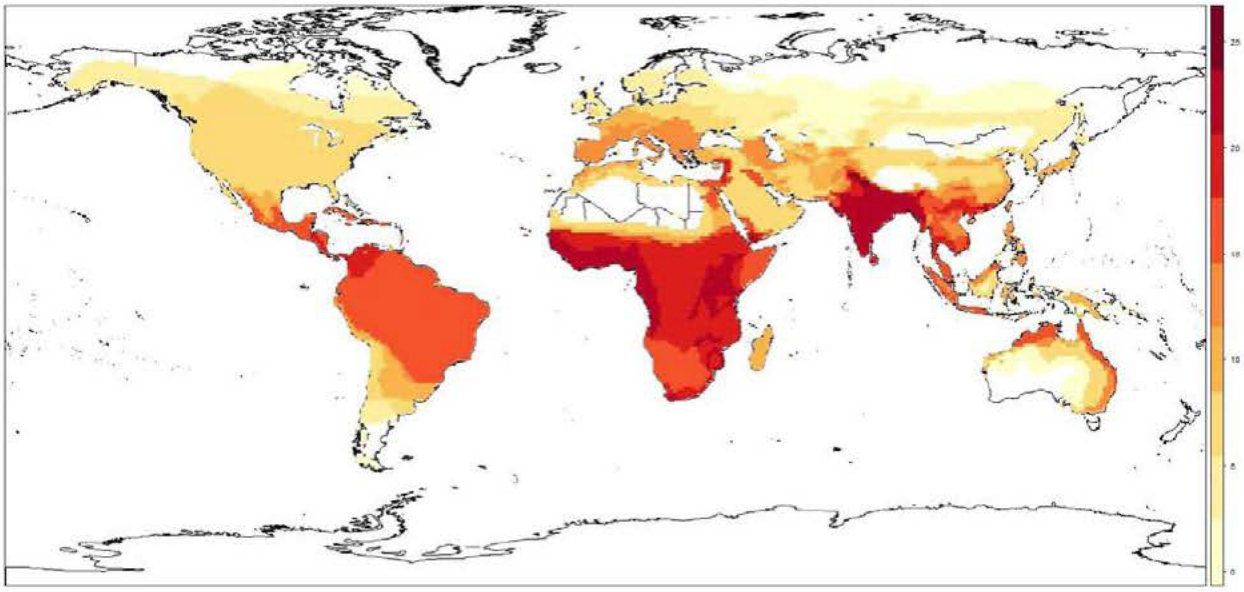


Fig. 14. Potential global virus richness within bat hosts of only those viruses shared with humans.

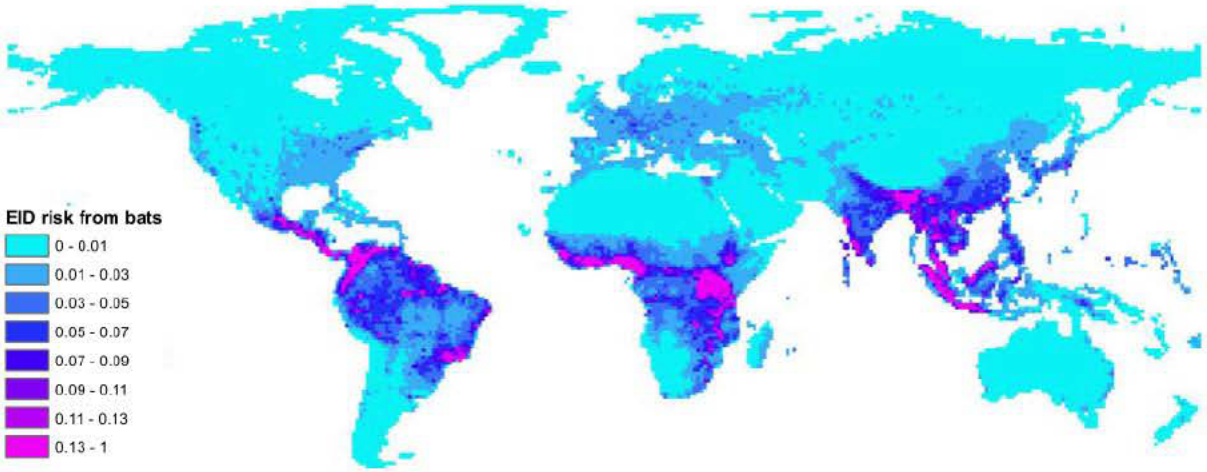


These models will allow us to refine and improve our sampling strategy in Year 4 – allowing us to better target both geographic areas and species for viral discovery during the last two years of our grant. Similarly the new viruses we are now discovering and charactering will be added to our database and will help feed the model with more detailed viral diversity data from each of our focal field sites. We are working to also improve the spatial resolution of these models.

Risk map for human EIDs from bats

We also extracted from the EID database we previously published (Jones et al. 2008), all human emerging infectious disease events associated with a Chiropteran host species (n=17).

To investigate the drivers of the spatial pattern of Chiropteran EID events, we compared the location of the events to socio-economic, environmental and ecological variables matched onto a terrestrial one-degree grid of the globe. Driving factors included human population density, human population growth, latitude, rainfall, and mammal richness. We then repeated the multivariable logistic regression analysis used in the original predicting of global EID hotspots model to construct a Chiropteran EID hotspots risk model, where the presence or absence of EID events was the dependent variable and all drivers plus a measure of spatial reporting bias by country were independent variables. The resulting map provides a visual display of the EID risk scores for Chiropteran-associated EID events on a 1-degree scale.



Expanding the geographic coverage of bat surveillance in Years 4-5

To date, we have sampled bats in nine countries: Bangladesh, Bolivia, China, Colombia, Malaysia, Mexico, Philippines, Thailand, and United States. In order to expand the geographic scope of our bat viral discovery program, we would like to request permission to add 3 countries to our field surveillance in Years 4-5 of this grant, Nigeria, Republic of Congo, and Israel. These three countries were chosen primarily to expand global coverage to areas likely to harbor a high diversity of bat viral pathogens (based on our models) but where knowledge of bat viral diversity is currently lacking or very poorly represented. These are also countries where EcoHealth Alliance has strategic partnerships with local contacts capable of facilitating field surveillance activities.

Adding Nigeria and Republic of Congo will be critical, as we currently have no African countries represented in our global sampling efforts. Further, both of these countries fall in the "hottest" of predicted bat viral hotspot areas in Central-West Africa as shown in the initial risk maps presented in this report. In Nigeria there is also an annual festival where locals hunt large numbers of bats seasonally, and there are some very interesting interactions that could facilitate viral spillover from bats to humans there. In Republic of Congo there is a very high tropical

diversity of bats and a paucity of information on bat viral diversity. In Israel, there have been recent reports of massive die-offs of fruit bats (*Rousettus aegyptiacus*) from an unknown cause. We suspect this could be viral and would like to investigate. Additionally, our data of viral diversity in bats from the Middle East is seriously lacking and we would like to fill in this important gap for our global modeling efforts.

Full details on species we would like to sample and number of individuals are outlined in our IACUC proposal. Briefly, we identified a small number primary species representing the most common bat families (Pteropodidae, Rhinolophidae, Vespertilionidae, Emballonuridae, Molossidae, and Hipposideridae) that we would like to target for our bat viral discovery work in each proposed country. We will target 50-100 individuals per species, with a maximum of 600 animals per species. We will collect a range of samples (blood, tissue, urogenital swab, fecal swab, oral swab) using minimally invasive techniques to maximize the likelihood of finding evidence for viral infection. Representative species for each bat Family are listed in the table below, all species are common and abundant.

	Family	**Representative species from Family**
Nigeria and Republic of Congo	*Pteropodidae*	*Eidolon helvum, Hypsignathus monstrosus, Rousettus aegyptiacus*
	Molossidae	*Chaerephon nigeriae*
	Hipposideridae	*Hipposideros caffer*
	Emballonuridae	*Taphozous mauritianus*
	Vespertilionidae	*Scotophilus dinganii*
	Rhinolophidae	*Rhinolophus clivosus*
Israel	*Pteropodidae*	*Rousettus aegyptiacus* (focus on this species)
	Vespertilionidae	*Myotis myotis*
	Rhinolophidae	*Rhinolophus clivosus*
	Emballonuridae	*Taphozous nudiventris*
	Molossidae	*Tadarida teniotis*

Contact information for our local counterparts are provided below for each country. As with all of our research under this grant, proposed sampling and processing of samples will be in strict

accordance with approved standards for animal safety under our IACUC permit (assurance #: A1250-03) with Tufts University and biosafety (as per the original grant application and re-outlined in this report). We have requested an IACUC amendment for the new countries Nigeria, Israel, and Republic of Congo. The IACUC committee chair will be able to approve these additions without going to a meeting of the IACUC committee. We expect to have news on this within the next 2 months.

Contact information for our local counterparts are provided below for each country.

Nigeria:
Professor Albert B. Ogunkoya,
Department of Veterinary Surgery and Medicine
Ahmadu Bello University, Samaru – Zaria
Kaduna State, Nigeria
Phone: (b) (6)
Email: (b) (6)

Republic of Congo:
Jean-Vivien Mombouli
Director of Research - Ministry of Health
Republic of Congo
Phone: (b) (6)
Email: (b) (6)

Israel:
Dr. Roni King, DVM,
Authority Veterinarian - Science & Conservation Div., Israel Nature and Parks Authority
3 Am Ve`Olamo St., Jerusalem 95463, Israel
Phone: (b) (6)
Email: (b) (6)

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kevin J. Olival	Senior Research Scientist EcoHealth Alliance
eRA COMMONS USER NAME (b) (6)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Colorado State University	B.S.	05/97	Biology
Columbia University	M.A.	10/03	Conservation Biology
Columbia University	Ph.D	05/08	Ecology and Evolutionary Biology
American Museum of Natural History	Post-doc	08/09	Molecular Parasitology

A. Personal Statement

I have been conducting research on bat evolution, ecology, population genetics, and viral discovery for the past 9 years. During my dissertation at Columbia University, I used host population genetics and phylogeography to understand the dynamics of Nipah virus in Southeast Asian fruit bats. As a post-doc at AMNH I discovered several novel malaria parasites in bats, and used molecular systematics to understand the evolution and origins of non-human *Plasmodium*. At AMNH I also developed a new method that used genetic, ecological and species life-history variables to predict viral diversity in bats. As an NIH Global Health Fellow, I expanded our knowledge of Nipah virus ecology in Bangladesh through population genetic analyses of the putative primary reservoir host, *Pteropus giganteus*, and field investigations on role of non-*Pteropus* fruit bats in Nipah virus circulation and zoonotic transmission. I have conducted phylogenetic and molecular evolution analyses of viruses for several studies. I have trained and led field research projects to conduct viral discovery and genetic sampling efforts throughout Asia, including Malaysia, Bangladesh, India, Vietnam, Cambodia, Thailand, and the Philippines. This included several expeditions to collect clinical samples from bats for viral discovery in Bangladesh and Malaysia, and a three-week long Ebola Reston investigation of bats in the Philippines. My latest research is focused on: 1) global viral discovery in bats; 2) integrating phylogenetic and macroecology methods to predict viral spillover in mammals and birds. In summary, the past decade of my research has been focused on the evolution and ecology of bats and their viruses/parasites, and my current focus of using phylogenetic methods to understand viral spillover in bats is highly complementary to the aims in our proposal.

B. Positions and Honors

Positions and Employment

1997-99 Assistant Lab Manager, ForBio Inc., Hawaii
1999-02 Research Associate, Kewalo Marine Lab, University of Hawaii, Honolulu, HI
2002-08 Columbia University, PhD program and EPA STAR Fellow
2008-09 Post-doctoral fellow, American Museum of Natural History, New York, NY
2009-11 NIH Fogarty US Global Health Fellow
2009- Visiting Research Scientist, American Museum of Natural History, New York, NY
2009- Adjunct Faculty, Columbia University, New York, NY

Other Experience and Professional Memberships

2000-02 Mentor, NSF Undergraduate Mentoring in Environmental Biology (UMEB) for Pacific Islander undergraduates, University of Hawaii
2002-08 Research Collaborator, Consortium for Conservation Medicine, New York
2003- Member, Henipavirus Ecology Research Group
2003 Lecturer in Disease Ecology, Columbia University Continuing Education course

2003-08 Visiting researcher – bat genetics, Veterinary Research Institute, Malaysia
2005 Visiting researcher – bat genetics, Institute for Ecology and Biological Resources, Vietnam
2005 Visiting researcher – bat genetics, Pasteur Institute, Cambodia
2005 Judge, NY Science and Engineering Fair
2006-07 Mentor, Conservation Genetics High School Internship Program, AMNH, New York
2006-10 Instructor, Columbia University Secondary School Summer Program, New York
2007 Steering Committee, Small Matters: Microbes and Their Role in Conservation, New York
2007 Symposium Organizer, Bat Hunting and Bushmeat, Phuket, Thailand
2009 Symposium Organizer, Bats and Disease, North American Symposium on Bat Research, Portland
2009 Symposium Organizer, Bat migration and disease, 1st Int'l Workshop on Bat Migration, Germany
2009 Organizer and Scientific Review Committee, Exploring the Dynamic Relationship Between Health and the Environment, AMNH Spring Symposium, New York
2009- Review Editor, *EcoHealth*
2010 Mentor and Scientific Review Committee, Student Conference on Conservation Science New York
2010- Key Personnel and Lead Country Liaison: Thailand, Bangladesh, and Vietnam - USAID PREDICT
2010- Lead Field Researcher, FAO-EHA investigation of Ebola Reston reservoirs in Philippines

Honors

1993-97 Colorado State University Distinguished Scholar Award
2003 NSF Graduate Student Fellowship, Honorable Mention
2005-07 Bat Conservation International Student Award and Scholarship
2007 Best Student Presentation, 1st International Southeast Asian Bat Conference, Phuket, Thailand
2004-07 US Environmental Protection Agency STAR Fellowship Award
2008 PhD Dissertation with Distinction, Columbia University

C. Peer-reviewed publications

Most relevant to the current application

1. Pulliam, JRC, H Field, **KJ Olival**, and HERG (2005). An alternative explanation of Nipah virus strain variation. *Emerging Infectious Diseases* 11(12): 1978-1979.
2. Daszak, P, R Plowright, JH Epstein, JH Pulliam, SA Rahman, HE Field, CS Smith, **KJ Olival**, S Luby, K Halpin, AD Hyatt, and H.E.R.G. (2006). The emergence of Nipah and Hendra virus: pathogen dynamics across a wildlife-livestock-human continuum. In: *Disease Ecology: Community structure and pathogen dynamics*, S. Collinge and C. Ray, Editors. Oxford University Press: Oxford. pp. 188-203.
3. Turmelle, A and **KJ Olival**. (2009). Correlates of viral richness in bats (Order Chiroptera). *EcoHealth* 6(4): 522-539.
4. Rahman, SA, SS Hassan, **KJ Olival**, M Mohamed, L-Y Chang, L Hassan, NM Saad, SA Shohaimi, ZC Mamat, MS Naim, JH Epstein, AS Suri, HE Field, P Daszak and HERG (2010). Characterization of Nipah virus from Naturally Infected *Pteropus vampyrus* Bats, Malaysia. *Emerging Infectious Disease* 16(12): 1990-1993.
5. (b) (4)

Additional recent publications of importance to the field (in chronological order)

1. Hadfield, MG, BS Holland, and **KJ Olival**. (2004) Contributions of *ex situ* propagation and molecular genetics to conservation. In: *Experimental approaches to conservation biology*, M.S. Gordon and S.M. Bartol, Editors. University of California Press: Berkeley. pp. 16-34.
2. **Olival, KJ** and H Higuchi. (2006) Monitoring the long-distance movement of wildlife in Asia using satellite telemetry. In: *Conservation Biology in Asia*, J McNeely, et al., editors. Society for Conservation Biology Asia Section and Resources Himalaya Foundation: Kathmandu, Nepal. pp. 319-339.

