An EcoHealth Approach: Prediction and prevention of emerging infectious diseases from wildlife

FINAL TECHNICAL REPORT

EcoHealth Alliance

University of Sao Paulo

MARCH 2015

Table of contents

1. Research team

Coordination
Peter Daszak, PhD – President, EcoHealth Alliance
Fernando Ferreira, PhD – Veterinary Faculty, University of Sao Paulo
Alessandra Nava, PhD – Visiting Researcher, FIOCRUZ ILMD Amazonas
Elizabeth Loh, MSc – Research Scientist, EcoHealth Alliance; PhD candidate, Durrell Institute of Conservation and Ecology, University of Kent

Virology
Columbia University, Center for Infection and Immunity (CII)
Simon Anthony, PhD – Virologist, Columbia University, CII

University of Sao Paulo, Institute of Biomedical Sciences (ICB II)
Edison Durigon, PhD – Laboratory director, ICB II
Angelica Campos, PhD – Postdoctoral scholar, ICB II
Dani Bruna, PhD – Postdoctoral scholar, ICB II

Laboratory technicians
Eliza Liang
Isa Navarette

Social Science Research
Ricardo Agum Ribeiro, MSc – Visiting Researcher, FIOCRUZ ILMD
Renato Samuel Duarte de Souza - UFAM (Amazonas Federal University) student
Hyago Gomes Pereira - UFAM student
Nelma Oliveira - UFAM student
Rosana Carvalho - UFAM student
Tatiana Mota - UFAM student
Rebeca Costa -UFAM student

Ecology
Moises Guimares – BSc, UNESP
Juliana Suieko-Shimabukuro – DVM, MSc
Elizabeth Loh – MSc, PhD candidate

Local Health Departments
Anavir Marcos Christofolli - DVM- former Health Center Chief in Teodoro Sampaio
Cesar Lessa - former DISA chief (Health District East) - Manaus

Field support
Miller Henrique Machado - Morro do Diabo state Park field guide
"Cido" Aparecido Gomes Maia – Representative of the Landless People Movement
Roberto Gomes Maia - Field assistant

2. The Research Problem

Land-use change is a clear threat to global biodiversity and ecosystem services (Groom, et al. 2006). It is also considered a key driver of emerging infectious diseases (EIDs) (Murray and Daszak 2013,Patz, et al. 2004). Environmental change can alter vegetation patterns, microclimates, and host species dynamics (e.g., abundance, distributions, and demographics) creating new and unprecedented contact opportunities between humans and wildlife. Nearly 75% of emerging infectious diseases in humans are of zoonotic origin, the majority of which originate in wildlife (Jones, et al. 2008). Given this, a better understanding of human-animal contact, as well as how fragmentation influences host diversity and viral diversity, is critical to understand how zoonotic infections emerge and spread.

Based upon previous work and strong relationships with local communities and municipal governments, our research team from the University of São Paulo (USP) and New York-based EcoHealth Alliance have used active surveillance of bat and domestic animal populations to detect pathogens that cause emerging and established zoonotic diseases. Further, in collaboration with local health departments, we have worked directly with local communities to quantify human-animal contact in order to better understand infectious disease risk. The focus of this project is to investigate the mechanisms underlying disease emergence by assessing the impacts of land use change, measured as forest fragmentation, on viral diversity and bat host assemblages.

We conducted project activities in two regions of Brazil: 1) the Atlantic Forest and 2) the Brazilian Amazon. In the Atlantic Forest, we are working in Pontal do Paranapanema. This area is located in the extreme western part of the Atlantic Forest in São Paulo State and is one of the most threatened biodiversity hotspots in the world (Myers, et al. 2000). The process of deforestation in the region is relatively recent beginning about 50 years ago, but only 17% of the original biome remains in a matrix composed mainly of pastures and sugar cane plantations. In addition, there is a recent history of agrarian reform in this region and many of the local communities with which we work were formerly part of the Movimento dos Trabalhadores Sem Terra (Landless Workers Movement). In this site, we conducted bat and domestic animal surveillance in 14 sites in and around Morro do Diabo State Park (**Figure 1**). Despite its environmental importance, this park is under intense economic and demographic pressure.

Figure 1. Forest fragments in Pontal do Paranapanema surrounded by a matrix of cattle pasture and sugar cane.

The Amazon Basin harbors the largest remaining area of contiguous forest on the planet, playing a key role in global carbon cycles and climate regulation, and housing nearly one-third of the planet's biodiversity. Yet over the last decades, approximately one-fifth of the Amazon rainforest has been converted into agricultural lands and cattle farms. Under the USAID PREDICT grant, EHA conducted active wildlife surveillance along an anthropogenic disturbance gradient in 3 sites in each of 3 land-use gradient levels: low (pristine forest landscape), intermediate (semi-disturbed landscape) and high (highly disturbed landscape). Sampling was focused on three high-risk mammal groups: rodents, bats and primates. To gain a more complete understanding of spillover risk at the landscape level, and to complement the wildlife surveillance work being carried out by PREDICT, our research team conducted a structured household questionnaire survey in each of the 3 land-use gradients where our PREDICT team is conducting wildlife surveillance (**Figure 2**). In each site, these surveys will be used to quantify human contact with potential wildlife reservoirs.

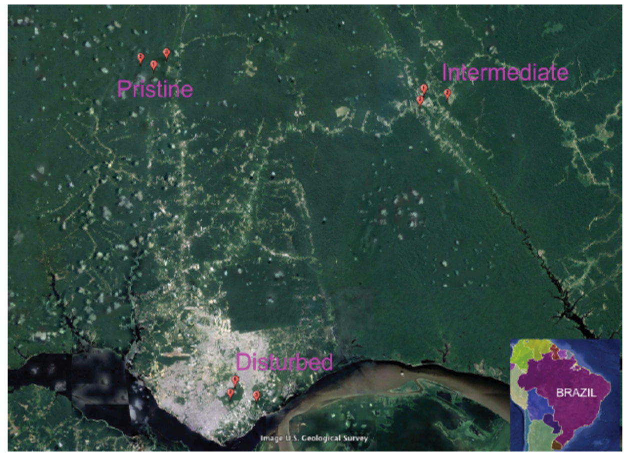


Figure 2. Sampling sites along a land-use disturbance gradient in and around Manaus.

