**Land-use change and microbial spillover as a coupled natural-human system**

**1. Project Summary**

***Overview*:** Land-use change is a major threat to global biodiversity and ecosystem services. It is also a key driver of disease emergence in humans, animals and plants, with around half of human diseases that originate in animals (zoonoses) linked to some form of land-use change. Despite this, the mechanisms by which land-use change influences host and pathogen community ecologies and the risk of cross-species transmission (“spillover”) remain poorly understood. This proposal addresses the **dynamic, complex social and ecological processes underlying cross-species pathogen transmission due to anthropogenic land-use change**. We will conduct empirical wildlife biodiversity, virological and human ecological field studies across a gradient of landscape disturbance in Brazil to analyze three major features of the theory of spillover risk in the context of land-use change: **1)** We will investigate the ‘**pathogen potential**’ **(Aim 1)** of study landscapes by establishing whether wildlife host community ecology is predictive of pathogen community ecology underland use change; **2)** We will investigate the ‘**contact potential**’ **(Aim 2)** of study landscapes through an intensive human ecology program to map human-animal contact, and examine if human-animal contact *rate* is predictable at the landscape scale under land use change; and **3)** We will investigate the ‘**transmission potential**’ **(Aim 3)** of study landscapes by analyzing whether the *type* of human-animal contact is predictable at the landscape level under land use change. To **empirically validate our model of spillover risk (Aim 4)**, we will combine the human-animal contact survey with a human virological survey to establish how 1), 2) and 3) combine into actual risk of zoonotic infection under land-use change. From these studies, we will **develop a generic framework of disease spillover due to land-use change as a coupled natural-human system (Aim 5)**, generating predictive models of relative spillover risk due to land-use change for use in management and informing land-use decisions.

***Intellectual Merit*:** Despite a large body of theoretical and empirical studies on established pathogens, very little is understood about the mechanisms that promote previously unknown pathogens to emerge into new hosts, and how land-use change influences the processes involved. Our non-exclusive hypotheses are that: 1) land-use change influences the community ecology of pathogens via impacts on the distribution and abundance of wildlife hosts and/or vectors, modifying the likelihood of cross-species transmission, and 2) human ecology in altered landscapes influences the contact rate between humans, livestock and wildlife hosts and/or vectors and promotes pathogen sharing (spillover). Our proposed work will test the relative contributions of these hypotheses via a detailed exploration into the three components of spillover (pathogen prevalence, contact and transmission) using a combination of wildlife, pathogen and human field studies and modeling. **This project will generate new knowledge to fill fundamental gaps in disease ecology, and help elucidate the full dimension of the impacts of global environmental change** on a dynamic coupled natural-human system.

***Broader Impacts:*** This study has implications for disease ecology, biodiversity science, conservation and public health. It will contribute to the professional development of postdoctoral scholars, graduate students, and field assistants in the USA and Brazil. PI Daszak directs a current NSF RCN grant (EcoHealthNET) which will be leveraged to train US and international graduate students in multi-disciplinary projects related to this proposal. Results from the proposed work will be published in high-impact journals, presented at conferences and to the public as part of EcoHealth Alliance’s non-profit outreach programs, to the ICSU Future Earth program, to the >25 conservation and health instutional partners of EcoHealth Alliance, to the IOM Forum on Microbial Threats, and to congress via the regular briefings we hold. Our data will also be made available to public health agencies, conservation and land-use planning bodies for the ongoing effort to reduce the risk and global burden of infectious diseases, protect ecosystems and influence land-use development decisions.

BLANK PAGE – PROJECT DESCRIPTION STARTS ON PAGE 1 (MAX. 20 PAGES) IN NEXT SECTION**2. Project Description**

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| **I. Introduction** |

Human activity has altered ecosystems on a global scale through deforestation, expansion of agriculture, pollution, eutrophication, depletion of marine fisheries and increased nitrogen fixation[1](#_ENREF_1),[2](#_ENREF_2). Anthropogenic influence on landscapes has increased most rapidly in the last half century to meet increased global demand for food and natural resources[3](#_ENREF_3). These changes have perturbed biotic systems and the environment, with direct and indirect impacts on human and wildlife populations[2](#_ENREF_2),[4](#_ENREF_4). This has had some positive outcomes (e.g. increased quality of life in many regions), but many negative (e.g. increased risk of drought, lack of food security, emerging diseases), which has led to increased appreciation and understanding of the natural capital and services provided by intact ecosystems[5](#_ENREF_5).

Emerging infectious diseases (EIDs) are a key threat to global public health, livestock, wildlife and ecosystem functioning[6](#_ENREF_6),[7](#_ENREF_7). Zoonotic EIDs (those originating in animals) cause millions of deaths each year, and some single emergence events (e.g. SARS) have cost the global economy tens of billions of dollars[8](#_ENREF_8) or caused species extinctions (e.g. chytridiomycosis)[9](#_ENREF_9). The World Economic Forum considers EIDs as “major” risks, with significant likelihood of both occurrence and economic threat over the next 10 years, comparable in scale to unsustainable population growth[10](#_ENREF_10),[11](#_ENREF_11). Predicting and preventing the emergence of novel diseases is considered a public health, and increasingly a conservation, priority[12](#_ENREF_12),[13](#_ENREF_13). **Yet, despite these impacts, our understanding of what causes diseases to emerge is rudimentary.** The underlying drivers tend to be changes in socioeconomic factors (e.g. increased travel and trade), demography (e.g. population expansion), agriculture (e.g. intensification of livestock production), medical science (e.g. increased antibiotic use) and environment (e.g. land use change, deforestation)[7](#_ENREF_7),[14](#_ENREF_14),[15](#_ENREF_15). However, a more detailed, mechanistic understanding of how these drivers promote cross-species transmission and lead to disease emergence is critical to predicting and combating EID threats[12](#_ENREF_12),[15-19](#_ENREF_15).