3. **Olival, KJ** and P Daszak (2005). The ecology of emerging neurotropic viruses. *Journal of NeuroVirology* 11: 440-445.
4. **Olival, KJ**, EO Stiner, and SL Perkins. (2007). Detection of *Hepatocystis sp.* in Southeast Asian Flying Foxes (Pteropodidae) using Microscopic and Molecular Methods. *Journal of Parasitology* 93(6): 1538-1540.
5. Epstein, JH, **KJ Olival**, JRC Pulliam, CS Smith, J Westrum, T Hughes, A Dobson, A Zubaid, SA Rahman, M Basir, HE Field, and P Daszak (2009). Management of *Pteropus vampyrus,* a hunted migratory species with a multinational home-range. *Journal of Applied Ecology* 46(5): 991-1002.
6. Murdock, C, **KJ Olival**, and SL Perkins. (2010). "Feeding preference of snow-melt mosquitoes (Culicidae: *Culiseta* and *Ochelerotatus*) show a link between cervid amplifying hosts for Jamestown Canyon Virus (Bunyaviridae: *Orthobunyavirus*) and humans." *Journal of Medical Entomology* 47(2): 226-229.
7. Khan SA, Epstein JH, **Olival KJ**, Hassan MM, Hossaini MB, Rahman KBMA, Elahi MF, Mamun MA, Haider N, Yasin G, Desmond J (2011). Hematology and serum chemistry reference values of stray dogs in Bangladesh. *Open Veterinary Journal* 1:13-20.
8. Smith, CS, JH Epstein, A Breed, R Plowright, **KJ Olival**, C de Jong, P Daszak and HE Field (2011). Satellite Telemetry and Long-Range Bat Movements. *PloS One* 6(2): e14696.
9. Bogich, T.L., Mazet, J. Morse, S., Karesh, W., Jones, K.E., Levy, M., Funk. S., Brito, I., **Olival, K.J.**, Hosseini, P., Epstein, J., Brownstein, J., Joly, D., and Daszak, P. (In Press) Using Mathematical Models in a Unified Approach to Predicting the Next Emerging Infectious Disease. In: *Conservation Medicine* 2nd Ed., A.A. Aguirre, R.S. Ostfeld and P. Daszak, Oxford University Press.
10. (b) (4)

D. Research Support
Ongoing Research Support

NIH 3R01TW005869-06S1 Daszak (PI) 09/01/09 – 8/31/11
NIH Ecology of Infectious Diseases (Fogarty International Center)
The Ecology, Emergence and Pandemic Potential of Nipah virus in Bangladesh – ARRA Supplement
To conduct fieldwork and phylogeographic analyses to understand the dynamics of Nipah virus in non-*Pteropus* bats in Bangladesh and model the geneflow and connectivity of known *Pteropus* bat reservoirs.
Role: US Global Health Fellow & Lead on ARRA research

1 R01AI079231 Daszak (PI) 09/18/08 – 08/31/13
NIAID Non-Biodefense Emerging Infectious Diseases
Risk of viral emergence from bats.
This project is to model hotspots for viral diversity and emergence in bats, to identify new viruses from bats, and to examine the pathogenicity and infectiousness for these novel pathogens.
Role: Key Personnel: study design, project implementation, and modeling

USAID EPT PREDICT Daszak (PI) 10/01/09 – 09/30/14
Modeling hotspots for disease emergence and conducting surveillance in wildlife in hotspots for new emerging zoonoses
Role: Key Personnel: Modeling disease risk and managing projects in Asian countries

Grant Number: 5R01AI079231-05

Principal Investigator(s):
PETER DASZAK, PHD

Project Title: Risk of Viral Emergence from Bats

Aleksei A. Chmura
Wildlife Trust
460 W 34th Street
17th Floor
New York, NY 10001

Award e-mailed to: (b) (6)

Budget Period: 09/01/2012 – 08/31/2013
Project Period: 09/18/2008 – 08/31/2013

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $518,980 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ECOHEALTH ALLIANCE, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI079231. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael A. Wright
Grants Management Officer

Additional information follows

SECTION I – AWARD DATA – 5R01AI079231-05

Award Calculation (U.S. Dollars)

Federal Direct Costs	$474,751
Federal F&A Costs	$44,229
Approved Budget	$518,980
Federal Share	$518,980
TOTAL FEDERAL AWARD AMOUNT	$518,980
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$518,980

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$518,980	$518,980

Fiscal Information:

CFDA Number:	93.855
EIN:	1311726494A1
Document Number:	RAI079231A
Fiscal Year:	2012

IC	*CAN*	*2012*
AI	8472302	$518,980

NIH Administrative Data:
PCC: M34A / **OC**: 414E / **Processed**: (b) (6) 08/07/2012

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI079231-05

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI079231-05

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award is not subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. Therefore, see the NIH Grants Policy Statement Section 8.6 Closeout for closeout requirements at: http://grants.nih.gov/grants/policy/#gps .

A final Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted through the eRA Commons (Commons) within 90 days of the expiration date.

Furthermore, unless an application for competitive renewal is submitted, a final progress report must also be submitted within 90 days of the expiration date. Institute/Centers may accept the progress report contained in competitive renewal (type 2) in lieu of a separate final progress report. Contact the awarding IC for IC-specific policy regarding acceptance of a progress report contained in a competitive renewal application in lieu of a separate final progress report.

NIH strongly encourages electronic submission of the final progress report and the final invention statement through the Closeout feature in the Commons. If the final progress report and final invention statement are not submitted through the Commons, a copy can be emailed or sent to the contacts listed below. Copies of the HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm.

Submissions of the final progress report and HHS 568 may be e-mailed as PDF attachments to the NIH Central Closeout Center at: DeasCentralized@od.nih.gov.

Paper submissions of the final progress report and the HHS 568 may be faxed to the NIH Central Closeout Center at 301-480-2304 or mailed to the NIH Central Closeout Center at the following address:

NIH/OD/OER/DEAS
Central Closeout Center
6705 Rockledge Drive, Room 2207
Bethesda, MD 20892-7987 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express mail delivery only)

The final progress report should include, at a minimum, a summary of progress toward the achievement of the originally stated aims, a list of significant results (positive and/or negative), a list of publications and the grant number. If human subjects were included in the research, the final progress report should also address the following:

Report on the inclusion of gender and minority study subjects (using the gender and minority Inclusion Enrollment Form as provided in the PHS 2590 and available at http://grants.nih.gov/grants/forms.htm).

Where appropriate, indicate whether children were involved in the study or how the study was relevant for conditions affecting children (see NIH Grants Policy Statement Section 4.1.15.7 Inclusion of Children as Subjects in Clinical Research at URL http://grants.nih.gov/grants/policy/#gps).

Describe any data, research materials (such as cell lines, DNA probes, animal models), protocols, software, or other information resulting from the research that is available to be shared with other investigators and how it may be accessed.

Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the final progress report.

Note, if this is the final year of a competitive segment due to the transfer of the grant to another institution, then not all the requirements stated above are applicable. Specifically a Final Progress Report is not required. However, a final FFR is required and should be submitted electronically as noted above. In addition, if not already submitted, the Final Invention Statement is required and should be sent directly the assigned Grants Management Specialist.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI079231-05

This award is issued in accordance with the FY2012 NIH fiscal policies described in NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-036.html.

INFORMATION ONLY: None of the funds in this award shall be used to pay the salary of an individual at a rate per year in excess of the amounts reflected in the following NIH Guide Notice: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-035.html .
****

No studies, work or any Federal funds can be expended in the countries of NIgeria, Republic of Congo and Israel until approved.
****

This award includes funds awarded for consortium activity with the Trustees of Columbia University. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

This award includes funds awarded for consortium activity with Australian Animal Health Laboratory/AUSTRALIA. Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants1.nih.gov/grants/policy/nihgps_2003/NIHGPS_Part12.htm#_Toc54600251, pages 224-227.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Michael A. Wright
Email: mawright@mail.nih.gov **Phone**: 301-451-2688 **Fax**: 301-493-0597

Program Official: Eun-chung Park
Email: epark@niaid.nih.gov **Phone**: 301-496-7453 **Fax**: 301-480-1594

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI079231-05

Facilities and Administrative Costs	*Year 5*
F&A Cost Rate 1	26.1%
F&A Cost Base 1	$169,458
F&A Costs 1	$44,229

EcoHealth Alliance

22 June 2012

Dr. Eun-Chung Park
Virology Branch, DMID, NIAID, NIH
6610 Rockledge Dr., Rm 4103
Bethesda MD 20892-7630

Dear Dr Park,

Please find our Year 4 progress report for grant #5R01AI079231, attached. Please let me know, if there is any other information you need. All details have been uploaded and submitted via the eSNAP system in eRA Commons.

Thanks again for your advice and guidance,
Yours sincerely,

Dr Peter Daszak
President, EcoHealth Alliance
460 West 34th St.
17th Floor
New York, NY. 10001
(e) (b) (6)
(t) (b) (6)

OMB No. 0925-0001

Grant Number R01AI079231-05	**Total Project Period** From: 09/18/2008 To: 08/31/2013
EIN: **Review Group:** 1311726494A1 IRAP	**Requested Budget Period:** From: 09/01/2012 To: 08/31/2013
Title of Project: Risk of Viral Emergence from Bats	**Due Date:** 07/16/2012 **Submitted Date:** 06/29/2012

Program Director/Principal Investigator: PETER DASZAK 460 West 34th Street New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** **Email Address:** (b) (6)	**Applicant Organization:** ECOHEALTH ALLIANCE, INC. ECOHEALTH ALLIANCE, INC. 460 W 34TH ST 17TH FLOOR NEW YORK , NY 100012317 **Department:** **Major Subdivision:**
Administrative Official: Aleksei Chmura 460 W 34th St., 17th Floor New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** 1.212.380.4465 **Email Address:** (b) (6)	**Signing Official:** Aleksei Chmura 460 W 34th St., 17th Floor New York , NY 10001 **Phone Number:** (b) (6) **Fax Number:** 1.212.380.4465 **Email Address:** (b) (6)
Human Subjects: ☒ No ☐ Yes **Research Exempt:** ☒ No ☐ Yes **Exemption No:** **FWA Number:** **Phase III Clinical Trial:** ☒ No ☐ Yes	**Vertebrate Animals:** ☐ No ☒ Yes **Animal Assurance Number:** **Inventions and Patents:** ☒ No ☐ Yes ☐ **Previously Reported** ☒ **Not Previously Reported**
Program Income: ☒ No ☐ Yes	

Budget Period	Anticipated Amount	Source
F&A Changes:		

Primary Project/Performance Site Location		
Organizational Name: ECOHEALTH ALLIANCE		
DUNS: 077090066		
Street 1: ECOHEALTH ALLIANCE, INC.	Street 2: 460 W 34TH ST	
City: NEW YORK	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 100012317
Congressional Districts: 08		

Additional Project/Performance Site Location		
Organizational Name: The Trustees of Columbia University		
DUNS: 621889815		
Street 1: 630 West 168th Street	Street 2: Box 49	
City: New York	County:	State: NY
Province:	Country: UNITED STATES	Zip/Postal Code: 10032
Project/Performance Site Congressional Districts: 15		

Additional Project/Performance Site Location		
Organizational Name: The Institute of Zoology		
DUNS: 227012275		
Street 1: Regent's Park	Street 2:	
City: London	County:	State:
Province:	Country: UNITED KINGDOM	Zip/Postal Code: NW14RY
Project/Performance Site Congressional Districts: 0		

Additional Project/Performance Site Location		
Organizational Name: Australian Animal Health Laboratory (AAHL)		
DUNS: 754307957		
Street 1: 5 Portarlington Road	Street 2:	
City: East Geelong	County:	State:
Province:	Country: AUSTRALIA	Zip/Postal Code: VIC 3219
Project/Performance Site Congressional Districts: 0		

Additional Project/Performance Site Location		
Organizational Name: International Centre for Diarrhoeal Disease Research, Bangladesh		
DUNS: 731524711		
Street 1: GPO 128	Street 2:	
City: Dhaka	County:	State:
Province:	Country: BANGLADESH	Zip/Postal Code: 1212
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: East China Normal University		
DUNS: 420945495		
Street 1: 3663 Zhongshan Beilu	Street 2:	
City: Shanghai	County:	State:
Province:	Country: CHINA	Zip/Postal Code: 200062
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Universidad Mayor de San Simon		
DUNS: 950009451		
Street 1: Centro de Biodiversidad y Genetica	Street 2: PO Box 538	
City: Cochabamba	County:	State:
Province:	Country: BOLIVIA	Zip/Postal Code:
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Fundacion Universitaria San Martin		
DUNS: 880303115		
Street 1: Kra 10 No 128-70 Torre 1	Street 2: apto 504	
City: Bogota	County:	State:
Province:	Country: COLOMBIA	Zip/Postal Code:
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Veterinary Research Institute		
DUNS: 000000000		
Street 1: D203, Block D, Paradesa Tropika	Street 2: Persiaran Meranti, Bandar Sri Damansara	
City: Kuala Lumpur	County:	State:
Province:	Country: MALAYSIA	Zip/Postal Code: 52200
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Universidad Nacional Mayor de San Marcos		
DUNS: 934842774		
Street 1: Museo de Historia Nacional	Street 2: Peruvian Wildlife Department, Apto 140434	
City: Lima	County:	State:
Province:	Country: PERU	Zip/Postal Code: 14
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location		
Organizational Name: Department of Veterinary Medicine		
DUNS: 000000000		
Street 1: Bureau of Animal Industry & FAO	Street 2: Eliptical Road, Quezon City	
City: Manila	County:	State:
Province:	Country: PHILIPPINES	Zip/Postal Code:
Project/Performance Site Congressional Districts: 00-000		

Additional Project/Performance Site Location			
Organizational Name: Western Michigan University			
DUNS: 062230560			
Street 1: 3431 Wood Hall		Street 2:	
City: Kalamazoo	County:		State: MI
Province:	Country: UNITED STATES	Zip/Postal Code: 49008	
Project/Performance Site Congressional Districts: 06			

Program Director/Principal Investigator:	Grant Number
PETER DASZAK	R01AI079231-05
Applicant Organization:	**Period Covered by this Report:**
ECOHEALTH ALLIANCE, INC.	09/01/2011 - 08/31/2012

Title of Project:

Risk of Viral Emergence from Bats

SNAP Questions:

Has there been a change in the other support of Senior/Key Personnel since the last reporting period?