Using results from intensive interviewing and behavioral characterization, we are exploring how this fundamental but poorly quantified measure varies with land-use practices and intensity of disturbance. By quantifying contact we are also providing a basis for determining which populations are at higher risk and what types of behavior change might be needed for mitigation strategies (see human-animal contact section below). Research conducted by our research team, in addition to research funded by USAID's PREDICT program (wildlife surveillance in the Amazon site), will contribute to a more complete understanding of potential risk in these two regions.

3. OBJECTIVES

General

To develop an EcoHealth approach integrating ecological, epidemiological and socio-demographic methodology to study the diversity of pathogens in bat and livestock populations, their linkages with ecosystem drivers and the modes of interaction of people, livestock and wildlife in Brazil.

Specific

1. To quantify the types and frequencies of human-animal contact and assess the influence of land-use change on different metrics of contact;
2. To use active surveillance and sampling of bat and domestic animal populations to detect emerging and endemic pathogens and their linkages with ecosystem drivers and human exposure;
3. To test the effect of land-use change (e.g. fragmentation) on pathogen diversity and disease risk;
4. To collect socio-demographic and baseline data on perceptions of disease, and perceptions of disease prevention and control in order to evaluate correct and incorrect knowledge about pathogens, vectors and reservoirs, and self-protective measures; and
5. To promote community awareness, primary prevention and practical solutions based on existing scientific knowledge that integrates the participation of rural communities and stakeholders to ensure the adoption and sustainability of such solutions.

4. Methodology

EcoHealth Alliance and University of Sao Paulo have implemented this study to enhance the understanding of ecological factors that drive zoonotic disease emergence due to land-use change. It has and will continue to provide detailed information about relative risk at a local level - a scale at which humans live and interact with wildlife and livestock. Characterizing known and unknown viral diversity and describing the relationship between viral diversity, host diversity, land-use change, and human ecology is critical for better understanding of the ecological processes behind zoonotic disease emergence so that disease outbreaks can be prevented.

Approximately 20% of novel emerging infectious diseases (EIDs) and 50% of emerging and re-emerging zoonotic diseases have been attributed to land-use change. Land-use changes are thought to affect the risk of cross-species transmission ("spillover") by perturbing the dynamics of pathogens in wildlife hosts and/or by bringing novel host-pathogen pairs (including humans) into contact for the first time (Murray and Daszak 2013).

Our project specifically aimed to evaluate how increasing land development influences 1) patterns of bat diversity; 2) corresponding patterns of viral diversity; and 3) patterns of human occupancy, abundance, and behavior that may influence contact rates with wildlife in changing landscapes.

The study spanned two distinct regions in Brazil – the Brazilian Amazon (around Manaus) and the Atlantic Forest (in Pontal do Paranapanema). Each region presents an excellent opportunity as a model system because each is highly biodiverse and under extensive pressure from land-use changes, factors that render them among the world's 'hotspots' of disease emergence risk.

Our project employed a systematic, gradient-based sampling scheme in our Atlantic Forest site containing fourteen field sites – three sites in continuous forest, four sites in large fragments (1000-2000ha), three sites in small fragments (<200 ha) and four sites in the human settlement communities bordering the Morro do Diabo state park. Host diversity measurements focused on bat communities, as this taxa has been implicated in several recent outbreaks of emerging zoonotic diseases including (SARS-CoV, MERS-CoV, and Nipah). At each of our sites, standardized methods were employed to characterize local species richness and diversity. From each animal captured within these surveys, blood, saliva, and rectal swabs were obtained. Urine and feces were also opportunistically collected.

A subset of samples were analyzed at the University of Sao Paulo, Institution of Biomedical Sciences (ICB II) by consensus PCR for the detection of known and new viruses belonging to up to 9 viral families. The remaining samples are currently being analyzed at the Center for Infection and Immunity at Columbia University. Field sampling at each site was conducted at least twice per year (in the wet season and in the dry season) for two years at each site to minimize the effect that seasonality might have on the likelihood of detection of both host and viral species.

In addition to viral surveillance and bat surveys, human-animal contact surveys were implemented to characterize human-animal contact at the landscape scale in both sites. Particular attention was paid to contact with bats, rodents, and primates, as well as other types of wild and domestic animals to which people are frequently exposed. While the core questions asked were the same across both research sites, the survey was adapted based on results from qualitative focal group research, then tailored to each region, local population, and setting in which it was implemented. Results from the surveys indicate how human-animal contact, a fundamental but poorly quantified measure in disease systems, varies with land-use practices and intensity of disturbance.

Framework

EcoHealth Alliance has developed a framework of sampling and analysis (building off Lloyd-Smith, et al. 2009) for the three focal areas of our project, representing three key components of spillover potential (pathogen, contact, and transmission potentials; see Figure 3 below), for an unknown pathogen with wildlife origins. Figure 3 also shows how both survey methodologies allowed us to capture each parameter critical for our analysis of disease emergence risk: 1) wildlife and viral survey (blue box) and 2) human-animal contact survey (green boxes), both undertaken across a land-use development gradient (brown shading), which represents the key driver (land-use change) of disease emergence under investigation in this study.