***A. Land use change and zoonoses – a model system.*** Land use change, including agricultural conversion, deforestation, logging and other extractive industries is the underlying driver of around half of all zoonotic EIDs[7](#_ENREF_7),[16](#_ENREF_16). Because zoonoses represent the majority (~60%) of all human EIDs[20](#_ENREF_20),[21](#_ENREF_21), and humans are more feasible to monitor in detail than wildlife, human zoonoses are **an ideal model system** with which to investigate the pre-conditions of cross-species transmission and disease emergence. Previous work suggests that increased interaction among wildlife, domestic and synanthropic animals and humans following land use change increases the frequency of cross-species transmission and enhances emergence[22-24](#_ENREF_22). However, the complexities of these relationships are enormous and poorly understood[25](#_ENREF_25). For example, reservoir population changes due to habitat fragmentation[26](#_ENREF_26), loss of predators[27](#_ENREF_27), and synchronous tree masting[28](#_ENREF_28" \o "Ostfeld, 1996 #28) all appear capable of altering Lyme disease prevalence in ticks and risk of human infection. Habitat restoration that reduces tick habitat may reduce Lyme disease risk[29](#_ENREF_29). Similarly, land-use change leads to reduced avian biodiversity, altered species composition, increased anthropogenic habitat for *Culex* mosquitoes, and heightened risk of West Nile virus[30-33](#_ENREF_30). A number of studies suggest that protecting biodiversity or limiting human influence in landscapes should reduce the risk of zoonotic disease emergence[26](#_ENREF_26),[34](#_ENREF_34), potentially representing a win-win for conservation and health. Yet this suggestion is controversial[35](#_ENREF_35),[36](#_ENREF_36), and given our poor understanding of the specific mechanisms involved, there is currently little uptake of this as a disease management or conservation option. Development of a new framework to understand **what drives a novel pathogen, of unknown ecology, carried by wildlife reservoirs to emerge in the first place** may lead to significant benefits to human, livestock and ecosystem health[**37**](#_ENREF_37).

***B. ‘Spillover’ - a dynamic coupled natural-human system.*** Unusual or infrequent pathogen transmission between species (“spillover”) is the defining characteristic of an emerging zoonosis. Conceptual models suggest three factors are critical to understanding spillover: **1) the prevalence of infection in the animal reservoir, 2) the rate at which humans come into contact with these animals, and 3) the probability that humans become infected when contact occurs**[37](#_ENREF_37). These components interact with and are influenced by diverse properties of natural and human systems, while the subsequent establishment and spread of pathogens depend on their modes of transmission and evolutionary constraints (e.g. phylogeny, mutation, adaptation)[37-39](#_ENREF_37). Under land use change, human ecology drives the contact *rate* among humans and reservoir hosts and/or vectors (e.g. how and when contact with wildlife occurs) and can influence the *type* of contact (e.g. hunting, butchering or cohabitation), and the likelihood of infection given contact. Additionally, human activities may influence the prevalence of infection in animal reservoirs by perturbing their abundance, distribution, and therefore their ability to transmit microorganisms within and among populations[40](#_ENREF_40). **Thus, the interaction of human ecology with biodiversity is fundamentally important to, but so far a neglected aspect of, zoonotic disease emergence due to land use change**[41](#_ENREF_41),[42](#_ENREF_42).

***C. Specific Aims:*** We aim to understand the mechanistic rules that govern pathogen spillover due to land-use change. We will empirically evaluate our general model of spillover risk that links pathogen potential, contact potential and transmission potential in dynamic landscapes. Our overarching question is: *How does human behavior and occupancy associated with land-use change impact wildlife pathogen community ecology, govern human-reservoir contact rate and type, and determine the subsequent risk of disease spillover?* From this, five specific Aims form the basis of this proposal:

**Aim 1) Quantify pathogen potential:** Is wildlife host community ecology predictive of pathogen community ecology under land-use change?

**Aim 2) Quantify contact potential:** Is human-reservoir contact *rate* predictable at the landscape level under land-use change?

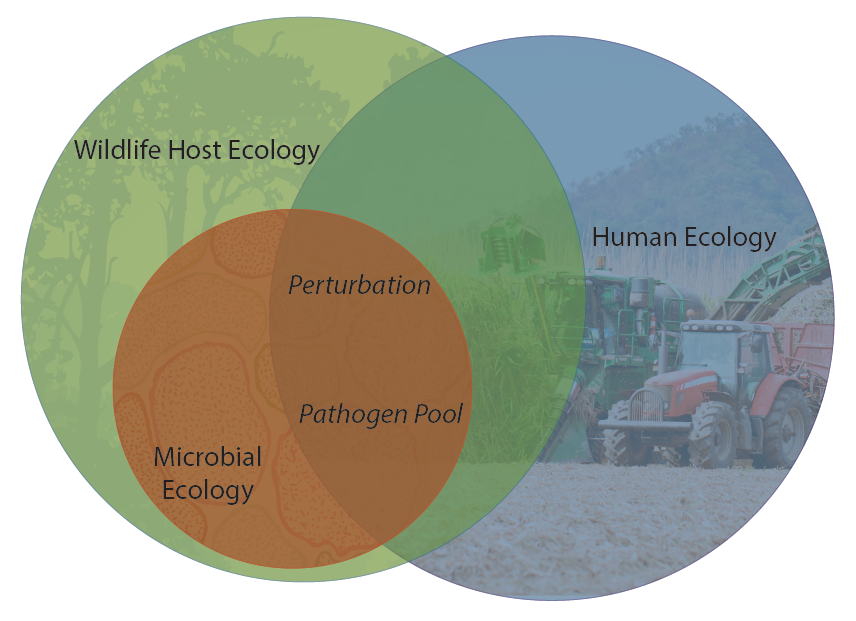
**Aim 3) Quantify transmission potential:** Is human-reservoir contact *type* predictable at the landscape level under land-use change?