☒ **No** ☐ **Yes**

Justification:

Will there be, in the next budget period, a significant change in the level of effort for the PD/PI or other Senior/Key Personnel designated on the Notice of Award from what was approved for this project?

☒ **No** ☐ **Yes**

Justification:

Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

☒ **No** ☐ **Yes**

Justification:

Changes in Select Agent Research? ☒ **No** ☐ **Yes**

Changes in Multiple PD/PI Leadership plan? ☒ **No** ☐ **Yes**

Change in human embryonic stem cell (hESC) line(s) used? ☒ **No** ☐ **Yes**

Justification:

Human Subject Education Requirement:

Has the Involvement of Human Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Has the Involvement of Animal Subjects changed since previous submission? ☒ **No** ☐ **Yes**

Publications:

Valid NIHMSID:	Citation ID:	Citation Source:	Citation Text:

All Personnel Report						
Program Director/Principal Investigator: PETER DASZAK			**Grant Number** R01AI079231-05			
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
PETER DASZAK	(b) (6)	PHD, BS	(b) (6)	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
PD/PI			(b) (6)	(b) (4), (b) (6)		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Thomas Briese		PHD	XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Jonathan H Epstein	(b) (6)	DVM	(b) (6)	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				(b) (4), (b) (6)		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Kate Elizabeth Jones		PHD	XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				(b) (4), (b) (6)		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
W. Ian Lipkin		PHD	XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-Investigator				(b) (4), (b) (6)		
Name:	**Commons ID:**	**Degree(s) Name:**	**SSN:**			
Bruce Andrew Mungall		PHD	XXX-XX-	**Months Devoted**		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**

Co-PD/PI				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Kevin J. Olival		PhD	(b) (6)	Months Devoted		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Staff scientist (Doctoral level)				(b) (4), (b) (6)		

Name:	Commons ID:	Degree(s) Name:	SSN:			
Gustavo F. Palacios		PHD	XXX-XX-	Months Devoted		
				to Project		
Role on Project:	**Supplement Support:**		**DoB:** (MM/YY)	**Cal**	**Acad**	**Sum**
Co-PD/PI				(b) (4), (b) (6)		

NIH-NIAID Progress report summary, June 2011- June 2012 (Year 4)

Grant Number: 5R01AI079231-04

Project Title: Risk of Viral Emergence from Bats

PI: Dr. Peter Daszak

Institution: EcoHealth Alliance

28 June 2012

Summary of Past 12 months:

Targeted sample collection for bat viral discovery

- We have collected over 10,739 samples from 89 bat species globally.
- Laboratory pathogen screening for a majority of samples is completed or currently underway, we have run thousands viral family-level PCRs this past year to identify non-biodefense pathogens.
- We continue to target our field sampling to include a large ecological and taxonomic diversity of bat taxa, e.g. sampling in Bolivia and Mexico has yielded including 51 species from 4 bat Families and over 2,200 samples.
- We are examining patterns of host-virus coevolution and viral diversification with this diverse dataset, with special focus on testing host characteristics that increase the likelihood of cross-species transmission of bat viruses.

Bat Viral Discovery and 'Deep Sampling'– Bangladesh Case Study

- Deep sampling has proven invaluable to ascertain the true extent of previously unknown bat viral diversity for known viral families.
- We have run over 16,000 PCR assays for 12 viral families, and discovered 63 putative novel viruses in Bangladesh alone.
- Non-bat viruses were detected in fruit bats as part of this study, including both avian and bovine coronaviruses.

Viral Discovery Curves – assessing total known and unknown viral diversity

- Viral discovery curves were used to examine the relationship between sampling effort and novel virus discovery for each different sample type and to determine how many samples are required to estimate total number of viruses in a species or sample type.
- When comparing our results of known viruses per host to those in the literature, we suggest that viral species richness in bats has been massively underestimated.
- Using this approach, we have identified 63 novel viruses that include 11 new Paramyxoviruses and 28 new Adenoviruses.

Other Research Highlights from Specific Countries

- In Thailand, we have continued active bat surveillance at high-risk interfaces and viral discovery using 454 next generation sequencing. Also, four samples collected from guano caves have resulted positive for Coronaviruses (CoVs), from two divergent viral groups.

- In Malaysia we have screened serum and fecal samples from 18 bats at CII using next-generation-sequencing (Illumina), with analysis and results underway.
- In China, we have tested over 300 samples using degenerate PCR protocols for three viral families, yielding the discovery of a new clade distinct but related to the SARS-like CoVs; and three novel bat Paramyxoviruses.
- In the United States (Hawaii and Puerto Rico), two new projects were developed. The first one, to assess how isolation of a species on islands and colonization bottlenecks affect viral diversity and diversification in bats. The second to assess what factors influence the degree of pathogen sharing in bats within one multi-species ecological community.

Predicting Cross-Species Transmission of Bat Viruses

- Using a logistic generalized linear model, we identified significant host and virus traits that predict whether or not a virus will be shared among bat species.
- Results showed that phylogenetic distance between bat host species is a strong predictor of viral sharing.
- Primates, rodents, bats and lagomorphs have significantly higher proportions of viruses shared with humans than other mammal Orders.

Modeling Bat Virus Spillover in Humans

- We have focused on integrating other global disease drivers and investigate explanatory power of anthropogenic activity upon the spatial pattern of virus sharing between humans and other animals.
- Using outputs from our hotspot model, we are exploring geographic areas likely to be most cost-effective in yielding novel bat viruses with devoted sampling efforts.

Modeling Risk of Pandemic Emergence of Bat Viruses Using Travel Data

- We mapped global pandemic risk from the emergence of all direct- and vector-transmitted zoonotic viruses using airline travel data, zoonotic disease hotspot risk maps, and per capita health care. We are currently refining these analyses to examine the pandemic risk for bat-borne zoonotic viruses alone.

Modeling Future Species Distributions of Bats and their Viruses

- Utilizing Ecological Niche Models and GCMs we modeled the future geographic change in bat species distribution and their associated viruses, with Paramxyoviruses as a case study. This work is ongoing.

Training and Outreach for Conducting Bat Virus Research

- To date, we have trained >230 people in-country in methods of bat capture, safe handling, species identification, proper use of Personal Protective Equipment (PPE), and minimally-invasive sample collection.
- In May 2012 we held a large hands-on training in Central Thailand for 32 people, which included veterinary and forest students from three universities in Thailand, staff and veterinarians from the Department of National Parks Thailand.
- EcoHealth Alliance with FAO co-edited and published a volume on best practices for investigating zoonoses from bats which is freely available online.

NIH-NIAID Progress report, June 2011- June 2012 (Year 4)

Grant Number: 5R01AI079231-04

Project Title: Risk of Viral Emergence from Bats

PI: Dr. Peter Daszak

Institution: EcoHealth Alliance

28 June 2012

Summary of Past 12 months:

Targeted sample collection for bat viral discovery

- We have collected over 10,739 samples from 89 bat species globally.
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- We are examining patterns of host-virus coevolution and viral diversification with this diverse dataset, with special focus on testing host characteristics that increase the likelihood of cross-species transmission of bat viruses.

Bat Viral Discovery and 'Deep Sampling'– Bangladesh Case Study

- Deep sampling has proven invaluable to ascertain the true extent of previously unknown bat viral diversity for known viral families.
- We have run over 16,000 PCR assays for 12 viral families, and discovered 63 putative novel viruses in Bangladesh alone.
- Non-bat viruses were detected in fruit bats as part of this study, including both avian and bovine coronaviruses.

Viral Discovery Curves – assessing total known and unknown viral diversity

- Viral discovery curves were used to examine the relationship between sampling effort and novel virus discovery for each different sample type and to determine how many samples are required to estimate total number of viruses in a species or sample type.
- When comparing our results of known viruses per host to those in the literature, we suggest that viral species richness in bats has been massively underestimated.
- Using this approach, we have identified 63 novel viruses that include 11 new Paramyxoviruses and 28 new Adenoviruses.

Other Research Highlights from Specific Countries

- In Thailand, we have continued active bat surveillance at high-risk interfaces and viral discovery using 454 next generation sequencing. Also, four samples collected from guano caves have resulted positive for Coronaviruses (CoVs), from two divergent viral groups.

- In Malaysia we have screened serum and fecal samples from 18 bats at CII using next-generation-sequencing (Illumina), with analysis and results underway.
- In China, we have tested over 300 samples using degenerate PCR protocols for three viral families, yielding the discovery of a new clade distinct but related to the SARS-like CoVs; and three novel bat Paramyxoviruses.
- In the United States (Hawaii and Puerto Rico), two new projects were developed. The first one, to assess how isolation of a species on islands and colonization bottlenecks affect viral diversity and diversification in bats. The second to assess what factors influence the degree of pathogen sharing in bats within one multi-species ecological community.

Predicting Cross-Species Transmission of Bat Viruses

- Using a logistic generalized linear model, we identified significant host and virus traits that predict whether or not a virus will be shared among bat species.
- Results showed that phylogenetic distance between bat host species is a strong predictor of viral sharing.
- Primates, rodents, bats and lagomorphs have significantly higher proportions of viruses shared with humans than other mammal Orders.

Modeling Bat Virus Spillover in Humans

- We have focused on integrating other global disease drivers and investigate explanatory power of anthropogenic activity upon the spatial pattern of virus sharing between humans and other animals.
- Using outputs from our hotspot model, we are exploring geographic areas likely to be most cost-effective in yielding novel bat viruses with devoted sampling efforts.

Modeling Risk of Pandemic Emergence of Bat Viruses Using Travel Data

- We mapped global pandemic risk from the emergence of all direct- and vector-transmitted zoonotic viruses using airline travel data, zoonotic disease hotspot risk maps, and per capita health care. We are currently refining these analyses to examine the pandemic risk for bat-borne zoonotic viruses alone.

Modeling Future Species Distributions of Bats and their Viruses

- Utilizing Ecological Niche Models and GCMs we modeled the future geographic change in bat species distribution and their associated viruses, with Paramxyoviruses as a case study. This work is ongoing.

Training and Outreach for Conducting Bat Virus Research

- To date, we have trained >230 people in-country in methods of bat capture, safe handling, species identification, proper use of Personal Protective Equipment (PPE), and minimally-invasive sample collection.
- In May 2012 we held a large hands-on training in Central Thailand for 32 people, which included veterinary and forest students from three universities in Thailand, staff and veterinarians from the Department of National Parks Thailand.
- EcoHealth Alliance with FAO co-edited and published a volume on best practices for investigating zoonoses from bats which is freely available online.

FULL REPORT, 5R01A1079231-04 NIAID, RISK OF VIRAL EMERGENCE FROM BATS

Targeted sample collection for bat viral discovery:

In Year 4 we continued to strategically collect samples from bats globally for viral discovery and to inform our hotspot modeling. All countries from which samples were collected have been approved for work under this NIAID award and our existing IACUC protocols. We continue to use field-sampling protocols for viral discovery that are minimally invasive and non-lethal. Our strategic and targeted sampling strategy includes careful taxonomic (maximizing higher-level taxonomic diversity of bats), geographic (to inform hotspot and risk models), and logistic (where approval to work has been granted and local capacity-building can be maximized) considerations. **In the past 12 months, we have collected over 10,000 samples from ~90 bat species globally (Table 1).** Laboratory pathogen screening for a majority of these samples is completed or currently underway. Over 10,000 PCRs screening samples using viral family-level protocols have been run.

Table 1. Summary of number of bat species and samples collected in last 12 months by country (July 2011-July 2012).

Country	# species	# samples
Bangladesh	4	6,748
Bolivia	32	1,108
China	19	362
Malaysia	10	460
Mexico	21	1,115
Thailand	10	691
USA*	7	255
Total	**89**	**10,739**

*USA includes Hawaii and Puerto Rico.

Bolivia and Mexico: Examples of Targeted and Diverse Sampling

As an example of our targeted approach to sampling a wide-diversity of bats, we present taxonomic data from field surveillance at two of our Latin American research sites (Bolivia and Mexico) below. The past 12 months of sampling has yielded a diverse set of bat taxa, including 51 species from 4 bat Families and over 2,200 samples from these two countries. This dataset includes robust samples sizes for some species (~250+ samples) i.e. deep sampling of particular species, as well as smaller numbers of samples from a wide diversity of species.

In both countries we have targeted species with different ecologies (frugivorous, insectivorous, sanguivorous), morphologies, and phylogenetic relatedness (Figures 1-2). This ecological and taxonomic diversity will allow us to examine patterns of host-virus coevolution and viral diversification across a large percentage of New World bat species, and in particular test host characteristics that increase the likelihood of cross-species transmission of bat viruses.

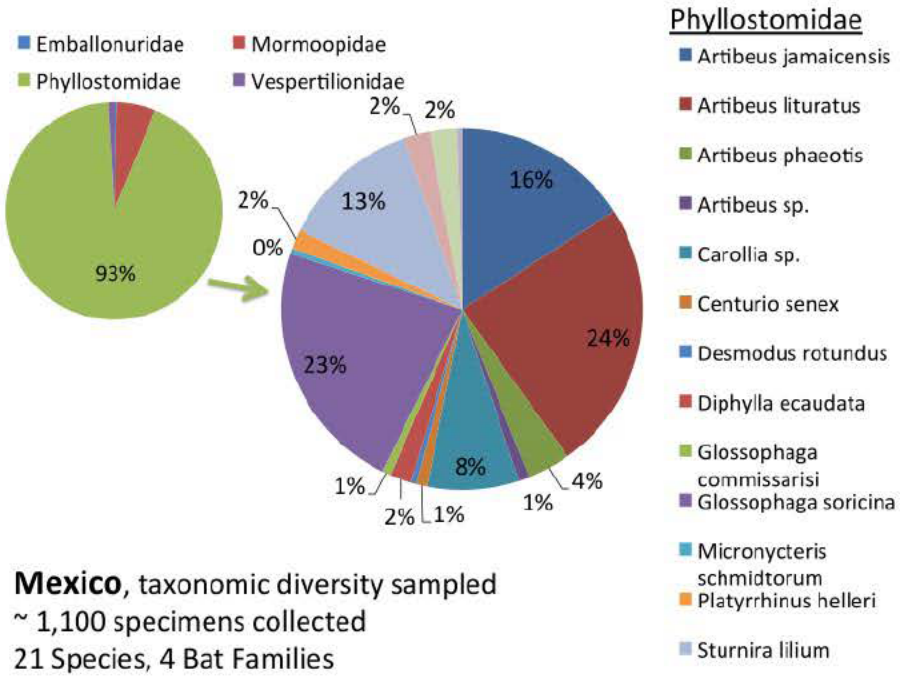

Figure 1. Taxonomic diversity of free-ranging bat species sampled from Mexico in the past 12 months. Species in the diverse family Phyllostomidae have been particularly targeted. In total over 1,100 samples were collected. Bats were collected across a habitat use gradient from pristine forest to urbanized areas. Samples and are being processed now.