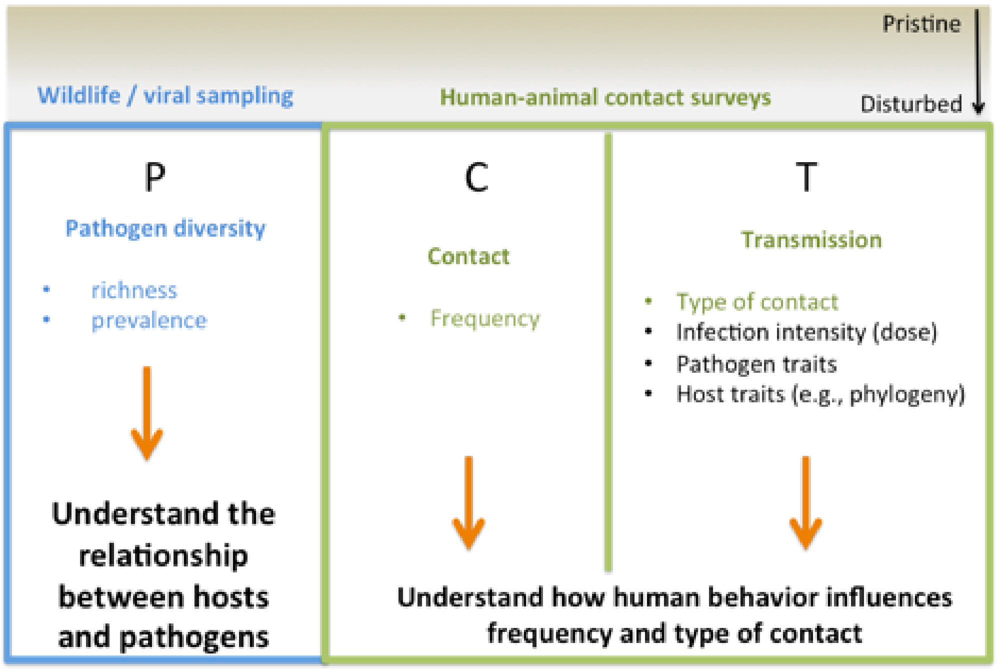


Figure 3. Schematic of Deep Forest survey components and sampling design. Descriptions of each box appear in the text above and below. P = pathogen potential; C = contact potential; T = transmission potential.

5. Project activities

5.1 Human-animal contact surveys

Human-animal *contact potential* is the capacity for a landscape to sustain spillover-relevant contact 'events' (e.g. eating or encountering wildlife) and is dominated by **contact frequency**. The household surveys asked respondents carefully designed questions from which an estimate of contact frequency for numerous different contact types could be inferred. In addition, these surveys allowed us to characterize anthropogenic activities in each of our sites (e.g. hunting pressure and agricultural practices), identify potential transmission routes, and assess potential risk of spillover into human communities. The survey consisted of six parts, including: 1) household information; 2) domestic animals; 3) wildlife; 4) animals eaten and/or hunted; 5) shared resources; and 6) beliefs, attitudes and knowledge about disease.

Transmission potential is the capacity for a landscape to promote factors that increase the likelihood of transmission given a contact event. Although numerous factors may influence likelihood of transmission given contact, we focused on the **type of contact** event (more 'risky' contact types are hypothesized to represent greater risk of spillover), where riskiness is assessed primarily via expert opinion, taking into consideration additional factors, such as duration or 'intimacy' of contact (e.g. eating vs. seeing). The household surveys asked respondents about a range of different direct and indirect contact types that represent potential risk factors for disease spillover. Examples include wildlife hunting, butchering, or consumption and seeing wildlife or their fecal material in the home. For analyses, the *contact potential* (see above) is combined with *transmission potential* to map landscape-level relative risk for each contact type.

5.1.1 Pontal do Paranapanema

The Morro do Diabo conservation area is located in the Pontal do Paranapanema basin in the western part of São Paulo state. Morro do Diabo State Park is the largest fragment in the region, comprising an area of 33,845.33 ha. Around the park, there are 63 small properties of agrarian reform settlements and 10 large farms with monoculture of sugar cane and livestock. Currently more than 3000 families have been settled in small properties (15-18 hectares) around the park and surrounding forest fragments. During the course of our project, we administered 200 surveys across a fragmentation gradient in this region including 50 households in the city of Teodoro Sampaio, 50 households in the settlements located around large fragments, and 100 households in the settlements located directly adjacent to the Morro do Diabo State Park. The surveys were conducted in people's homes.

5.1.2 Amazonia

Our survey sites in the Amazon region are located along a land-use gradient spanning approximately 50km from the city of Manaus. Our sites included one site located in preserved primary forest (pristine), one site in secondary forest (intermediate) and one site in a highly deforested area (urban). Our urban sites were located in a forest fragment, managed by the Federal University of Amazonas, situated in the city of Manaus. The intermediate sites were located in Rio Preto da Eva, specifically in the Beija-Flor indigenous community. This community is made up of different ethnic groups of the Sataré-Mawe, Tukano, Dessano, Twiuca, Apurinâ, Baniwa, Arara, Marubo, and Mayuruna, which are distributed among three communities: Beija flor I, Beija Flor II and Beija Flor III. During the course of our project, we administered 503 surveys across a land-use gradient including 303 households in the city of Manaus, 100 households in the pristine site, and 100 households in the intermediate site. The surveys were conducted in people's homes.

5.2 Bat sampling in Pontal do Paranapanema

We conducted bat surveillance in 14 sites in and around Morro do Diabo State Park including 4 small fragments (<200 ha), 3 large fragment (1000-2000ha), 3 continuous forest sites (located within 33,845ha of continuous forest) and four sites within the human settlements. To characterize local species richness and diversity, we deployed eight ground-based mist nets, one canopy net and a harp trap. Mist nets were deployed at sunset and remained open for a total of 6 hours. We collected blood, saliva, and rectal swabs from each bat and urine and feces were also opportunistically collected. In total, we sampled 1407 animals (Table 1) and recorded 25 bat species.