**Aim 4) Model validation:** Can patterns of actual human zoonotic infection be predicted by our general model for known pathogens, and therefore extrapolated to unknown pathogens?

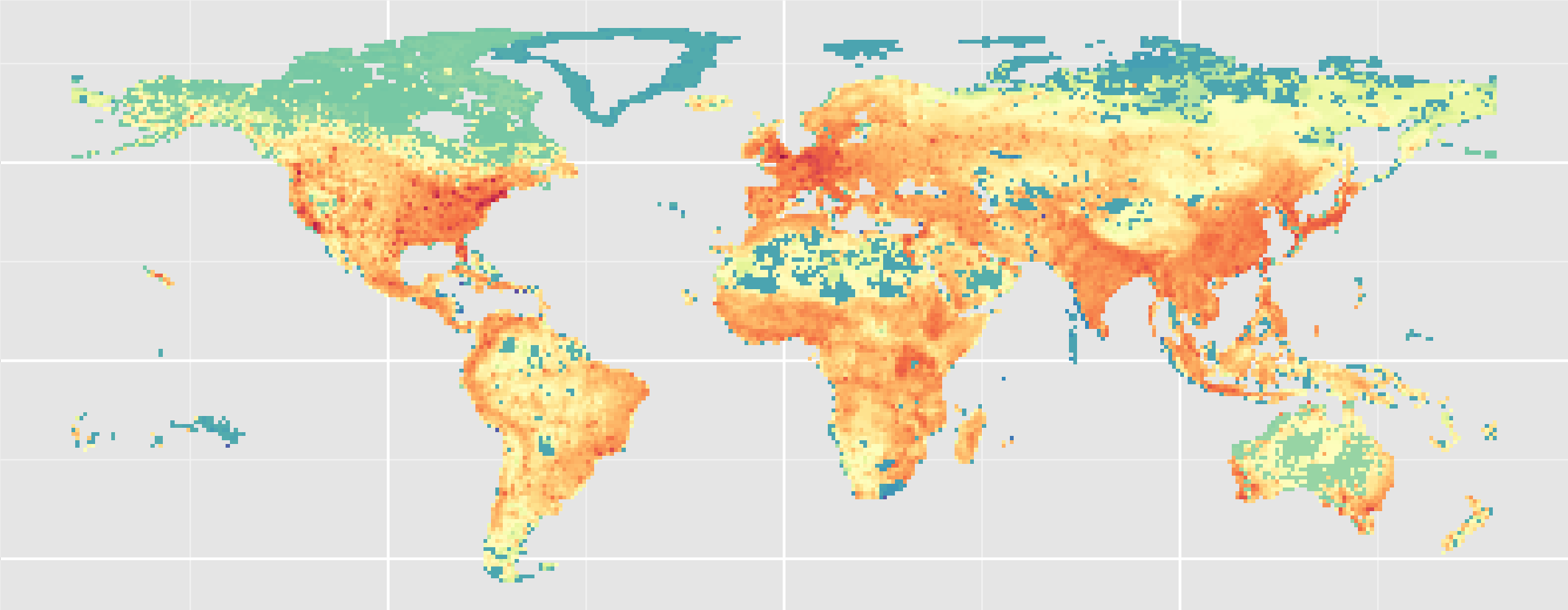
**Aim 5) Model application:** Can we integrate these relationships to derive predictive models of zoonotic spillover under land use change?

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| **II. Results from past work** |

Our collaborative team has over a decade of experience producing and analyzing primary data and global datasets of anthropogenic change, pathogen distribution and traits, and zoonotic disease emergence. Our team of Principal Investigators includes the President (Daszak) and Senior Ecologist (Murray) of an interdisciplinary research institute (EcoHealth Alliance) that specializes in the ecology of emerging diseases from wildlife, a leading human ecologist/anthropologist (Jones, Stanford University) working on social network structure and disease emergence, a lead scientist and Director of Brazil’s premier science-based public health research institution (Luz, FioCruz Amazonia), and an early-career virologist (Anthony, Columbia) working in one of the world’s premier virus research facilities (Lipkin, Centre for Immunity and Infection, Columbia University). This team has published high-impact papers highlighting the role of wildlife diseases in endangering biodiversity and human health[43-46](#_ENREF_43), identifying the wildlife origin of MERS[47](#_ENREF_47) and SARS-CoV[48](#_ENREF_48),[49](#_ENREF_49); investigating the underlying drivers of Nipah virus[*50*](#_ENREF_50)*,*[*51*](#_ENREF_51) and Hendra virus[52](#_ENREF_52) emergence; analyzing the risk of zoonotic influenza reassortment[53](#_ENREF_53" \o "Fuller TL, 2013 #52) and spread[54](#_ENREF_54); designing new approaches to predict the risk of disease spillover from wildlife[6](#_ENREF_6),[12](#_ENREF_12),[14](#_ENREF_14),[16](#_ENREF_16); analyzing social network structure as a risk of disease spread[55](#_ENREF_55),[56](#_ENREF_56); and elucidating the dynamics of pathogens in key wildlife populations[35](#_ENREF_35),[57-62](#_ENREF_57). Our team has worked on challenging international disease ecology projects in over 20 countries, with over a decade of experience working in Brazil, the site of the proposed work. In addition we have conducted the following preliminary studies to develop and begin to test our central hypotheses:

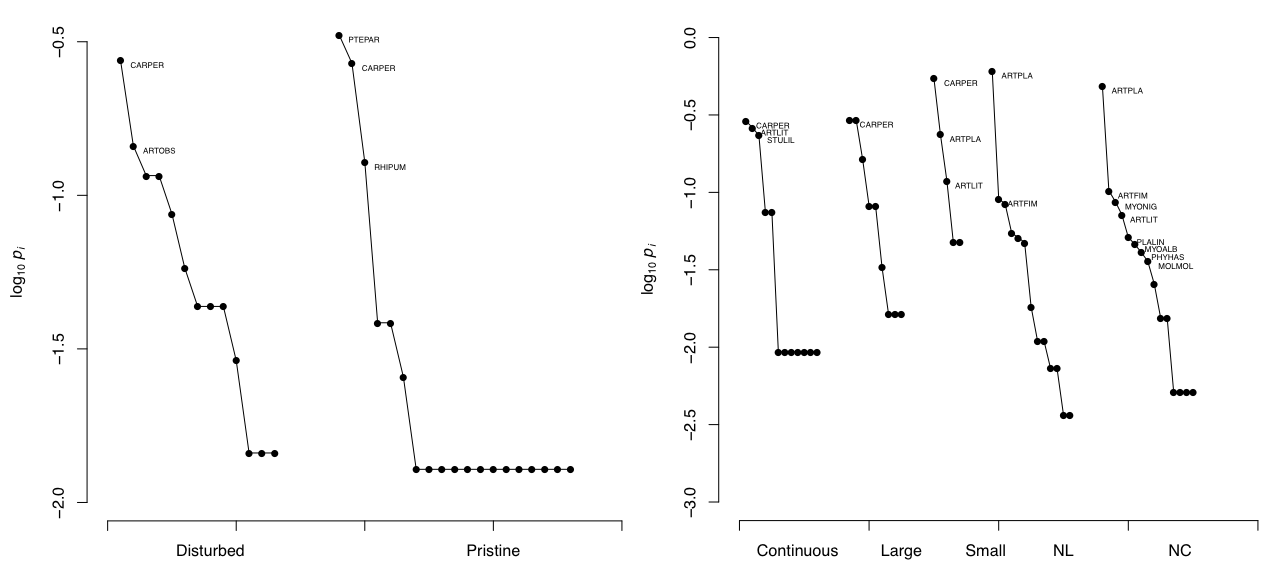
***A. Conceptual model for land use change and disease emergence.*** We recently analyzed the literature that links disease emergence with land-use change to identify two central processes of interest[25](#_ENREF_25): 1) the disruption of disease dynamics in multi-host, multi-pathogen systems (**the ‘perturbation’ hypothesis**), and **2)** the exposure of potential hosts to novel pathogens (the **‘novel pathogen pool’** hypothesis)**.** Both can increase the risk of disease emergence by influencing cross-species transmission rates (spillover); they are not exclusive processes, and may be confounded when considering the mechanisms of disease emergence in dynamic landscapes. This is because human ecology – the presence, distribution and behavior of people - is the common denominator for both. **Untangling the two processes is central to a better understanding of spillover and disease emergence under land-use change** (Fig. 1). Testing for a signature of ‘perturbation’ relies on detecting changes in host and pathogen diversity (species richness and relative abundance) attributable to land-use change that increases the risk of cross-species transmission via changes in prevalence of pathogens and contact patterns within and among wildlife, vectors, domestic animals and humans. A signature of ‘novel pool’ mechanisms relies on detecting novel host and pathogen species in changing landscapes that increases the likelihood of novel or increased contact and pathogen transmission between wildlife, vectors, domestic animals or human hosts.

**Figure 1**. Schematic diagram of the factors that promote disease emergence due to land-use change.

***B. Global-scale analysis of emerging disease drivers.*** We have developed a strategy to analyze the relationships among large-scale socioeconomic, and ecological factors and disease emergence, while adjusting for ascertainment bias. To do this, we developed a unique global database of the time and place of first observed emergence “event” and biological traits for all > 500 pathogens to emerge in humans since 1940. Adjusting for per country reporting effort, we used logistic regression to determine the association between observed emergence events and a variety of global datasets of socioeconomic and ecological factors. Our results demonstrate that zoonoses from wildlife are significantly associated with disease reporting effort, human population density and wildlife diversity. We mapped the risk associated in the model with various environmental and demographic predictors to produce the first map of global emerging disease “hotspots”[16](#_ENREF_16) (Fig. 2). Since then, we have improved every aspect of our models. We have incorporated data on new events, a suite of novel and improved predictor data sources, and more robust modeling techniques from epidemiology and ecology, and are currently validating these new models and maps. **Our recent efforts confirm that land-use is among the most commonly implicated drivers of zoonotic disease emergence, and that extent of urban land per pixel is one of the most influential predictors of disease risk** (negative correlation), even adjusting for human population density. In addition, higher levels of wildlife biodiversity (mammal species richness) are positively associated with disease risk in most scenarios. One hypothesis for this is that higher host species richness might correlate with higher pathogen diversity, increasing spillover potential for each human-reservoir contact, consistent with the “novel pool” hypothesis outlined above. Another key predictor is human population density, though its positive association is less than the direct correlation we would expect under a hypothesis that disease emergence events vary proportionally with human population density. The factor could be a proxy for the human-reservoir contact rate, or for anthropogenic influence on the landscape modifying the disease ecology of natural systems, consistent with the “perturbation” hypothesis above. None of these mechanisms can be further resolved from our macro-ecological analyses, and none have been empirically tested in the field at finer spatial scales. These results provide the macro-scale motivation and scientific underpinning for us to pursue the finer-scale ecological and demographic projects under this proposal.