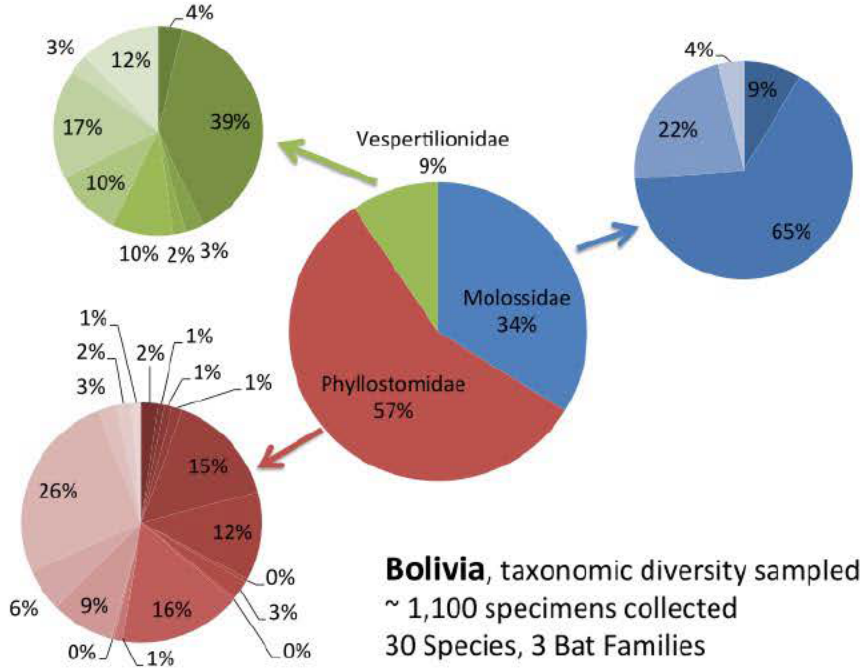

Figure 2. Taxonomic diversity of free-ranging bat species sampled from Bolivia in the past 12 months. Note species names not shown for simplicity; over 30 species from 3 families

were sampled. In total over 1,100 samples were collected. Samples are being processed for PCR now. Samples will be tested in batches as they relate to ecological characteristics of the host species.

Bat Viral Discovery and 'Deep Sampling'– Bangladesh Case Study:

We continue to use a multi-staged strategy to maximize our viral discovery of bat pathogens. This includes: **1) assessing the breadth of viral diversity** across a range of bat families and species using conserved or degenerate PCR primers for >10 viral families (as detailed in last year's report); 2) **unbiased viral discovery using next-generation-sequencing** technologies; and 3) most recently, **using deep or exhaustive sampling for a few key host species** – utilizing 100s or 1000s of individual samples and 1000s of PCR tests across multiple viral families for each host species. In Year 4 we have allocated significant resources to the later, deep sampling, approach. This has proven invaluable to **ascertain the true extent of previously unknown bat viral diversity for known viral families**, and to inform optimal sample collection (sample type and sample size) for viral discovery using rarefication curves. This intensive effort represents a new approach to understanding wildlife viral diversity that has not been previously utilized by other research groups.

We have run over 16,000 PCR assays for 12 viral families, and discovered 63 putative novel viruses in Bangladesh alone (Table 2). Here we use an operational definition and consider a virus "novel" when the genetic distance between the putative new virus and nearest existing virus is greater than the interspecific distance of known, sister viral species currently accepted by the International Committee on Taxonomy of Viruses (ICTV) for each viral family. Further characterization of these putative novel viruses is underway.

Table 2. Summary of 'deep sampling' pathogen discovery results for five bat taxa from Bangladesh (2011-2012). Number of positive samples (above diagonal), number of PCR tests run (below diagonal), and number of new viruses discovered (in parentheses) for each viral family. A total of 63 new viruses have been discovered from this sample set.

	Pteropus giganteus	*Megaderma lyra*	*Rousettus leschenaulti*	*Cynopterus spp.*	*Macroglossus sorbinus*	TOTAL
RNA Viruses						
Paramyxovirus	25/1200 (10)	3/168 (1)	0/190 (0)	0/188 (0)	0/3 (0)	**11**
Astrovirus	36/1200 (8)	NT	NT	NT	NT	**8**
Coronavirus	75/1200 (2)	22/168 (2)	5/190 (2)	3/188 (1)	0/3 (0)	**7**
Hantavirus	0/1200 (0)	0/168 (0)	0/190 (0)	0/188 (0)	0/3 (0)	**0**
Influenza A	0/1200 (0)	NT	NT	NT	NT	**0**
Flavivirus	NT	0/168 (0)	0/190 (0)	0/188 (0)	0/3 (0)	**0**
Enterovirus	NT	0/168 (0)	0/190 (0)	0/188 (0)	0/3 (0)	**0**
Filovirus	NT	0/168 (0)	0/190 (0)	0/188 (0)	0/3 (0)	**0**
DNA Viruses						
Herpesvirus	206/1200 (TBD)	Pending/168	Pending/190	Pending/188	0/3 (0)	**0**
Polyomavirus	11/1200 (4)	0/168 (0)	20/190 (5)	0/188 (0)	0/3 (0)	**9**
Adenovirus	175/1200 (14)	40/168 (7)	12/190 (4)	4/188 (2)	1/3 (1)	**28**
Bocavirus	Pending/1200	0/168 (0)	0/190 (0)	0/188 (0)	0/3 (0)	**0**
Total PCR tests	10,800	1,680	1,900	1,880	30	
Total New Viruses	38	10	11	3	1	**63**

Phylogenies of these new viruses including known species within each family show a surprising level of diversity (Figures 3 – 5). For example, novel Paramxyoviruses were found scattered across the phylogeny, including three new viruses intermediate between existing Morbilliviruses and ancestral to the known Henipaviruses and several new groups of Rubulaviruses (Figure 3). These results are complementary to work recently published by Drexler et al. (2012). However, here we show for the first time that this level of novel viral diversity can be found within a single host species.

Another interesting finding is that non-bat viruses were detected in fruit bats as part of this study. This includes both avian and bovine coronavirus (Figure 4), as well as an avian adenovirus and human bocavirus. These non-bat viruses were detected in individual large fruit bats (*P. giganteus*) that were sampled. We hypothesize that the bats may have picked up these viruses from contaminated water systems, where human, avian and bovine faecal contamination are known to occur. We are currently investigating how cross-species transmission events might be a proxy for measuring disturbed ecosystems that may increase risk of disease emergence. We have also initiated sampling of water bodies used by bats in Bangladesh.

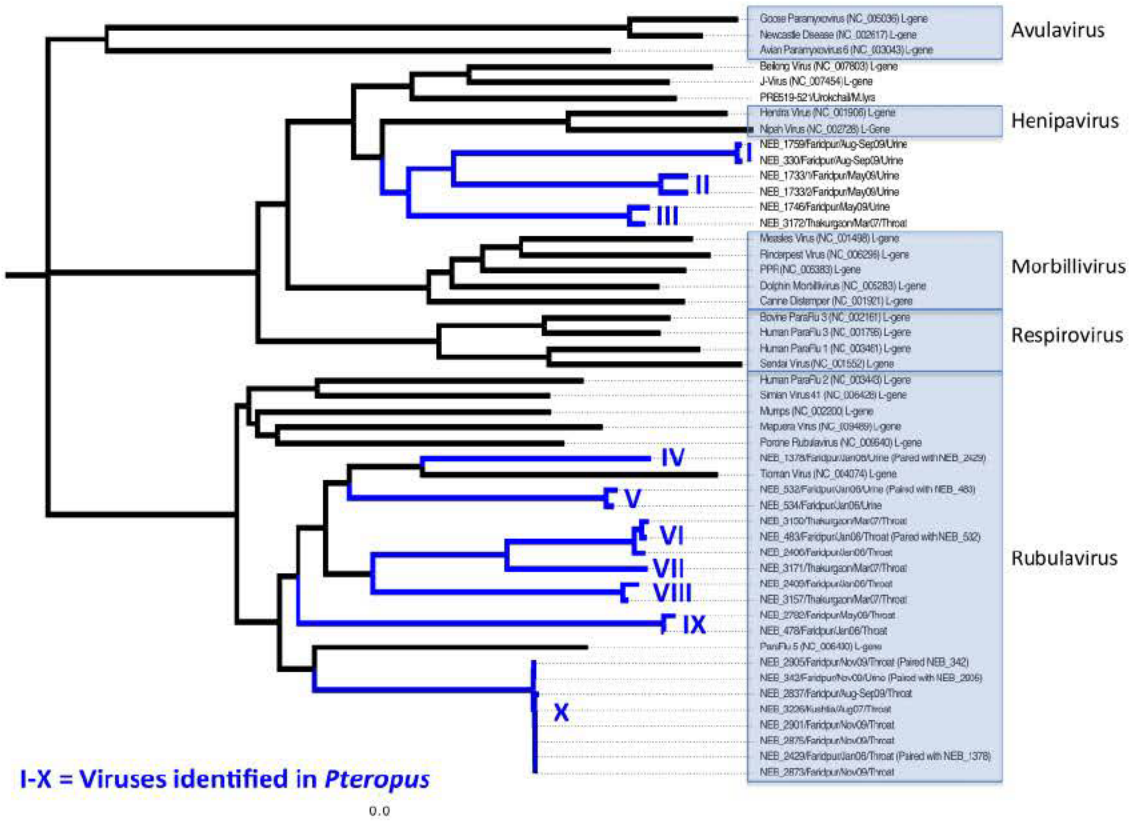

Figure 3. Ten novel Paramxyoviruses discovered from one bat species (*Pteropus giganteus*) in Bangladesh using deep sampling approach and conserved family-level primers. Novel viruses were found scattered across the phylogeny of Paramyxoviruses, including three new

viruses (clades I-III) intermediate between existing Morbilliviruses and the ancestral to the known Henipaviruses. Additionally several new clades of viruses that nest within the genus *Rubulavirus* were identified (clades IV-X).

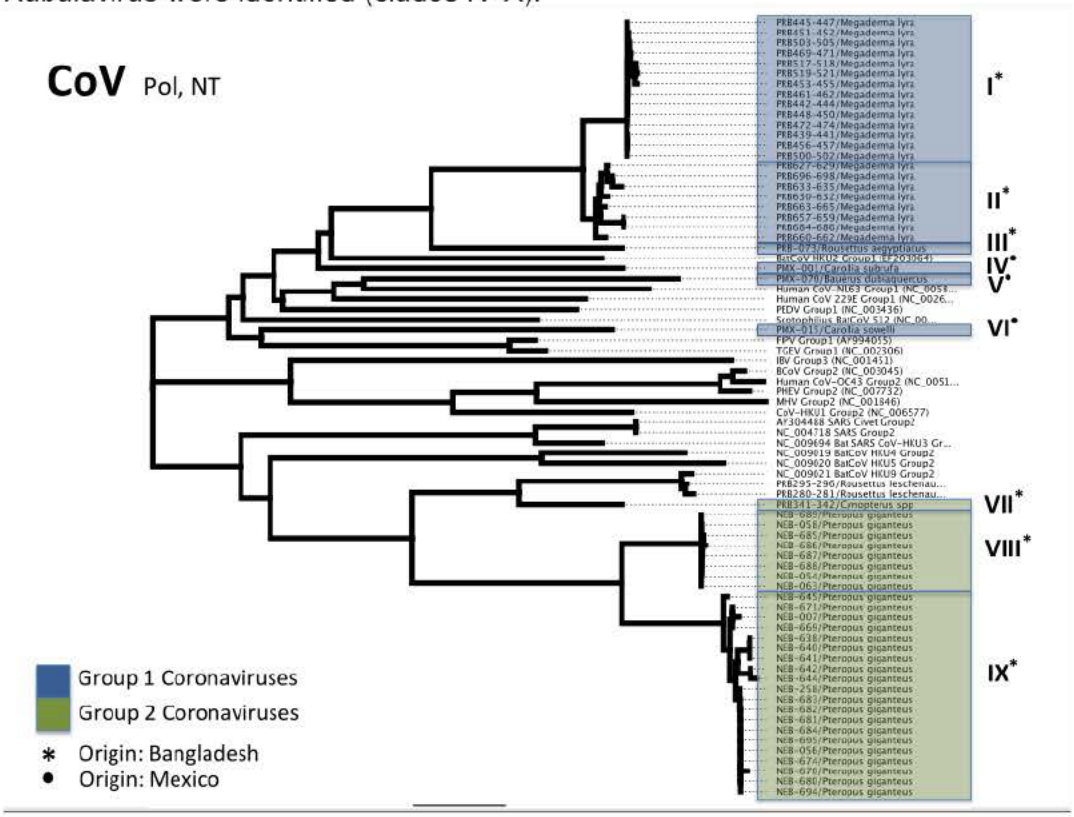

Figure 4. Nine novel Coronaviruses from two bat species in Bangladesh (*Pteropus giganteus* and *Megaderma lyra*) that were deeply sampled. Figure also includes additional positive samples recently detected from Mexican bats.