Table 1. Total number of captures by gradient level and season.

	Dry	Rainy	Total
Settlements	310	293	603
Continuous	396	96	492
Large	62	63	125
Small	132	55	187

5.3 Livestock sampling in Pontal do Paranapanema

A total of 184 domestic animals were sampled in the settlement communities bordering the park including: 114 cattle, 5 horses, 37 chickens, 18 dogs, and 10 pigs. These samples are awaiting laboratory diagnostics.

5.4 Laboratory diagnostics

5.4.1 University of Sao Paulo

During the course of the project, two post-docs were trained in consensus-PCR techniques. A total of 972 samples from 335 bats were screened at ICB II for nine viral families including Astrovirus, Coronavirus, Hantavirus, Herpesvirus, Paramyxovirus, Alphavirus, Arenavirus, Filovirus and Flavivirus

5.4.2 Columbia University

A total of 1177 samples were tested from 392 bats for the coronavirus family, using consensus PCR. An additional 1000 samples were screened for influenza viruses. Laboratory diagnostics is ongoing at Columbia University. All remaining samples will be screened for coronaviruses, influenza viruses, and herpesviruses. We expect all viral screening to be complete by August 2015.

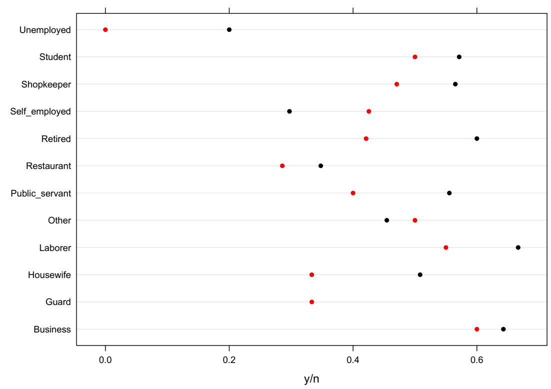
6. Project Results

6.1 Human animal contact surveys

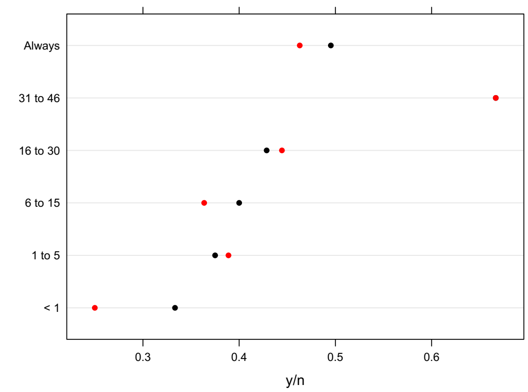
The surveys from both sites were analyzed together to determine how contact varies by different subgroups and whether there are specific populations at higher risk for disease spillover. Aggregated data has been shared with the health department in Teodoro Sampaio at their request, so they can also look at contact rates and behaviors relevant to endemic diseases such as malaria. We found that women report consuming wildlife more frequently than men, with little effect from occupation. We also found that the length a person has resided in a given area positively correlate with the frequency at which they consume wildlife (Figure 4).

Figure 4 (below) illustrates the effects of different socio-economic factors on whether or not a respondent reports eating wildlife, one metric of human-animal contact. According to our results, it appears that women (black dots) generally tend to eat wildlife more frequently than men (red dots) with little effect from occupation (panel a), and respondents who have resided in an area longer tend to eat wildlife more frequently than those that have just moved to an area (panel b).

a.



b.



In addition to determining how contact varies by different subgroups, we also examined gradient level effects on different metrics of contact. We found that self-reported wildlife consumption patterns differed considerably among gradient levels (Figure 5), which may be partially reflective of cultural differences and laws and their enforcement regarding wildlife protection. Generally speaking, there was less wildlife consumption reported in the disturbed sites compared to the intermediate and pristine sites. This finding has implications for the risk of spillover from wildlife as a result of wildlife consumption in different cultural contexts and illustrates why all potentially high-risk behaviors should be explored across geographic and cultural zones.

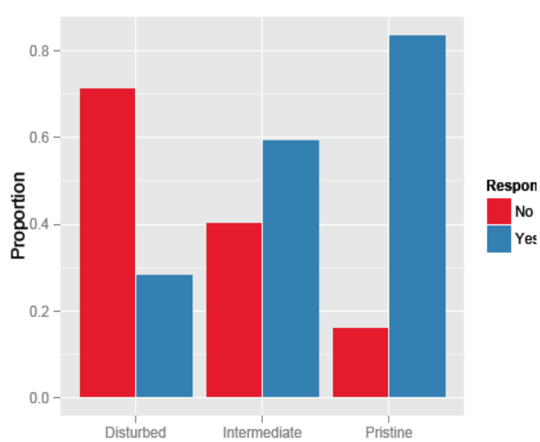


Figure 5. Proportion of respondents reporting wildlife consumption across three gradient levels in Manaus.

Figure 5 illustrates the effect of the gradient level on one high-risk behavior, the **consumption of wildlife**, just one example of a specific *contact type* (direct contact) of interest. Self-reported wildlife consumption patterns differed considerably among gradient levels and may be partially reflective of cultural differences and laws and their enforcement regarding wildlife protection. Generally speaking, there was less wildlife consumption reported in the disturbed sites compared to the intermediate and pristine sites. This finding has implications for the risk of spillover from wildlife as a result of wildlife consumption in different cultural contexts and illustrates why all potentially high-risk behaviors should be explored across geographic and cultural zones.

Mapping Human-animal Contact

In order to understand how human-animal contact relates to landscape-level risk of disease spillover, we also took into account the spatial distribution of both landscape disturbance and the human population size that inhabits our study landscapes. Methodologically, we developed a four-step process (Figure 6, below). The results show that even though wildlife consumption is generally higher in pristine areas (see above), the overall contact rate with animals due to consumption does not follow the same pattern, with population size driving the overall rate of human animal contact at the landscape level. Importantly, the results show that wildlife consumption based human-animal contact varies spatially across the landscape gradient.