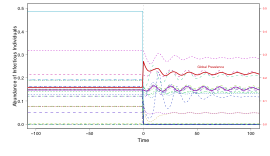
**Figure 2**. Global model of zoonotic EID risk.

***C. The ‘Deep Forest’ Project.*** We have developed a novel project framework, and begun initial field experiments, to test mechanisms of disease emergence inferred from our ‘hotspots’ model above. The work in the **current proposal will expand the scope of these initial trials to 1) more effectively include the human ecology component and 2) account for completely modified landscapes**. The Deep Forest Project (DFP) is designed to evaluate how increasing land-use development affects 1) patterns of biodiversity, and 2) corresponding patterns of viral diversity. Each DFP study region contains 9 field sites along an urban-to-rural land-use gradient: 3 sites in low (pristine forest landscape), intermediate (semi-disturbed landscape) and high (highly disturbed landscape) land-use areas. In the last 2 years, we have selected sites on three continents: South America (Brazil), Asia (Malaysian Borneo) and Africa (Uganda). These regions make excellent model systems as they are highly biodiverse, are under extensive pressure from land-use changes, and are within predicted ‘hotspots’ of disease emergence risk.

During the last 12 months,under prior USAID and IDRC funding, we have conducted standardized wildlife surveys in two regions of Brazil (Amazon near Manaus; Atlantic Forest near Teodoro Sampaio) to characterize local host species richness and abundance. We have conducted sampling for a viral diversity study on three mammal groups: rodents, bats and primates, collecting over 3,000 blood, saliva and fecal swab samples with urine and feces also opportunistically collected. These three orders together represent 70% of mammalian biodiversity[63](#_ENREF_63), and each is reservoir of a series of important pathogens[64](#_ENREF_64). Samples have been imported for viral discovery at Columbia University (co-investigator Anthony) using consensus PCR for the detection of pathogen species within 12 high-risk viral genera/families selected because of their importance for human health. A subset of the samples will be analyzed with metagenomic deep sequencing, which allows the detection of the entire community of pathogens within the samples. This study is ongoing, with field surveys conducted twice per year (once in the wet season and once in the dry season) at each site to minimize the effect that seasonality might have on the likelihood of detection of host and pathogen species. We are using our ‘virodiversity’ framework (standardized analysis of viral diversity - see below) to robustly compare communities of both hosts and pathogens as a function of land-use change from sites across the gradient (**Fig. 3**). The Brazilian Deep Forest sites will form the basis of the current proposed work.

**Figure 3.** Change of community structure and diversity of bats from disturbed to pristine (right - Amazon) and a fragmented landscape (left – Atlantic forest). All communities are dominated by few but different species. We will use a virodiversity framework (standardized analysis of viral diversity) to compare host and pathogen community diversity among gradients.

***D. WAIFW matrix model for multi-host systems under land-use change.*** We have developed an Susceptible-Infected-Susceptible (SIS) Who Acquires Infection from Whom (WAIFW) matrix modeling approach to simulate prevalence of a model pathogen under a scenario of land-use change. We asked the question ‘What happens to the dynamics of pathogens when a pristine community is disrupted by land use change and becomes a fragmented community?’ The model (equation 1) represented here:

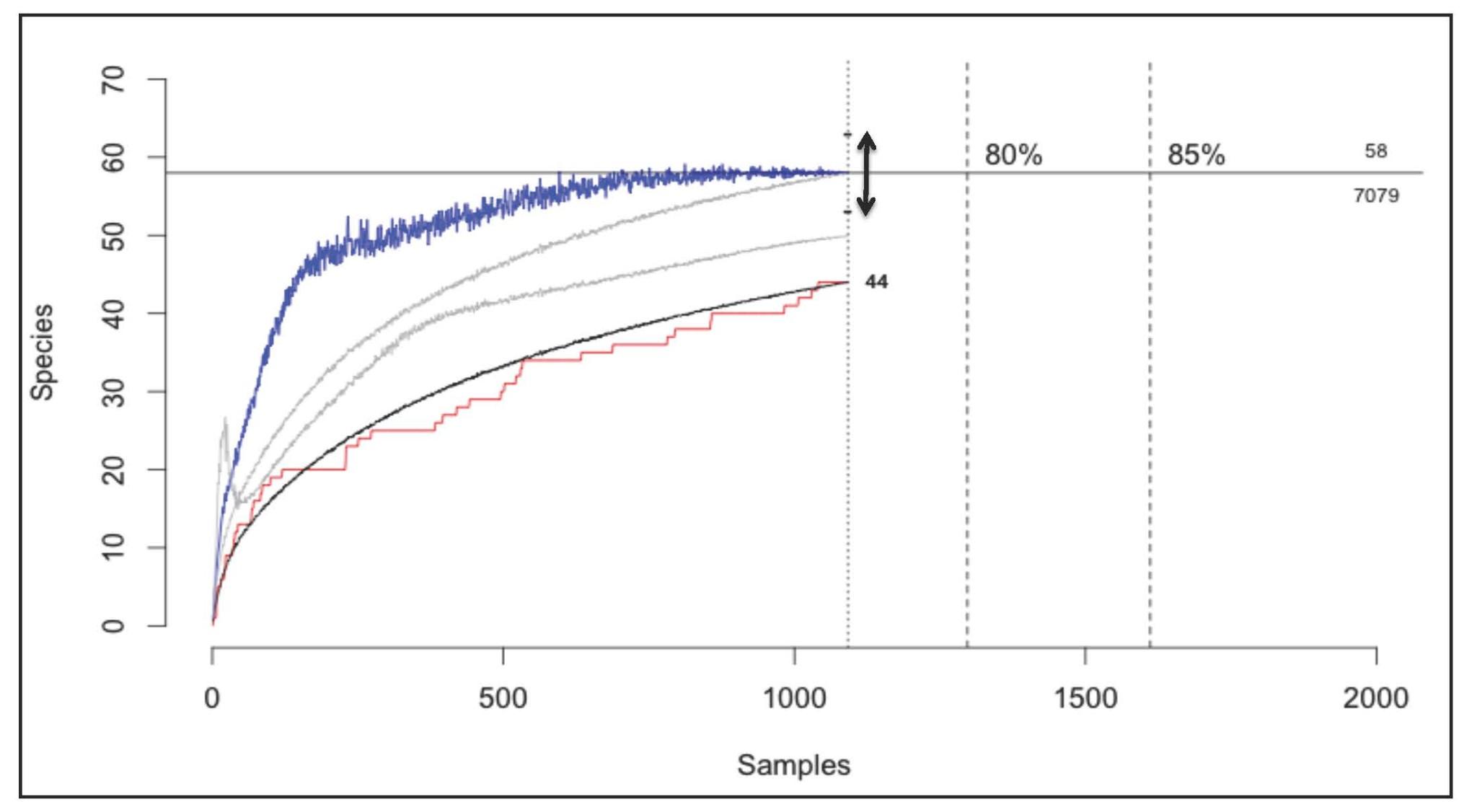
 **Figure 4.** Global prevalence of rabies (red line) predicted from the WAIFW model (1).