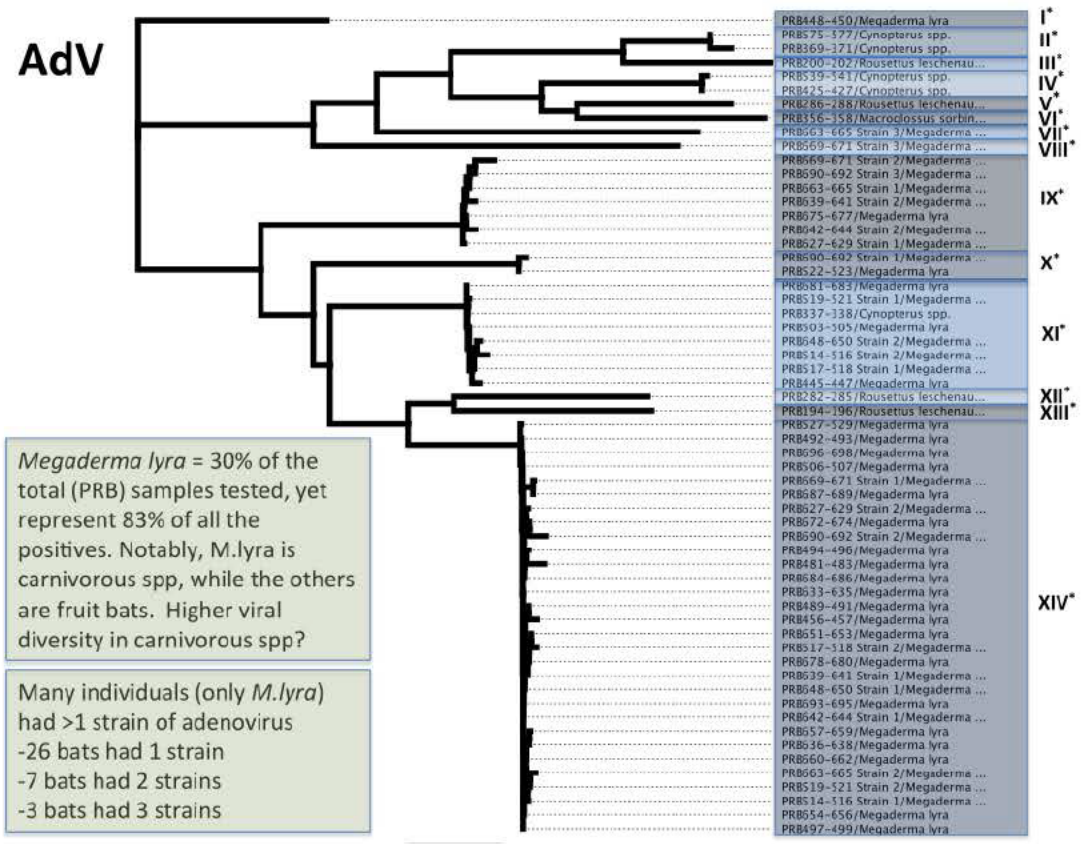

Figure 5. Fourteen novel Adenoviruses (AdV) from 3 deeply sampled bat species in Bangladesh (*Megaderma lyra, Rousettus leschenaultii,* and *Cynopterus sphinx*). An unusually high diversity of AdVs was found in the carnivorous species, *M. lyra*.

Viral Discovery Curves – assessing total known and unknown viral diversity:

We have used a new approach borrowed from the field of biodiversity studies to examine species accumulation (or rarefication) curves to assess the extent of viral diversity in our deeply sampled bat species, with a focus on Pteropodid bats. As per above, using our conserved viral family-level primers, we have identified 63 novel viruses that include 11 new Parmyxoviruses and 28 new Adenoviruses (Table 2). In just a few host species we have catalogued an unprecedented number of new viruses using this exhaustive sampling approach.

Our new approach of using viral discovery curves show the relationship between sampling effort and novel virus discovery for each different sample type. In addition to detecting a large number of previously unknown viral species, this approach allows us to estimate the total number of viruses (both detected and undetected) likely to be present in the sample, and characterize the viral community assemblage across host species and populations. This approach also allows us to determine how many samples are required to estimate total number of species in a species or sample type. **These new results compared to known viruses per host published in the literature suggest that viral species richness in bats has been massively underestimated.** Below we show viral accumulation curves for one RNA viral family (Figure 6, Paramyxoviruses) and one DNA viral

family (Figure 7, Adenoviruses).

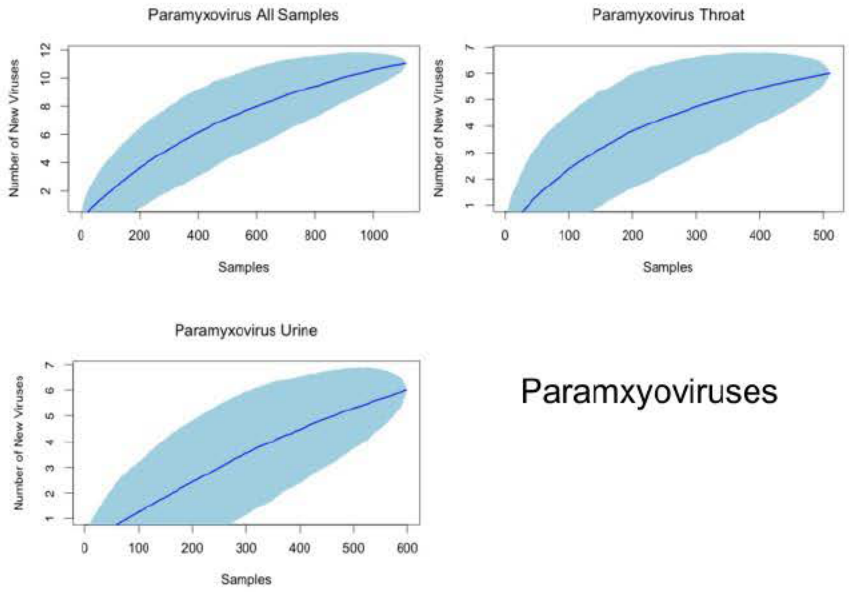

Figure 6. Viral accumulation curves by sample type (throat sample vs. urine sample, and total) for novel Paramxyoviruses (RNA) from bats in Bangladesh. Blue cloud represents 95% confidence interval using a jackknife estimator. The paramyxovirus curves do not appear to have reached an asymptote, suggesting more intensive sampling will continue to yield more new samples in *P. giganteus*. However at >1000 samples tested already, finding these additional rare viruses may not be cost effective.

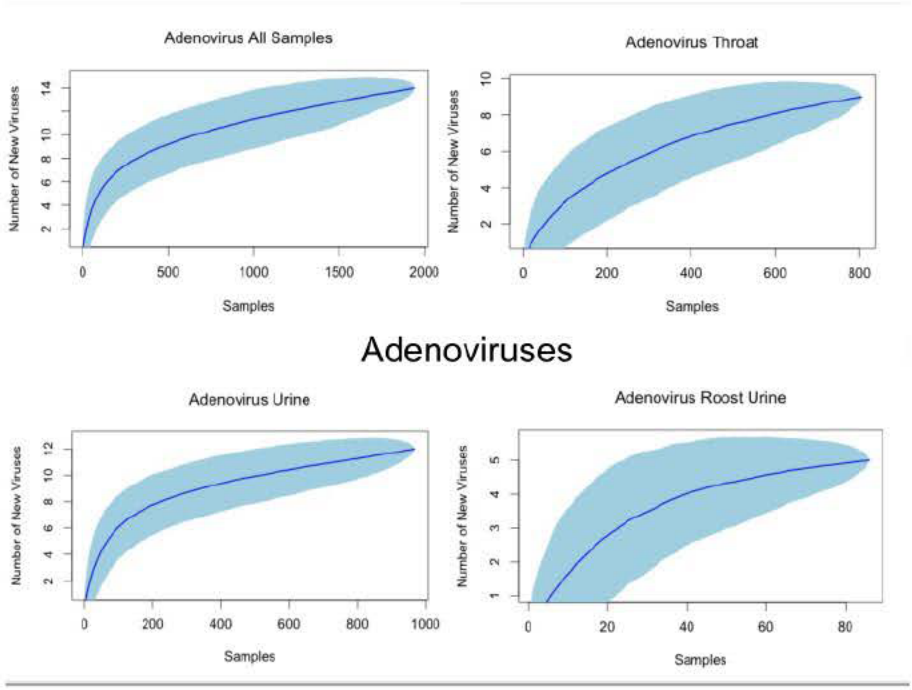

Figure 7. Viral accumulation curves by sample type (throat vs. urine vs. pooled roost urine samples, and total) for novel Adenoviruses (DNA) from bats in Bangladesh. Note the steep slope to the curve for the first ~200 samples tested, suggesting that more than half of all detectable viruses were found in this initial effort. Subsequent sampling of 1,500 more samples only yielded 6 new viruses after that point. This information is of great value for targeting limited resources to maximizing the discovery of novel pathogens in wildlife host populations, and can inform optimum numbers of samples that needs to be tested to find new viruses for a given virus family.

Other Research Highlights from Specific Countries:

Thailand Bat Pathogen Metagenomics

Our research activities in Thailand this past year has included active bat surveillance at high-risk interfaces (bat guano harvesting caves) and unbiased viral discovery using 454 next generation sequencing.

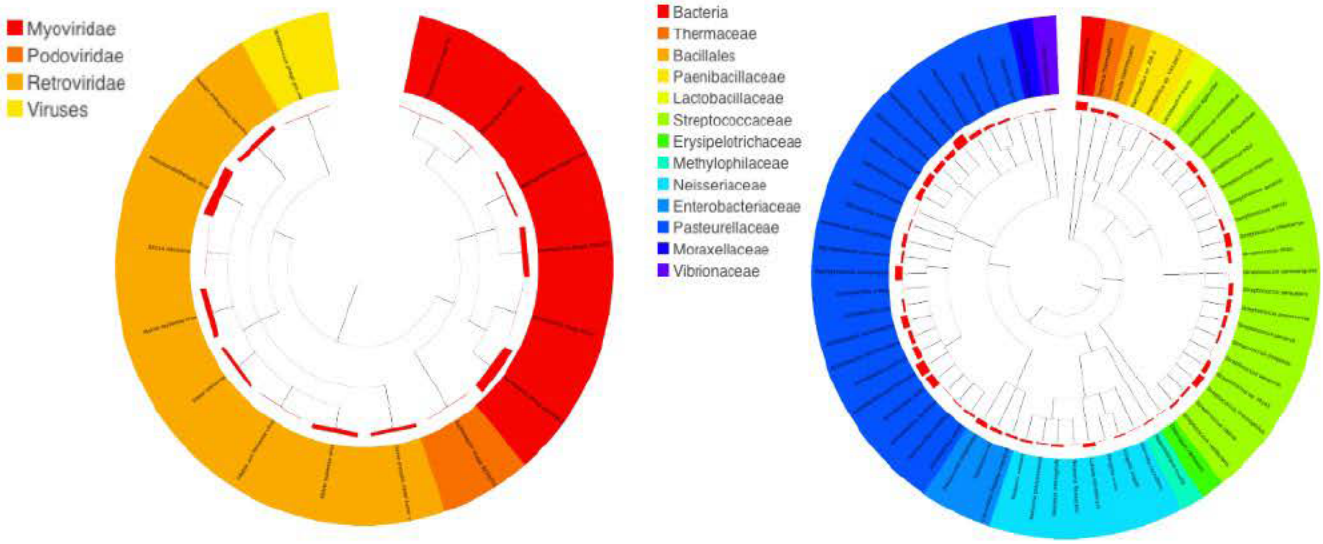

Figure 8. Results from metagenomic survey (454 Jr. Roche) of bat saliva sample from *Pteropus lylei* in Thailand. Sequence reads that match with bacterial pathogens and symbionts to the right, and those that match with known virus reads to the left (red, orange, yellow figure). Three viral phage families were detected including Myoviridae, Podoviridae and an unclassified phage family, and a large number of retroviruses were also detected in this sample (orange). The width of the red bar in each tree shows the number of reads that mapped to a given virus or bacterial group.

We spent significant effort this past year optimizing and developing appropriate extraction and post-extraction total nucleic acid amplification techniques. We found that *phi29* DNA polymerase with whole transcriptome amplification (WTA) technique worked best for the low nucleic acid amounts present in clinical bat samples (e.g. oral swabs). From this one sample we obtained 88,916 unique reads with 396±122 bp mean sequence length that were summited to the MG-RAST metagenomics analysis server. The result of comparison against non-redundant protein database using a maximum e-value of 1e-5, a minimum identity of 70%, and a minimum alignment length of 15 are shown in Figure 8.

Novel Bat CoVs from Guano Caves in Thailand

We recently completed a study screening guano harvested from a cave in Ratchaburi, Thailand. During the last year, samples of guano were collected every two weeks and screened for Coronaviruses and Histoplasmosis. Four samples were PCR positive for Coronaviruses, and phylogenetic analysis reveals two divergent CoV viral groups (Figure 9). This study also included an extensive interview of guano harvesting workers (exposed population) and local villagers (control population) to gauge the level of contact they had with bat guano, and any medical conditions over the last year. No significant correlation was found between guano contact and history of illness from this survey. We are currently preparing a manuscript entitled "Assessing pathogen risk from bat guano collection in Ratchaburi, Thailand" that summarizes these findings.

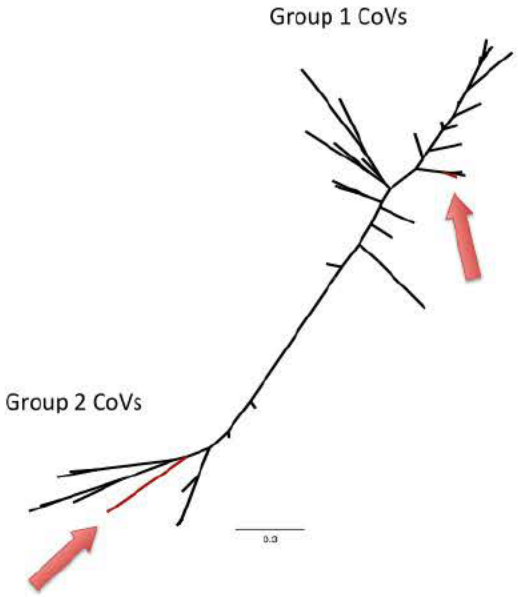

Figure 9. Group 1 and group 2 CoVs detected in guano harvested in Thailand (new viruses represented by red branches). Maximum likelihood tree based on ~400bp of the RdRp gene, including bat CoVs from Genbank. One group of viruses clusters with a clade including Kenya bat coronavirus BtKY39 (toward top of tree) and three other positive samples represent a distinct viral lineage most closely related to CoV from Rhinolophus spp. in Australia. Genbank numbers and taxa names removed from figure for simplicity.

Malaysia

We screened serum and fecal samples from 18 bats at co-investigator Lipkin's laboratory – the Center for Infection and Immunity (CII) at Columbia University using next-generation-sequencing (Illumina). Libraries from these samples were prepared and processed; we are awaiting results and analysis of these metagenomic data. Additionally PCR reactions were run using consensus level screening at summarized in Table 3; we are awaiting these test results.

Table 3. Consensus, family-level PCR testing recently completed on Malaysian bat samples; awaiting result.