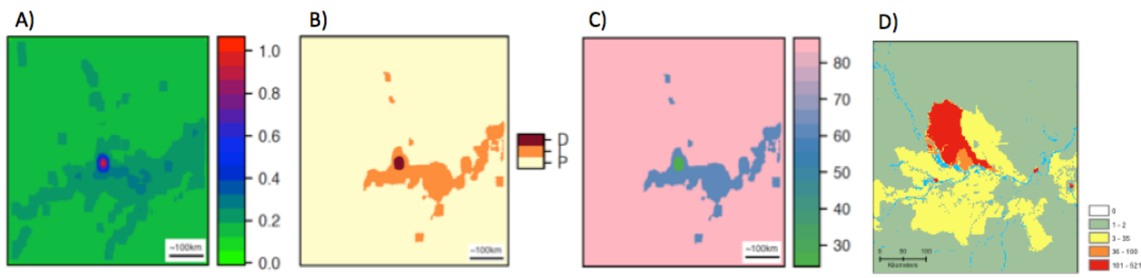


Figure 6. Steps showing the creation of relative human-animal contact rate maps at the landscape level for our Amazon site. The contact type illustrated here is wildlife consumption as reported in the DFHC survey. A) Step 1: Raw landscape disturbance index (range 0 = pristine, 1= highly disturbed). B) Step 2: Reclassified LDI to match disturbance gradient levels used for wildlife and viral sampling and DFHC surveys (P = pristine, I = intermediate, D = disturbed). C) Step 3: Percentage of respondents reporting wildlife consumption mapped to gradient scale. D) Step 4: Final result illustrating relative human-animal contact rate (scale is an index of consumption contacts per grid cell) at the landscape scale, derived by multiplying the proportion of respondents reporting wildlife consumption by human population size per pixel.

6.2 Laboratory diagnostics

University of Sao Paulo laboratory results:

- A total of 972 samples from 335 bats were screened at ICB II for nine viral families including Astrovirus, Coronavirus, Hantavirus, Herpesvirus, Paramyxovirus, Alphavirus, Arenavirus, Filovirus and Flavivirus,
- 172/335 bats were screened from the settlement communities
- 163/335 were screened from the forested areas
  23 animals were positive for the following viruses: 12 Coronavirus positives, 2 Paramyxovirus positives, 1 hantavirus positive, 3 herpesvirus positives, and 4 astrovirus positives.
- Of the 23, 16 individuals were from the settlements, and 6 were from forested areas (Figure 8).

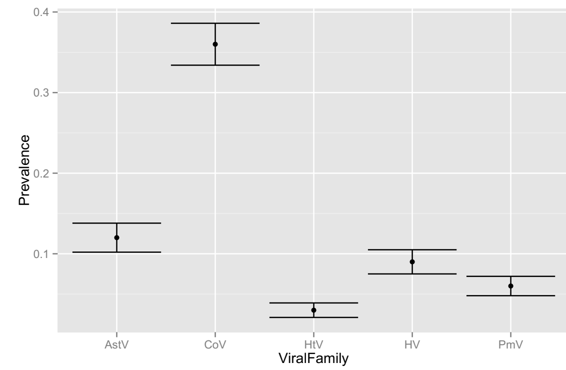


Figure 7 shows the prevalence of each viral family.

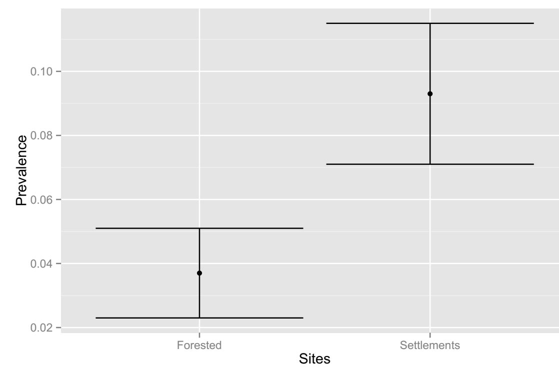


Figure 8 shows the overall viral prevalence in settlement communities is 9.3% (16/172 bats) compared to 3.7% in forested areas (6/163 bats).

A chi-square test of independence was performed to examine the relationship between total viral prevalence and level of forest disturbance. The relationship between these variables was significant ($X^2 = 4.31$, df=2, $p<0.05$) indicating total viral prevalence was significantly higher in the bat populations in human settlement communities compared to those bats in forested areas, which could have significant implications for spillover risk.

Viruses detected at USP:

- The following known coronaviruses were detected:
  - A strain of BtCoV/KCR260/Car_per/CRC/2012 (Betacoronavirus) was found in 2 Flat-faced fruit-eating bats (*Artibeus planirostris*) and 1 Wagner's bonneted bat (*Eumops glacinus*)
  - A strain of Mex_CoV-11b was found in 1 Flat-faced fruit-eating bat (*Artibeus planirostris*)
  - Infection with CoVs is often asymptomatic, however, they can be responsible for a wide range of respiratory and enteric diseases of medical and veterinary importance. The genus Betacoronavirus includes viruses that are of significance to public health such as SARS and MERS, however the betacoronavirus above is not considered to be closely related to either of these viruses. Therefore, at this time there is no evidence to suggest these viruses poses a threat to human health.

- The following known paramyxoviruses were detected:
  - Canine distemper virus was detected in 1 Velvety free-tailed bat (*Molossus molossus)* and 2 Black Myotis (*Myotis nigicans*)
  - Canine distemper virus (CDV) is a morbillivirus that is the etiological agent of one of the most important viral diseases affecting canids and an expanding range of other carnivores.