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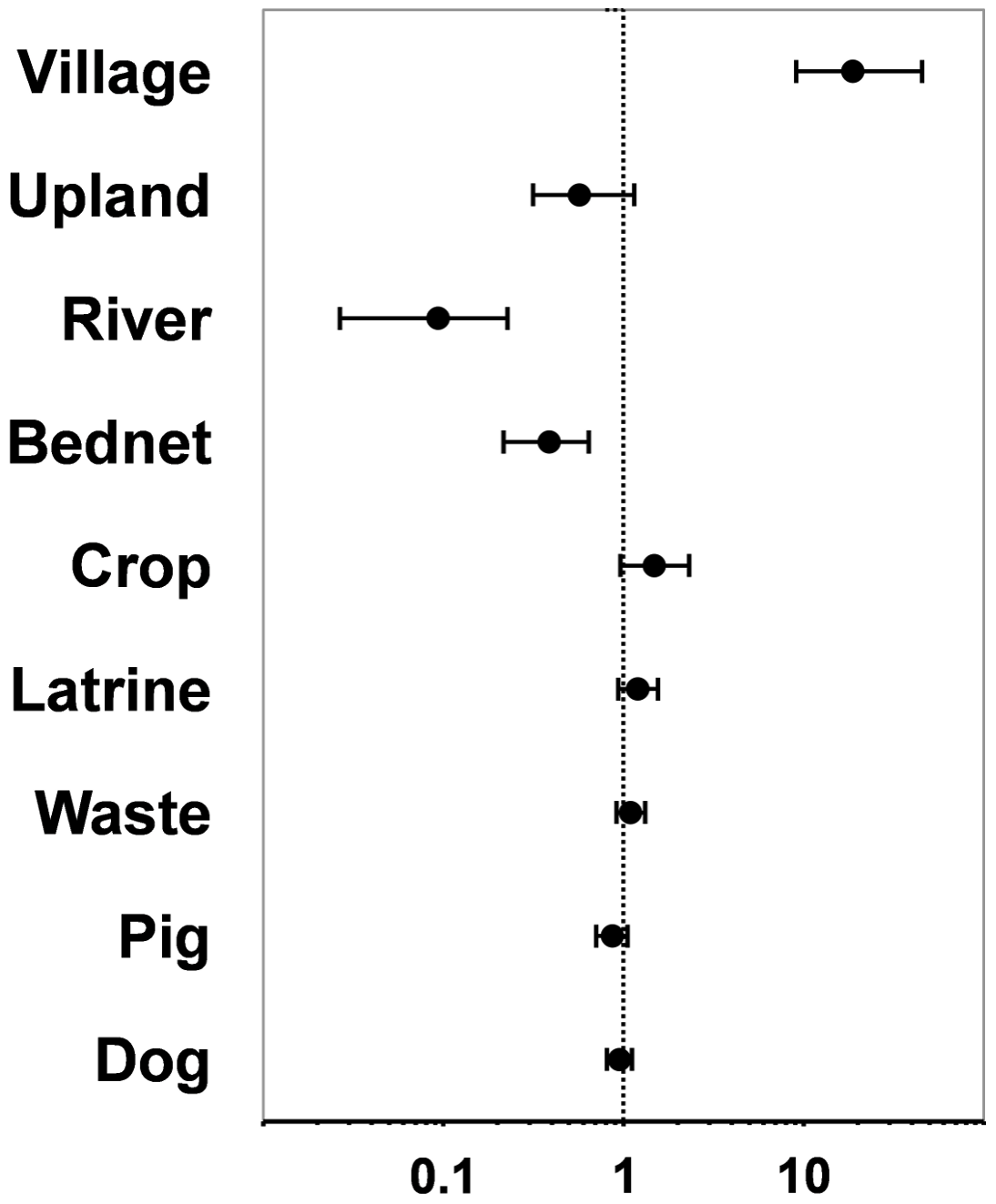