# of samples submitted	Diagnostic methods	Viral families/ genus screening
Serum samples from 25 bats	Reverse transcriptase assay	Retroviruses – Gammaretrovirus and Lentivirus
Pooled cDNA of throat, urine and rectal swab samples from 25 bats	Consensus PCR and CII PCR (for influenza A)	Astroviruses (2 protocols), coronaviruses (2 protocols), filoviruses, flaviruses, paramyxoviruses, hantaviruses, herpesviruses, bocaviruses, poxviruses (2 protocols), papillomaviruses, adenoviruses, polyomaviruses and influenza A

China

In China we have discovered a number of novel bat viruses this past year. We have tested over 300 samples using degenerate PCR protocols for three viral families. For a subset of these samples run in 2011, we found 2% positive for Coronavirus, 25% positive Astrovirus, and 8% positive Paramyxoviruses (Table 4). Newly detected viruses shown in phylogenies below (Figures 10-XX). We also continue to strategically collect field samples, and in the past 12 months collected 362 bat samples from 19 species.

Table 4. Samples tested in past 3 months in China using 3 viral family protocols.

	Astrovirus	Coronaviruses	Paramyxoviruses
Presumptive Positive	42	4	13
Percent Positive	25%	2%	8%
Total Sampled	167	167	167

New coronaviruses were detected in *Miniopterus, Myotis* and *Rhinolophus* species, including 3 *Rhinolophus* positive samples that form a new clade distinct but related to the SARS-like CoVs (Figure 10). Molecular analysis using the full spike receptor domain confirms the phylogenetic position of these samples (Figure 11). Interestingly we found that all three of these bat SARS-like CoVs do not share identical ACE2 binding "hot spot" amino acids with the human-CoVs causing the 2003 SARS outbreak (Wu et al. 2011).

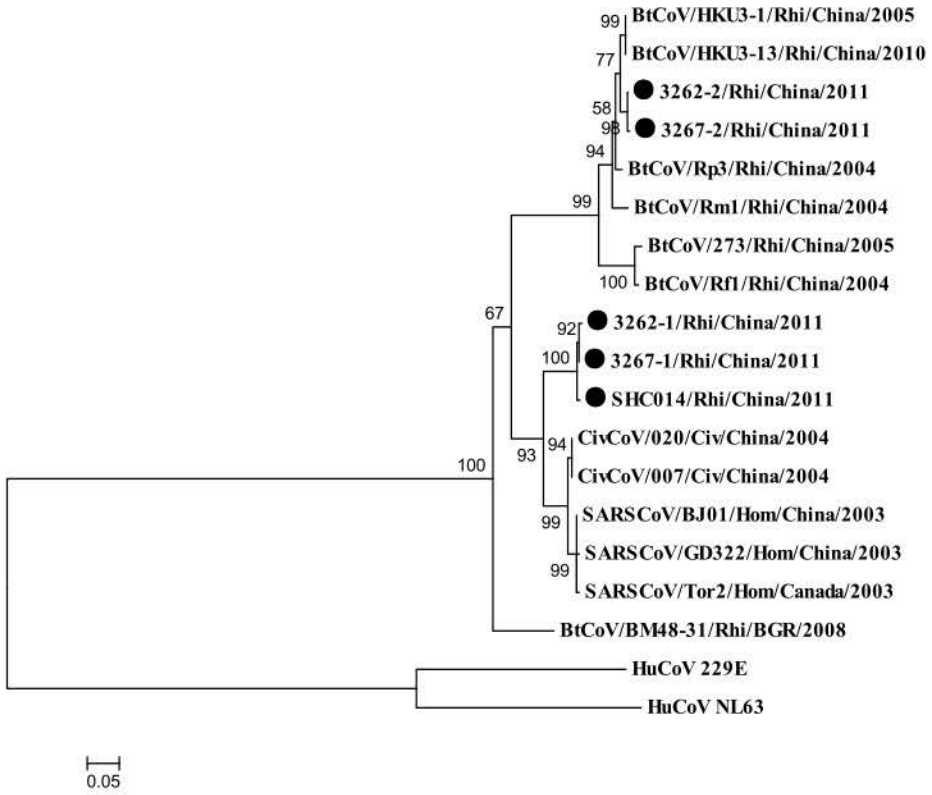

Figure 10 (above). Phylogeny of new CoV discovered in China (black circles). Tree based on 277 amino acids of CoVs spike receptor binding domain (Corresponding to SARSCoV Tor2 aa.319-596)

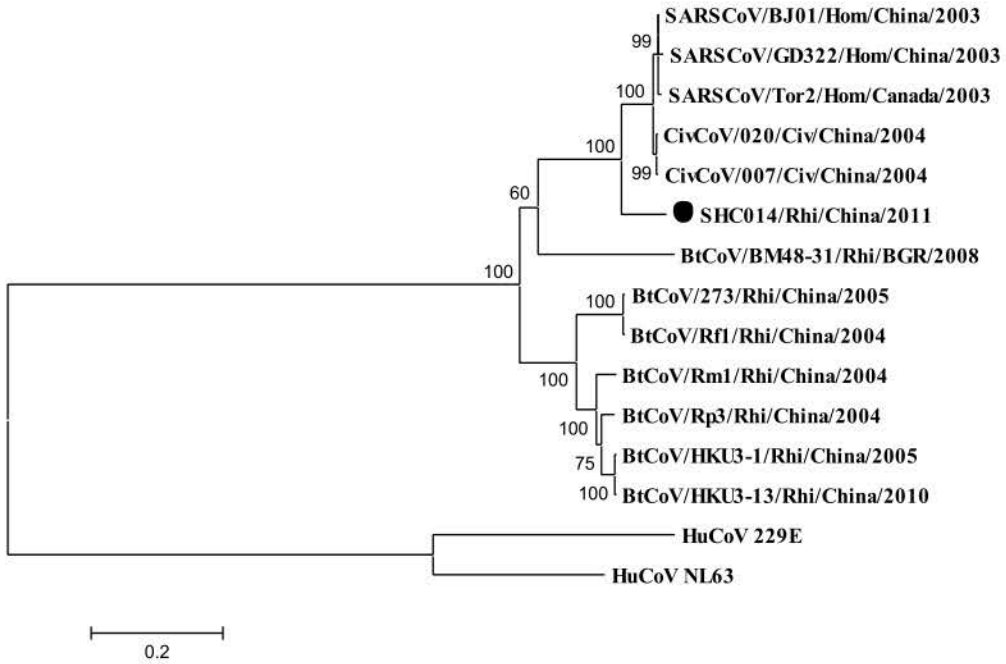

Figure 11. Coronavirus phylogeny of complete CoVs spike protein (~1256 amino acids) showing one new virus found in China (black circle). Full analysis confirms phylogenetic position virus that is basal and sister to other known SARS-like CoVs from China.

We also discovered three novel paramyxoviruses from *Rhinolophus* and *Hipposideros* bat species in China. These viruses are genetically similar to each other (~95% aa identity), but only show a ~65% identity with the nearest other known Paramxyoviruses. J-virus and Beilong virus were identified as the closest known matches. See Table 5 and Figure 12 below.

Table 5. Species, location, and pairwise genetic identity of putative new Paramxyoviruses from China. Table includes distance to nearest known Paramxyovirus (J-virus and Beilong virus). Based on 450 amino acids of the L protein.

Origin Species	Sampling Time	Sampling Site	Seq->	CP14ch	FK28	LD28	J-virus	Beilong virus
Rhinolophus affinis	2008	Hainan	CP14ch	ID				
Hipposideros larvatus	2009	Guangxi	FK28	0.947	ID			
Hipposideros larvatus	2008	Hainan	LD28	0.984	0.963	ID		
			J-virus	0.659	0.639	0.644	ID	
			Beilong virus	0.638	0.623	0.623	0.685	ID

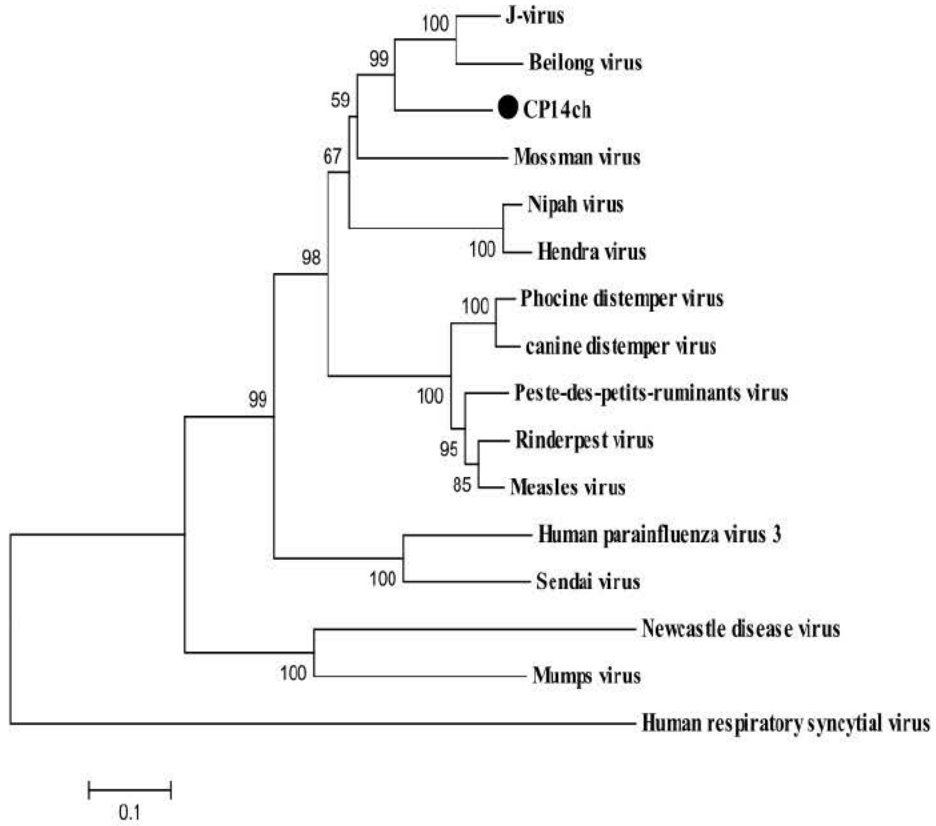

Figure 12. Paramyxovirus phylogeny based on 450 aa of the L protein, showing new virus from China (CP14ch). Sample was collected from *R. affins*, and J-virus and Beilong virus were identified as the closest other known Paramyxoviruses.

United States

We implemented two new projects in the insular USA (Hawaii and Puerto Rico) this year to test important hypotheses in disease ecology and biogeography of bat viruses. Samples have been collected from both sites and testing across viral families is now underway for samples collected in Puerto Rico.

1. Island biogeography of bat viruses. Island biogeography predicts that species diversity will increase with increasing island size. Similarly, it's been shown that species that have small founder populations (e.g. the number of individuals that start a new population), particularly island species, will often loose their parasites and pathogens during that colonization bottleneck (Torchin and Mitchell 2004). Dispersal of bats over large oceanic distances is a relatively rare event, and number of migrants surviving such a migration decreases with increasing distance (Heaney 2007). One open question is: How does isolation of a species on islands and population bottlenecks due to colonization events affect viral diversity and diversification in bats?

To test the above theories on founder effects and island biogeography of bat viruses, we sampled bats on the islands of Hawaii and Puerto Rico. Our work with the endangered Hawaiian Hoary bat (Hawaii's only native terrestrial mammal) is being done in collaboration with the USGS based in Hawaii. To date, about 20 fecal samples have been collected from live captured bats on the island of Hawaii. Trapping success has been slower than expected as these are relatively rare species, and we are awaiting an additional 10 samples to begin viral screening at CII at Columbia University. In April 2012, we collected 235 samples from 6 bat species at a single site in Puerto Rico. For both the Hawaii and Puerto Rico samples, we will compare these samples with the known diversity of pathogens in the same or sister taxa distributed on the mainland to see if viral diversity has decreased with island colonization events.

2. Puerto Rico, determinates of host virus sharing within a single environment.

Bat species exhibit a wide range of ecologies, roosting and foraging behaviors, and life-history traits that may influence the diversity of viruses found in each species (Turmelle and Olival 2009). At a community level, these differences may affect the opportunity for contact and thus, pathogen spillover risk between species. In Puerto Rico, we sampled six species that co-occur in the same cave at Cueva de los Culebrones (Figure 13) each with widely different ecologies and roosting behaviors.

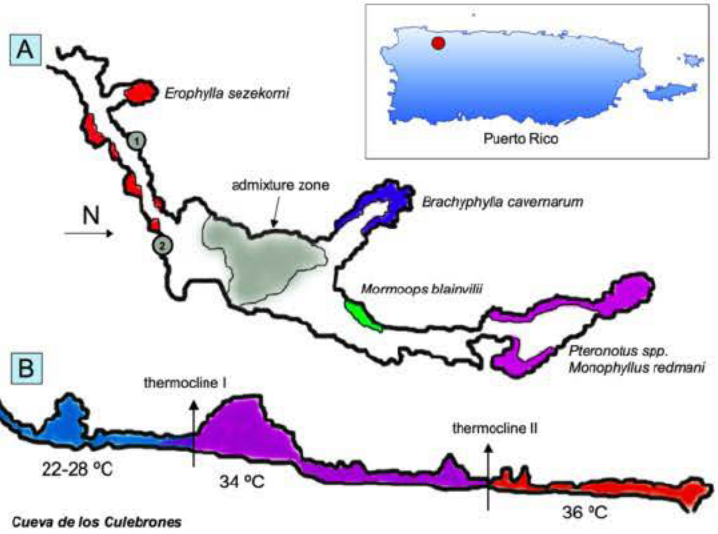

Figure 13. A) Horizontal transect of the cave, showing preferred roosting areas by species. B) Vertical transect showing the temperature profile of the cave. Inset map: Cave location in PR.

At this site we will test the hypotheses that roosting proximity within the cave environment, during foraging (e.g. comparing frugivores vs. insectivores), and interaction at the roost sites (species are segregated by species within the cave, see Figure 14) may influence the degree of pathogen sharing in this ecological community. We have extracted nucleic acid from the 235 samples collected at this site in April and are beginning viral testing in July 2012.