- The following known hantavirus was detected:

- A strain of Juquitiba virus Oln_9845 was detected in 1 Flat-faced fruit-eating bat (*Artibeus planrostris*)
- Juquitiba virus is an agent of Hantavirus Pulmonary Syndrome (HPS). HPS is a severe, sometimes fatal respiratory disease in humans caused by infection with a hantavirus. Hantaviruses are among the most important zoonotic pathogens of humans, and are primarily associated with rodents.

Columbia University laboratory results:

- Laboratory diagnostics is currently ongoing at Columbia University.
- A total of 1177 samples were tested from 392 bats for the coronavirus viral family. Four distinct coronaviruses were detected in 25 animals, of which 3 are known viruses and 1 is new.
- A total of 1000 samples were screened for the influenza viral family. One influenza virus was detected in 7 animals.
- The following known Coronaviruses were detected:
    - A strain of BatCoV/Trinidad/1FY2BA/2007 was found in 13 Seba's short-tailed bats (*Carollia perspicillata*) and 1 Flat-faced fruit-eating bat (*Artibeus planirostris*)
    - A strain of BatCoV/Mex_CoV-4 was found in 1 Great fruit-eating bat (*Artibeus lituratus*), 4 Flat-faced fruit-eating bats (*Artibeus planirostris*), and 2 Fringed fruit-eating bats (*Artibeus fimbriatus*)
    - A strain of BatCoV/Mex_CoV-5 was found in 2 Flat-faced fruit-eating bats (*Artibeus planirostris*)
- The following known influenza virus was detected:
    - H18N11 influenza virus was found in 7 Flat-faced fruit-eating bats (*Artibeus planirostris*)

The following new coronavirus was detected:

- IDRC/CoV-1 was detected in 2 Little yellow-shouldered bats (*Sturnira lilium).*
- Infection with CoVs is often asymptomatic, however, they can be responsible for a wide range of respiratory and enteric diseases of medical and veterinary importance. However, at this time there is no evidence to suggest that the four coronaviruses above pose a threat to human health.

7. Project Outputs

7.1 Research*:*

- The Deep Forest Booklet (research protocol)

Publications:

- E.H. Loh, K.A. Murray, A. Nava, A. Aguirre, P Daszak. 2015. Biodiversity and disease risk in fragmented landscapes: evaluating the links between land use change and infectious disease emergence. In "Tropical Conservation: Perspectives from Local and Global Priorities" A.A. Aguirre. Oxford University Press (in prep).

- Abstract:

  During the past decade, tropical forests have continued their long-term reduction in extent, largely due to human activities such as agricultural expansion, deforestation, and conversion of natural habitats to other land uses. Land-use change is a clear threat to global biodiversity and ecosystem services. It is also considered a key driver of emerging infectious diseases (EIDs). In this chapter, we (i) review what is currently known about these linkages, focusing on the influence of human-driven ecological changes on host diversity and disease risk, (ii) propose a new hypothesis on potential host and pathogen responses to habitat fragmentation, and (iii) provide recommendations on long-term studies and research efforts to improve current understanding of these relationships, with potential implications for both biodiversity and human health.
- E.H Loh, KJ Olival, C Zambrana-Torrelios, TL Bogich, C Kreuder-Johnson, JAK Mazet, WB Karesh, P Daszak. 2015. Targeting transmission pathways for emerging zoonotic disease surveillance and control. Vector-borne & Zoonotic Diseases (in press).
- Abstract:

  We used literature searches and a database of all reported emerging infectious diseases (EIDs) to analyze the most important transmission pathways (e.g. vector-borne, aerosol droplet transmitted) for emerging zoonoses. Our results suggest that at the broad scale, the likelihood of transmission occurring through any one pathway is approximately equal. However, the major transmission pathways for zoonoses differ widely according to the specific underlying drivers of EID events (e.g. land-use change, agricultural intensification). These results can be used to develop better targeting of surveillance for, and more effective control of newly emerged zoonoses in regions under different underlying pressures that drive disease emergence.
- E.H. Loh, K.A. Murray, C. Zambrana-Torrelio, P.R. Hosseini, W.B. Karesh, P. Daszak. 2013. Ecological Approaches to Studying Zoonoses. Microbiology Spectrum 2013.

Conference Abstracts:

- Campos, Angelica. 2013. Detection of Emerging Viruses in Bat Populations from Morro do Diabo State Park and surrounding human settlements, São Paulo, Brazil. XXIV Brazilian Virology Congress.
- Loh, EH. 2014. Effects of forest fragmentation on viral diversity and bat species richness in and around Morro do Diabo State Park , São Paulo Brazil. 3rd Annual Congress on Disease Ecology.

Further research:

- Samples collected during this project are also being used for Masters projects at the Center for Infection and Immunity at Columbia University.

7.2 Media

- National Geographic Documentary featuring our research project aired on national television in Brazil during February 2014. https://www.dropbox.com/sh/f03r8vljszfjmx8/AABCdGrTcLlFHsw18duKyPfwa?oref=e&n=18932040

7.3 Capacity:

- Trained 29 individuals from government partners, universities and local health departments in wildlife and livestock surveillance, household survey implementation and diagnostic techniques.
- Provided support and supplies to enable diagnostic testing of hundreds of wildlife samples for viruses of pandemic potential
- Held several workshops in both sites with participants from local government departments, local schools and communities, and universities to help build working relationships and strengthen interdisciplinary communication.
- Established formal collaboration between EHA, USP and Fiocruz via an official Memorandum of Understanding (MOU) demonstrating the establishment of a dedicated, sustainable in-country team.
- Renato Samuel, a member of our social science research team, received a research scholarship from FIOCRUZ under the coordination of our team's anthropologist Ricardo Agum.
- Project coordinator, Elizabeth Loh, will be submitting her PhD dissertation in 2015 based on research conducted during this project, which we expect to result in an additional 3 publications submitted before the end of 2015.
- Project coordinator Alessandra Nava is now on the board of the International Association of Ecology and Health
- Research team members Ricardo Agum and Alessandra Nava have joined the FIOCRUZ ILMD Disease Ecology group.