is the carrying capacity of host species ***i***, informed by empirical abundance in communities representing various stages of land-use change, a WAIFW matrix for both inter- and intraspecific transmission between susceptibles of species ***i*** and infected individuals from species ***j***, represents a species-specific intrinsic rate of growth, represents species specific recovery rate, and representing species-specific disease induced mortality for species ***i,j*** in . The results indicate that land-use change alters host community structure and can result **in marked increases in both short and long-term global prevalence (Fig. 4; red line) of a target multi-host pathogen, providing support for the perturbation hypothesis and changing pathogen potential of landscapes under land use change**. In the proposed work, we will improve the model by introducing more robust host community data from our biodiversity surveys, examine varying land-use change scenarios, introduce additional pathogens identified via our pathogen discovery and description effort, and develop a spatial framework to probe the variation of prevalence (as an indicator of pathogen potential) across dynamic landscapes.



***E. Measuring ‘Virodiversity’ –diversity of unknown viruses within a wildlife population.*** We have developed a framework to robustly assess the viral community from wildlife hosts. In a recently published proof-of-principal study, we combined virological and ecological techniques to describe the virodiversity of the bat *Pteropus giganteus[65](#_ENREF_65" \o "Anthony, 2013 #64)*. In one analysis, we used consensus family-level PCR assays to discover 44 viruses from seven viral families. We estimated asymptotic viral richness from observed detections using three statistical models[65](#_ENREF_65) (Fig. 5), which rely on the frequency of rarely occurring species to measure completeness of discovery. Our estimate of the number of viruses in a single bat host (58) is more than 20 times greater than the mean (2.71 viruses per bat species) recently reported in the literature[64](#_ENREF_64). This illustrates how truly little is known about the viral pool in wildlife. The identification of co-existing viruses is also important to a description of virodiversity because of the positive and negative associations that can occur between them[66-71](#_ENREF_66). In our virodiversity study[65](#_ENREF_65), we reported a large number of intra and inter-familial co-occurrences in *P. giganteus* and showed that as many as five different viruses can exist in a single sample. This revealed information about the carrying capacity and composition of discrete viral niches within a host species, and also the number of different viruses that could potentially spillover to a new host from a single exposure event. **This study provided the first ever estimate of total (known and unknown) viral richness in a single wildlife species and the sampling effort required to detect any proportion of it.** This approach will allow us to compare virodiversity among host populations in different geographic regions and ecological settings (i.e. across our land-use gradient) given incomplete sampling.

**Figure 5.** Viral diversity estimation from incomplete sampling. Total viral richness estimated was estimated to be 58 viruses and the required sampling effort to discover all 58 was estimated to be 7079 samples (i.e., ~7 times our effort).

**F. Risk factors for zoonotic infection in Amazonia**

Our team (PI Luz) has devised and demonstrated a strategy to quantify the prevalence and risk factors for zoonotic infections in our Brazilian human population, using Mayaro virus as a model system[46](#_ENREF_46). Mayaro virus is a zoonotic arbovirus with wild primate reservoirs and a mosquito vector. Using a combination of household surveys to collate covariate predictor information (e.g., age, gender, use of bed nets, settlement type, type of waste disposal system), serological assays from human blood spot samples, and cutting edge statistical models suited to observational data (information theory-based multimodel inference (MMI)), the study was able to effectively demonstrate the key risk factors for infection of Mayaro virus. MMI revealed the most relevant covariates (residence area, bed-net use, crop-plot ownership, closed latrine), allowed quantification of covariate relative importance and effect sizes for inference (Fig. 6). We will expand on this effort using similar methodology to validate our general model and understand risk factors for human infection of multiple zoonotic pathogens and how risk of infection varies with human-animal contact, prevalence in reservoirs, land-use change and other covariates.

**Figure 6.** Risk factor analysis for Mayaro virus in Brazil.

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| **III. Description of proposed research** |

***A. General Model, Research Framework, Study Sites***

***General Model:*** Three pillars, termed **pathogen potential, contact potential** and **transmission potential**, derived from the spillover model of Lloyd-Smith *et al*.[37](#_ENREF_37), underpin and link the components of our proposal through a model in the general form: S ∝ P x C x T, where S is spillover and we define P, C, and T thus: 1) **Pathogen potential, P**, is the community of pathogens within the community of hosts[72](#_ENREF_72) available to be transmitted to humans, represented by the viral diversity (combination of viral richness and abundance (prevalence)) in hosts in the community. **Aim 1** will investigate this parameter via wildlife sampling and how it changes with land-use development using presence and prevalence of target pathogens in bat and rodent communities as model wildlife systems; 2) **Contact potential, C**, is the *rate* of human-animal contact with wildlife (or their fomites) of potential zoonoses and/or their vectors. **Aim 2** will investigate this parameter, which is comprised of the coupled dynamics of two highly complex and interacting ecologies, that of reservoir hosts (and potentially vectors) and that of humans. The base unit of contact potential is a ‘contact event’ between humans and animals during which infection could conceivably occur. The key element of understanding contact potential in natural settings is to evaluate, quantify and characterize human ecology across dynamic landscapes. We will achieve this with a comprehensive human ecology study, comprising structured surveys, focal subject follows, and transect surveys. 3) **Transmission potential, T**,is the probability that a single animal-human contact ‘event’ results in a pathogen being transmitted, i.e. the likelihood of infection given contact. **Aim 3** will investigate this parameter by evaluating the *type* of contact (e.g. hunting, butchering, cohabitation, insect bite) occurring in landscapes. Since **T** is governed not only by the characteristics of specific contact events (e.g., type of contact), but also attributes of each pathogen within the reservoir pathogen community (e.g. mode of transmission, phylogeny), and by factors governing receptivity (e.g. human susceptibility), we will investigate **T** with reference to specific pathogens grouped by their modes of transmission. In **Aim 4**, we will validate this general model linking 1, 2, and 3 with an empirical study of zoonotic infection in humans, verifying directly how human-animal contact rate and type of contact filter the risk of infection from wildlife populations. In **Aim 5**, we will integrate the components of the study through modeling into a general model that can be used for mapping and forecasting spillover risk under scenarios of land-use change.