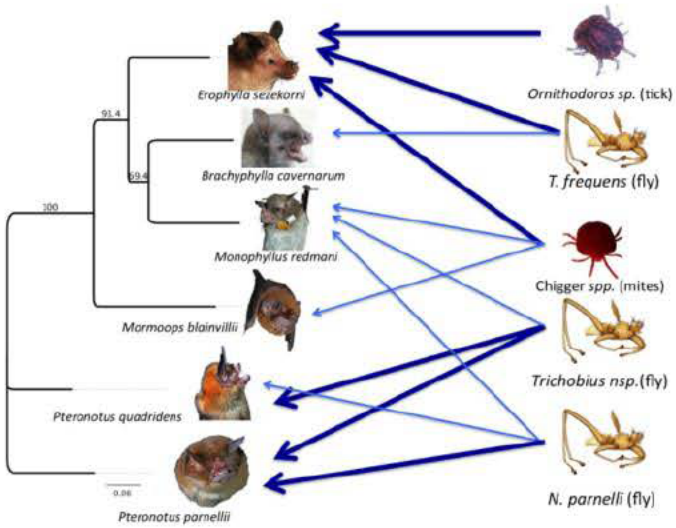

Figure 14. Blood-feeding vector associations for each bat species in the cave that may inform models for any potential vector-borne viruses detected at Cueva de los Culebrones in Puerto Rico.

Predicting Cross-Species Transmission of Bat Viruses:

Using a database of all mammalian viruses and their known hosts that we built from the literature the past two years, we have been examining the host and viral traits that may explain cross-species virus transmission in bats. Using a GLM logistic regression approach, we modeled the probability of a host being infected with bat viruses. Data from 131 bat species and 49 unique viruses known to infect those species (all viruses currently recognized as species by ICTV), were included in this model. Results from our analysis of all known bat viruses support previous empirical work from individual viruses (e.g. bat rabies) that have shown host phylogeny is a significant predictor of cross species transmission (Streicker et al. 2010).

Table 6. Results from logistic generalized linear model (GLM), identifying significant host and virus traits that predict whether or not a virus will be shared among bat species. Those variables in bold were significant.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.8292	0.19639	-19.498	<0.001***
Phylogenetic Distance to other bat hosts	-1.1709	0.07469	-15.676	<0.001***
Number of disease publications per host	0.43199	0.06618	6.528	<0.001***
Number of publications per virus	0.50347	0.08172	6.161	<0.001***
Species is hunted (IUCN)	-0.51859	0.19374	-2.677	0.007**
Species in artificial habitat (IUCN)	0.24513	0.17613	1.392	0.163
Virus is segmented	-0.85348	0.22687	-3.762	<0.001***
Virus is vector-borne	-1.0137	0.14334	-7.072	<0.001***

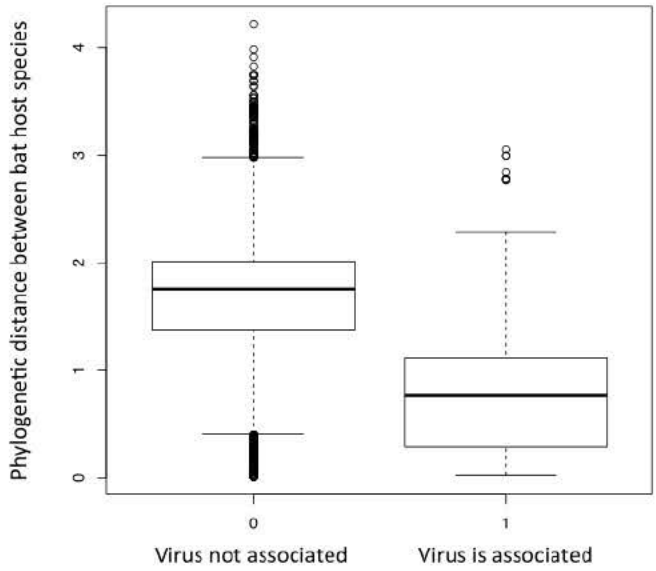

Figure 15. Phylogenetic distance between bat host species is a strong predictor of viral sharing between bat species. In other words, evolutionarily more closely related host species are more likely to share viruses (probability of being associated = 1) than distantly related bat species. Pairwise host phylogenetic distance from a maximum likelihood best tree using cytochrome B mtDNA.

Similarly, using a data collected from the literature for all known mammalian virus-host associations (1,775 assocciations, 400+ viruses, and 588 mammalian host species), we show that the taxomic groups of most concern for spillover into humans are primates, rodents and bats (Figure 16).

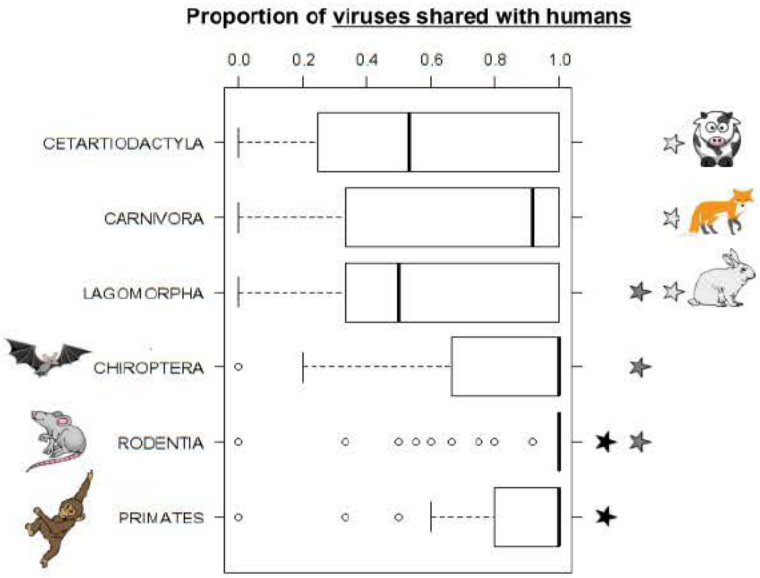

Figure 16. Species level analysis of the proportion of unique viruses shared with humans across mammalian orders. **Primates, rodents, bats and lagomorphs have significantly higher proportions of viruses shared with humans than other mammal Orders.** Stars represent groups that are not significantly different from one another using a Tukey test.

Modeling Drivers of Bat Virus Spillover in Humans:

Over the last 12 months, we have continued to refine our bat viral hotspot models. Specifically, we have focused on integrating other global drivers and investigate explanatory power of anthropogenic activity upon the spatial pattern of virus sharing between humans and other animals. We map the spatial hotspots of zoonotic bat viruses, and show virus sharing is positively associated with human population density, occurrence of bushmeat hunting and livestock counts, even after correcting for the strong influence of environment. Here we used host bat ranges to create proxy distributions of human-shared bat viruses, and use spatial regression models to ask which factors are positively associated with this sharing.

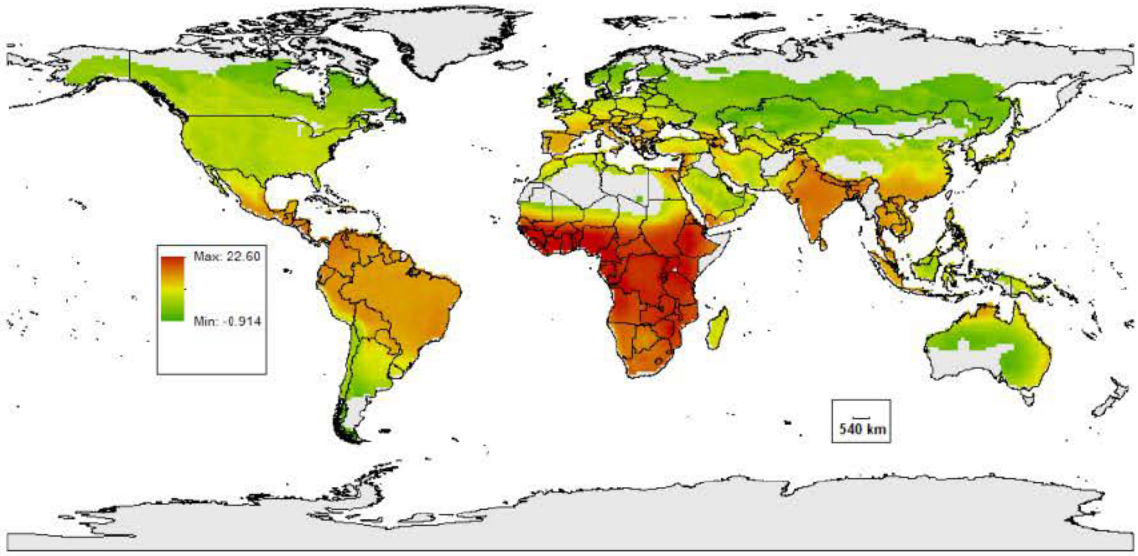

Figure 17: Fitted model, risk map for zoonotic bat-borne viruses. Red and orange are areas of higher risk for bat zoonoses.

Table 7. Coefficients and confidence intervals for predictors of bat-human virus sharing. Coefficients for log-transformed covariates can be interpreted as the increase in viruses predicted by an e-fold (2.718) increase in the covariate. All variables in bold were significant in the model, and covariates are listed in decreasing order of significance.

Model Component	Coefficient	95% confidence interval for coefficient	P value
(intercept)	3.299	2.369 to 4.228	3.466e-12
log(bat species richness)	2.872	2.795 to 2.949	< 2.2e-16
log(annual rainfall range)	0.629	0.543 to 0.716	< 2.2e-16
log(bat publication authors)	-0.157	-0.191 to -0.122	< 2.2e-16
log(GDP per capita)	-0.350	-0.437 to -0.263	3.109e-15
log(human density)	0.079	0.056 to 0.102	1.547e-11
bushmeat activity	0.579	0.404 to 0.754	8.443e-11
log(pig stocks)	0.026	0.015 to 0.038	9.542e-06
log(sheep stocks)	0.023	0.011 to 0.034	1.049e-04
arcsin(% crop/pasture cover)	0.106	-0.027 to 0.239	0.119
monthly temperature range	-0.008	-0.035 to 0.019	0.563

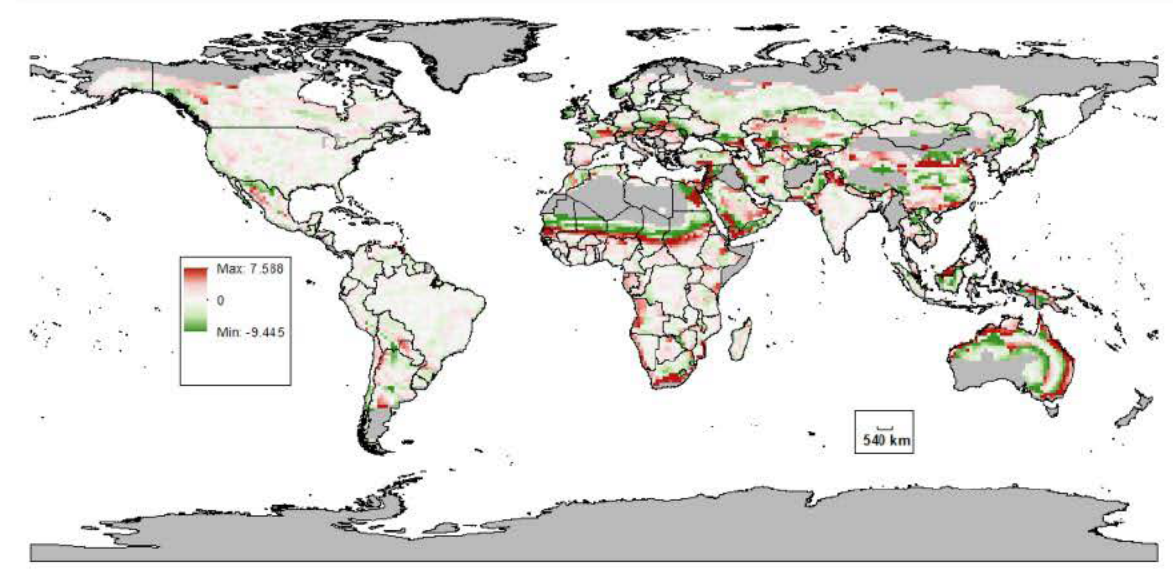

Figure 18. Map showing residuals of bat virus hotspot model. Positive residuals values (red) are where the model under-predicts the actual bat viral diversity, and negative values (green) are where the model over-predicts bat viral diversity. We are using this modeling technique to explore geographic areas (e.g. negative values in red) that are likely to be more cost-effective in yielding novel bat viruses with devoted sampling efforts.

Modeling Risk of Pandemic Emergence of Bat Viruses Using Travel Data:

We modeled the global vulnerability from the emergence of all directly transmitted zoonotic viruses using airline travel data from the International Air Transport Association, zoonotic disease hotspot risk maps (Jones et al. 2008), and per capita health care (as a correction, i.e. probability of detecting a disease before it gets on an airplane). We are currently refining these analyses to examine the risk of emergence for bat-borne zoonotic viruses alone, and are integrating other variables that predict the risk of spread. Our basic model in the two figures that follow is outlined below.

$$\phi_j = \sum_{all\ i} \frac{C_{ij} \cdot E_i}{H_i}$$

- Calculating index
  - E_i = Zoonotic disease hotspots
  - C_{ij} = Est. Number of passengers
  - H_i = Healthcare spending per capita
    - i = source of risk
    - j = destination of risk

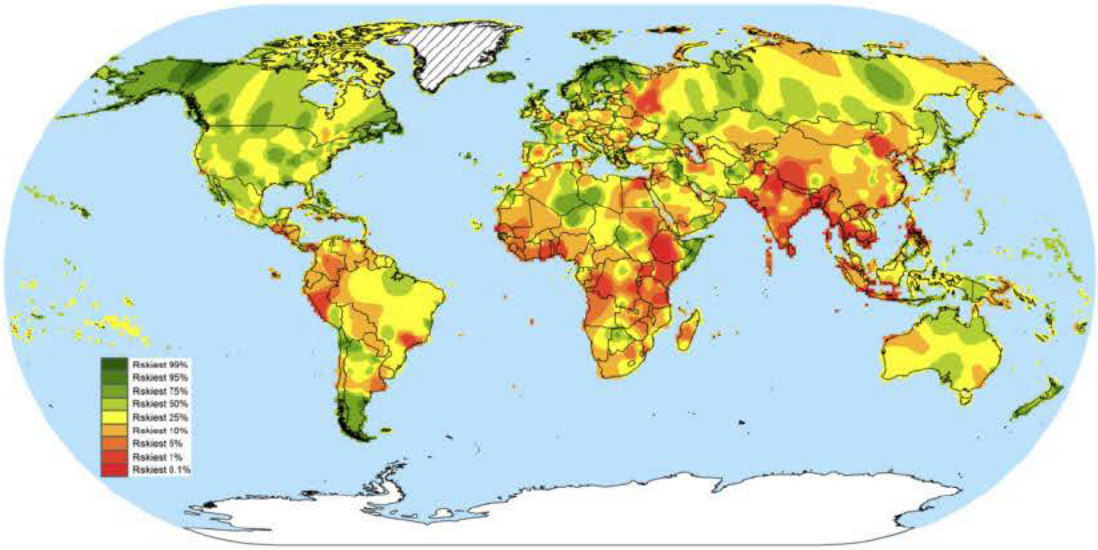

Figure 19. Global vulnerability **outflow** map, areas of greatest risk of pandemic origin for a zoonotic disease from wild animals. Subset models are currently being developed to look at risk of bat virus pandemic outflow specifically.