7.4 Development of an EcoHealth Network:
Throughout the course of our project, we have developed a dynamic collaborative network of researchers and students interested in EcoHealth Approaches to Health. We have strengthened collaborations with project stakeholders in both sites including local and provincial health departments, government departments, protected area management, universities, and the general public.

We have worked in Pontal do Paranapanema for over a decade during which we have established a strong network of interdisciplinary collaborations with conservation organizations, protected area management, and the local health department in Teodoro Sampaio. However, since we had not previously worked in

Manaus, we invested significantly more time early on in the project setting up research meetings, meeting with researchers from different disciplines and exploring how joint efforts could result in scientific and social benefits.

To strengthen the social dimensions of our project, we established academic links with FIOCRUZ Amazonia and two departments at UFAM - the Faculty of Social Sciences and the Department of Biology.

Dr. Sergio Luz, Director of FIOCRUZ Amazonia guided us in recruiting and hiring Project Anthropologist Ricardo Agum and assisted in the development of both the quantitative and qualitative survey methods. This collaboration resulted in the establishment of an official MOU between FIOCRUZ, EHA and USP. In addition, Project Coordinator Alessandra Nava and Project Anthropologist Ricardo Agum are now both part of FIOCRUZ Disease Ecology Group. We have already submitted several grant proposals, which was a direct result of this collaboration.

We worked with Professor Marcelo Gordo and his lab from UFAM's Department of Biology to gain a better understanding of what types of animals are present and how people use and interact with the forest. Prof Gordo has worked in UFAM fragment for over a decade and his perspective was invaluable in determining not only the types of animals found in each site, but also the range of activities occurring in the forest that result in human-animal interactions.

Lastly, during the project we established a very strong relationship with the Faculty of Social Sciences. This partnership resulted in the training of six students as interviewers who accompanied the DISA health agents to administer the survey. They were instrumental in achieving adequate sample sizes (over 500+ surveys).

7.5 Parallel funds and in-kind support:

- During the course of the project, EHA's PREDICT grant supported laboratory diagnostics and the salaries of laboratory coordinator Danielle Durigon, project coordinator Alessandra Nava, and project coordinator Elizabeth Loh.
- Our research team has secured matching in-country funding from various departments of the Brazilian government including the Ministry of Culture and CNPQ/FAPEAM to continue our research totaling over $180,000 reais including:
    1. Project title: Pardo Vision
       Funding source: Ministry of Culture
    2. Project title: Politics of Malaria
       Funding source: CNPQ/FAPEAM
    3. Project title: Ecosystem Health in the Central Amazon: effects of anthropogenic change on the epidemiology of infectious diseases.
       Funding source: CNPQ/FAPEAM

- University of Sao Paulo: The Faculty of Veterinary and Preventative Medicine provided funding for PI Professor Fernando Ferreira's full salary during the course of the project.
- UFAM Faculty of Social Sciences: provided six students to administer the surveys in the Manaus site.
- Department of Health (DISA) Manaus: facilitated access to the communities by allowing our interviewers to accompany their local health agents during community visits.
- FIOCRUZ Amazonia: provided facilities and supplies for our survey work and hosted several meeting with our collaborators.

8. Project Outcomes

During the course of our project, we have developed an innovative, strategic framework to enhance the understanding of ecological factors that drive zoonotic disease emergence due to land-use change. This approach involved interdisciplinary collaborations (Figure 10) and can also be used to help understand the risk of spillover for known pathogens.

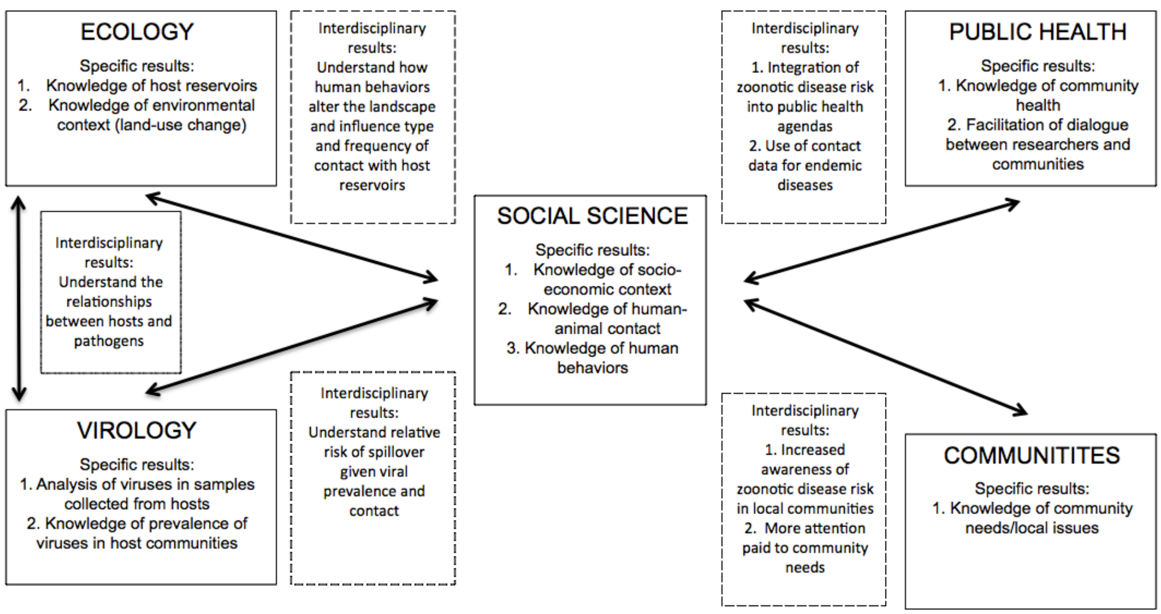


Figure 10. Conceptual diagram of stakeholder involvement, illustrating the relationships between the different disciplines, their specific results, and specific interdisciplinary outputs.

One of the most significant outcomes of our project is the strengthened relationship between two prominent Brazilian research institutions, Fiocruz ILMD and University of Sao Paulo, and EcoHealth Alliance. As a direct result of our project, two members of our research team (Alessandra Nava and Ricardo Agum) are now officially part of the FIOCRUZ ILMD Disease Ecology group.

Another significant project outcome is the integration of topics including zoonoses, biodiversity and ecosystem health into the public health agenda in Teodoro Sampaio. This was particularly challenging because these topics were relatively novel to many stakeholders and required numerous meetings and workshops to introduce such issues. Another challenging obstacle was gaining the trust and acceptance of the communities in Manaus. The involvement of representatives from the local health department in Manaus –DISA Leste – was paramount in gaining access to the communities where we conducted our household surveys emphasizing the importance of local buy-in in both of our sites in Brazil.

9. Overall Assessments and Recommendations

In a very short time frame (3 years) our project has accomplished a significant amount of research and capacity building in two distinct regions of Brazil, in part by leveraging in-country support and other donor funds. EHA's PREDICT-1 grant focused on detection and discovery of zoonotic diseases at he wildlife-human interface. The Deep Forest project is a unique and targeted study emphasizing rigorous and systematic sampling methodology to address the ecological factors that drive zoonotic disease emergence due to land-use change. In Manaus, the Deep Forest project is evaluating how increasing land-use development affects 1) patterns of biodiversity, 2) corresponding patterns of viral diversity and 3) patterns of human occupancy, abundance and behavior. In Brazil, USAID is funding the first two components of this project: patterns of biodiversity and corresponding patterns of viral diversity, while IDRC is funding the social component evaluating patterns of human occupancy, abundance and behavior. To gain a better understanding of the social dimensions of spillover risk, we conducted over 500 household surveys in the same sites where PREDICT was conducting concurrent wildlife sampling.

The household surveys in both sites were an ambitious undertaking, resulting in over 700 household surveys conducted between both sites. This data will be combined with USAID's wildlife and viral data in Manaus and our data from Pontal to estimate the relative risk of novel and existing viral spillover due to land-use change. While the aim of this project is to estimate the relative risk of spillover of *unknown* pathogens from wildlife in dynamics landscapes, <u>we also believe that this approach could be scaled up and used to help understand the risk of spillover for known pathogens in other landscapes</u>. For example, since human-animal contact is a risk factor for many known diseases (e.g., Ebola), quantifying bushmeat consumption and contact rates at the landscape scale, as shown in Figure 6, could also help focus surveillance or mitigation activities to reduce the risks of spillover from bushmeat consumption. The same systematic approach could be applied to better identify the risks attributable to other hypothesized drivers of disease emergence.

At a regional level, our project has contributed to development in several significant ways. In terms of capacity-building, our team has trained over 29 individuals from various government departments, protected area employees, public health officials, and local settlers, several of which are still actively involved in ongoing research in the region, which we attribute to the training and opportunities they received from participating in the project.

Further, in Brazil there has been a recent history of agrarian reform. Many of the people from the settler communities were formerly part of the MST (Movimento dos Trabalhadores Sem Terra) who received small plots of land around Morro do Diabo State Park in recent decades. Historically the landless poor have been socially excluded and have been denied full civil rights as Brazilian citizens. Through our community workshops, we were able to bring together local community members and representatives from the health department and Morro do Diabo State Park to discuss the most important health issues from the community perspective. The Health Department in Teodoro Sampaio is now integrating community needs and concerns into the public health agenda, with representatives from the local settler communities involved in public health issues, which we consider to be a successful outcome of our project.

Our project hypothesized that landscapes with more viruses available (deeper viral pool) will contribute to greater spillover risk, holding the other risk factors constant (contact types and contact rates). We will empirically relate the depth of the viral pool (from which novel pathogens may emerge) to wildlife diversity and abundance, with final analysis and results pending completion of viral testing by August 2015. Once available for analysis, our wildlife and viral results will be combined with the models presented above to estimate the relative risk of novel viral spillover due to land-use change. However, further research is critical to better understand the transmission component of spillover risk. In order to better understand what spillover is actually occurring between people and animals, we recommend further research involving the collection of samples from people living in the areas where we have been conducting active wildlife and viral surveillance. Through our partnership with FIOCRUZ, we have unprecedented access into these communities, with local health teams already poised to undertake this work. Because of our new established partnership with FIOCRUZ, an arm of the Brazilian health Ministry, we are in the unique position to start this work immediately and bypass many obstacles associated with IRB approval, which is notoriously difficult to obtain in Brazil.

10. References

Groom MJ, Meffe GK, Carroll CR, others. Principles of conservation biology. Sinauer Associates Sunderland, Massachusetts, USA; 2006.

Murray KA, Daszak P. Human ecology in pathogenic landscapes: two hypotheses on how land use change drives viral emergence. Current opinion in virology 2013;3:79-83.

Patz JA, Daszak P, Tabor GM, Aguirre AA, Pearl M, Epstein J, Wolfe ND, Kilpatrick AM, Foufopoulos J, Molyneux D and others. Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. Environ Health Perspect 2004;112:1092-8.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. Nature 2008;451:990-3.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature 2000;403:853-858.

Lloyd-Smith JO, George D, Pepin KM, Pitzer VE, Pulliam JR, Dobson AP, Hudson PJ, Grenfell BT. Epidemic dynamics at the human-animal interface. science 2009;326:1362-1367.