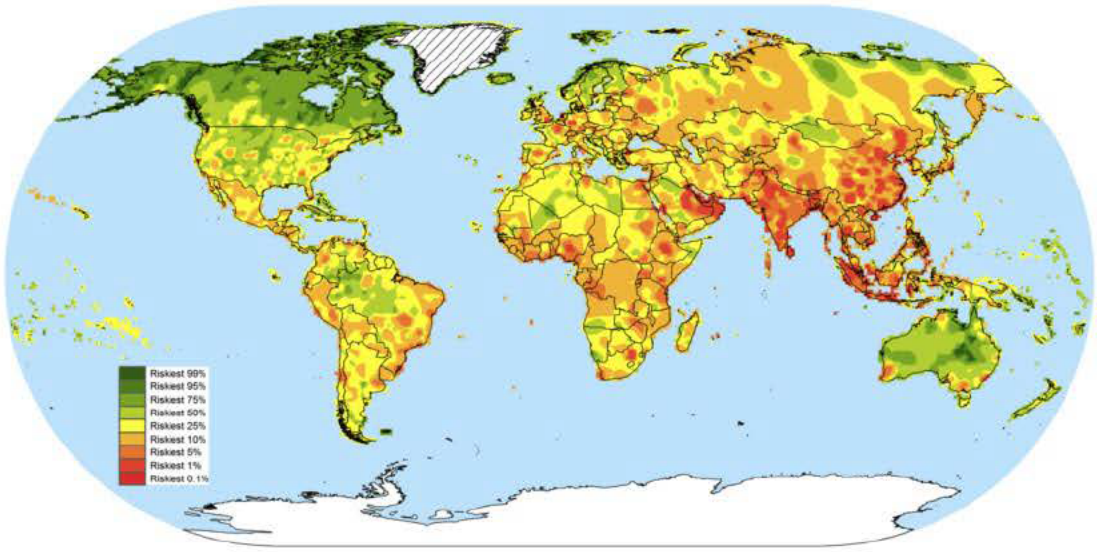

Figure 20. Global vulnerability **inflow** map, areas of greatest risk for pandemic zoonotic disease introduction.

Modeling Future Species Distributions of Bats and their Viruses:

Using Ecological Niche Models and ensemble methods that aggregate across Global Climate Models (GCMs) we recently modeled the future geographic change in bat species distribution and their associated viruses. Our initial model was based on bat species known to harbor Paramxyoviruses, including those hosts we have recently identified with novel Paramxyoviruses. These future projections of geographc range, for host and disease, will allow us to build temporally-explicit, predictive bat virus hotspot models; this work is ongoing.

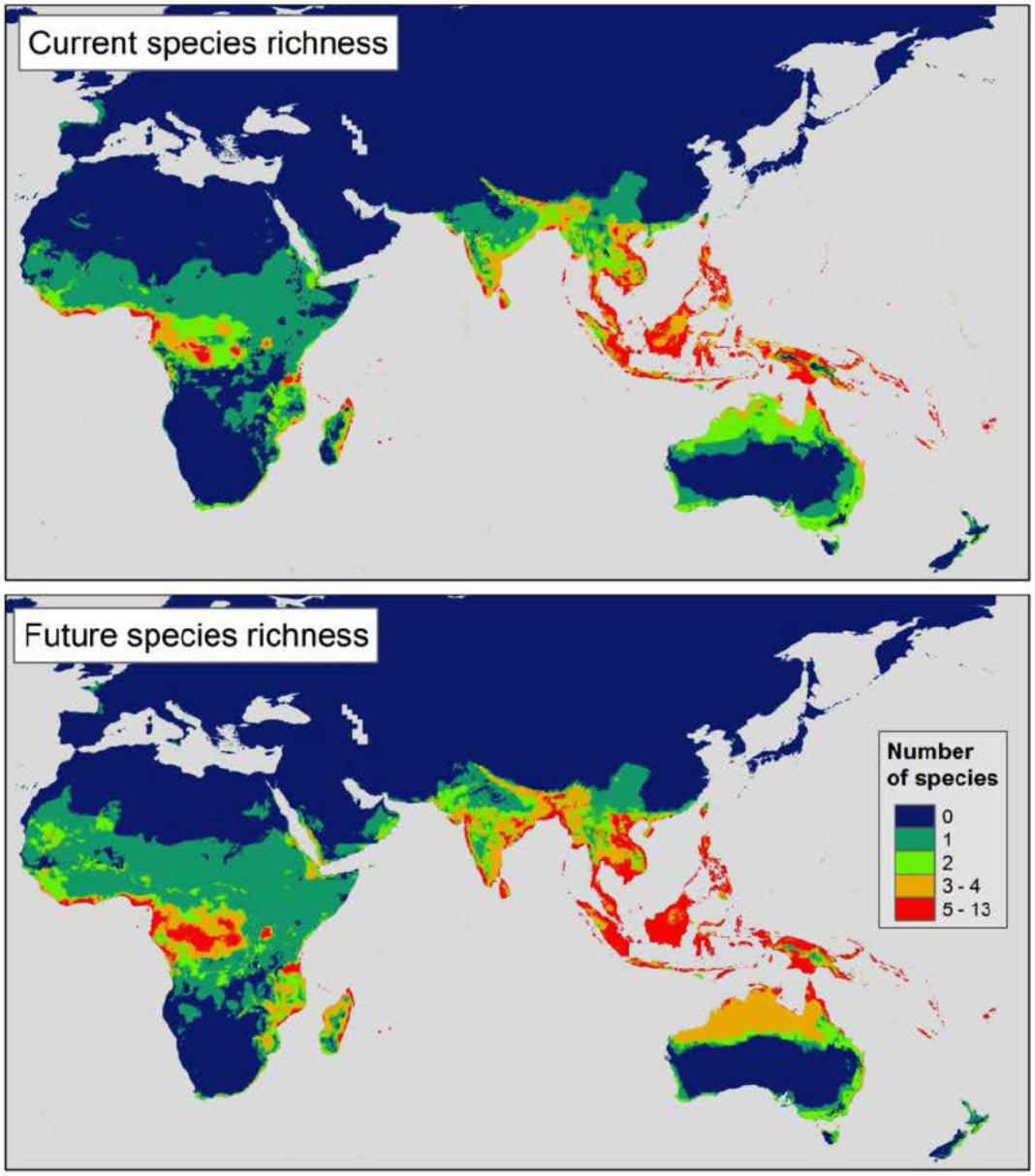

Figure 21. Top: Species richness map for current distribution of bat species known to harbor Parmxyoviruses. Bottom: Species richness map for midcentury (2050's) using A2 climate change scenario models, based on 50% Global Climate Model agrement using ensembal methods.

Training and Outreach for Conducting Bat Virus Research:

Over the past few years in our global efforts to collect bat virus specimens supported under this award and with other complementary funding sources, we have trained >230 people in-country in methods of bat capture, safe handling, species identification, proper use of Personal Protective Equipment (PPE), and minimally-invasive sample collection (Table 8).

Table 8. Total number of in-country personnel trained to date in best practices for conducting bat virus research. Training includes overview of EcoHealth Alliance protocols on safe capture, handling, species identification, PPE, and sample collection.

Country	Number personnel trained
Australia	4
Bangladesh	5
Bolivia	18
China	2
Colombia	54
Malaysia	56
Mexico	26
Philippines	25
Thailand	32
UK	1
USA	8
Total	**231**

In May 2012 we held a large hands-on training at three sites in Central Thailand for 32 people that included veterinary students and forestry students from three universities in Thailand, staff and veterinarians from the Department of National Parks Thailand, and Forestry Rangers.

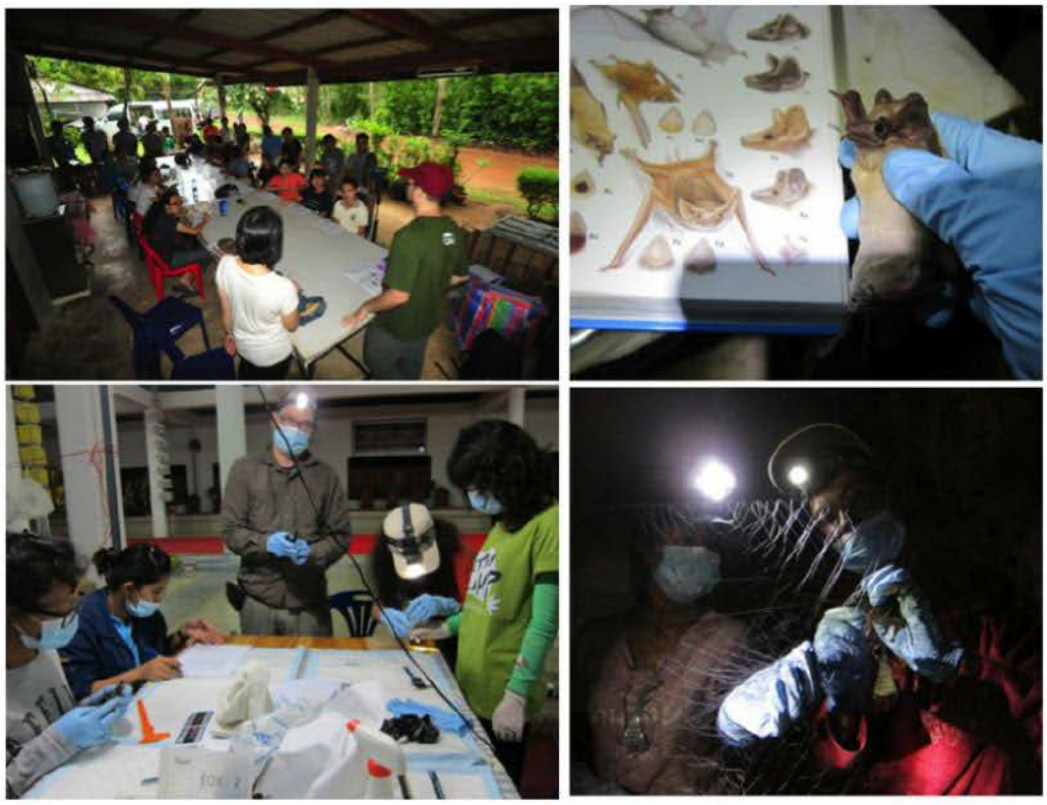

Figure 22. Clockwise from top left, Dr. Kevin Olival from EcoHealth Alliance and Dr. Supaporn Wacharapluesadee from Chulalongkorn presenting overview of safety issues to team at field site; demonstrating live-animal morpological bat species identification using field guides; Dr. Prateep Duengkae demonstrating safe use of Personal Protective Equipment while removing a bat from mist net; and Dr. Olival demonstrating safe bat handling and sampling to a small group.

This past year, in collaboration with the Food and Agriculture Organization, CSIRO, and other agencies, EcoHealth Alliance co-edited and published a volume on best practices for investigating zoonoses from bats (Newman et al. 2011). The publication includes eight chapters that cover a wide range of subjects including: an overview or emerging infectious diseases in bats, viral discovery techniques, safe bat handling, satellite telemetry, and

population monitoring, NIAID is formally acknowledged in this publication, and the book is freely available at: www.fao.org/docrep/014/i2407e/i2407e00.pdf.

Thirteen papers submitted or in press from this award over past 12 months:

1) Bogich, T. L., K. J. Olival, P. Hosseini, C. Zambrana-Torrelio, E. Loh, F. S., I. Brito, J. H. Epstein, J. S. Brownstein, D. O. Joly, M. A. Levy, K. E. Jones, S. S. Morse, A. A. Aguirre, W. B. Karesh, J. A. K. Mazet, and P. Daszak. (2012). Using Mathematical Models in a Unified Approach to Predicting the Next Emerging Infectious Disease. Pages 607-618 in A. A. Aguirre, R. S. Ostfeld, and P. Daszak, editors. New Directions in Conservation Medicine: Applied Cases of Ecological Health. Oxford University Press, Oxford.

2) (b) (4), (b) (6)

3) Daszak P, Lipkin WI. (2011). The search for meaning in virus discovery. Current Opinion in Virology 1: 620-623.

4) (b) (4), (b) (6)

5) (b) (4), (b) (6)

6) [redacted] (b) (4), (b) (6)

7) [redacted] (b) (4), (b) (6)

8) Newman, S. H., H. E. Field, C. E. d. Jong, and J. H. Epstein, editors. (2011). Investigating the role of bats in emerging zoonoses: Balancing ecology, conservcation and public health interests. Food and Agriculture Organisation of the United Nations., Rome

9) Olival, K. J. (2012). Correlates and evolutionary consequences of population genetic structure in bats. Pages 267-316 *in* G. F. Gunnell and N. Simmons, editors. Evolutionary History of Bats: Fossils, Molecules, and Morphology. Cambridge University Press, Cambridge.

10) Olival, K. J., J. H. Epstein, L. F. Wang, H. E. Field, and P. Daszak. (2012). Are bats unique viral reservoirs? Pages 195-212 *in* A. A. Aguirre, R. S. Ostfeld, and P. Daszak, editors. New Directions in Conservation Medicine: Applied Cases of Ecological Health. Oxford University Press, Oxford.

11) [redacted] (b) (4), (b) (6)

12) Smith CS, Epstein JH, Breed AC, Plowright RK, Olival KJ, de Jong C, Daszak P, Field HE. (2011). Satellite telemetry and long-range bat movements. PLoS ONE 6: e14696.

13) Smith KM, Anthony SJ, Switzer WM, Epstein JH, Seimon T, et al. (2012) Zoonotic Viruses Associated with Illegally Imported Wildlife Products. PLoS ONE 7(1): e29505.

Key Activities Planned for Year 5:

- Continue and expand viral family-level pathogen discovery on all collected samples.
- Continue with targeted field sampling in taxa and geographies of high-value for pathogen discovery and modeling.
- Substantially increase number of bat samples screened using 454 pyrosequencing.
- Viral isolation and characterization of newly identified Paramxyoviruses and other new viruses using pipeline at the Australian Animal Health Laboratory (AAHL) using bat cell lines (e.g. (Crameri et al. 2009)).
- Continue to integrate test results from laboratory /pathogen discovery with global risk models. Refine and update bat virus hotspot risk model by integrating new drivers and data sets.
- Refine residual models to identify areas to target for cost-effective future sampling. Use data from deep sampling to better target specific routes of transmission and species of greatest risk for human exposure to novel bat pathogens.

- Expand deep sampling and pathogen detection on new bat taxa to better quantify shared viral diversity across species in the same ecological community. Refine species accumulation curve and optimal sampling analyses.

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