***Study Sites:*** The study will take place in two (for comparison) regions of Brazil: 1) the Brazilian Amazon, and 2) in the interior Atlantic Forest in São Paulo state – both sites at which we have been working for multiple years (Fig. 7). Wildlife surveys and our human ecology program will take place in both regions, while the human infection study will occur in the Manaus region only. In the Brazilian Amazon our established sites are distributed along a land-use gradient spanning approximately 50 km from the city of Manaus. Our sites are located in a preserved primary forest (pristine), in forest within a highly fragmented, developing landscape (intermediate) and in a forest patch within a highly deforested area (urban). **A fourth site will be added in a completely transformed land-cover type (e.g., oil palm, sugar cane, pasture), making 4 levels of land-use development gradient**. Each site has 3 independent replicates making a total of 12 sampling sites along the disturbance gradient. Our urban sites are located in a forest fragment, managed by the Federal University of Amazonas, situated in the city of Manaus. Human migration to and from Manaus is relatively low with only 14% of the population from other states. Most of the population in this area descends from early European settlers, chiefly the Portuguese, and assimilated indigenous peoples (mostly Tupi and Guarani, but also many other ethnic groups). The intermediate sites are located in Rio Preto da Eva, specifically in the Beija-Flor Indigenous Community. The population in the intermediate sites is comprised of different ethnic groups including Sataré-Mawe, Tukano, Dessano, Twiuca, Apurinâ, Baniwa, Arara, Marubo, Mayuruna, which are distributed among three communities: Beija flor I, Beija Flor II and Beija Flor III. Our pristine sites are located in UFAM Fazenda approximately 50 km north of Manaus. Nova Canaã is the closest community to our pristine sites in UFAM Fazenda. The population in this area is very diverse, with the majority of people coming from different rural areas of Amazonia state, a small proportion with indigenous origins. Wildlife sampling will take place only at the completely transformed site because we have already sampled wildlife from the other sites along the gradient.

**Figure 7.** Our two study regions in Brazil.

In the Atlantic Forest, our field sites are along a land-use gradient in and around Morro do Diabo State Park, which contains the largest preserved area of inland Atlantic Forest in São Paulo State (~33,000 ha). Scattered fragments on farms and rural agrarian settlements total an additional 15,000 hectares. In this region, our pristine sites are located inside Morro do Diabo State Park, situated near the city of Teodoro Sampaio. This park protects the biggest remnant fragment of Atlantic Forest in Brazil. The intermediate sites are located in smaller fragments scattered around the park (100-200ha) and the disturbed sites are located in the formerly ‘landless’ settlement communities bordering the forest. **Again, we will add a fourth gradient level with three replicates in completely transformed landscape in this region.** To date, approximately 320 families of small producers have been settled around MDSP, each family with a plot of 15 hectares. Half of the lot is normally used for subsistence agriculture (corn, cotton, manioc, rice, beans), and the other half for small-scale milk production. In addition, more than 1,500 additional families are camped along highways and ranches awaiting land titles. As a consequence, new colonies of farming families are cultivating land along the borders of forest fragments. As for the Manaus sites, wildlife sampling will take place in the Atlantic Forest only at the completely transformed site because we have already sampled wildlife from the other sites along the gradient. In both study regions, the human ecology program will be implemented at all four sites along the gradient.

***B. Aim 1: Pathogen potential:* Is wildlife host community ecology predictive of pathogen community ecology** underland use change?

***Quantifying pathogen potential:*** While non-host specific factors (i.e., environment) might explain some of the variation in pathogen diversity (richness and abundance) at relatively large spatial scales[73](#_ENREF_73), **we hypothesize that within similar landscapes wildlife host community ecology will be related to pathogen community ecology**[74](#_ENREF_74). Hence, the pathogen potential of landscapes will be governed by the presence, diversity and relative abundance of the reservoir host species. We will assess pathogen potential in our two pristine landscapes as a baseline for Aim 1. Overlaying this, habitat fragmentation and degradation are major products of land-use change[4](#_ENREF_4), and could result in a number of potential outcomes pathogen diversity via impacts on their hosts. For example, the distribution of host species has been linked to life-history traits, habitat and environmental suitability, island biogeography, and species interactions (e.g., competition)[75](#_ENREF_75). Land use change could influence each of these factors, resulting in fundamental shifts in host community structure (species diversity, richness and relative abundance)[76](#_ENREF_76). For example, species resilient to land-use change might be relatively fecund, habitat generalists, good dispersers and dominant competitors. As landscapes are increasingly impacted, some less resilient host species may thus disappear, taking their microbes with them. Other resilient species might appear (in addition to domestic and semi-domestic animals and vectors), introducing new pathogens into the system, or increasing their abundance[77](#_ENREF_77). Relative abundance of host species might also change, potentially affecting pathogen prevalence, spillover risk, and increasing or decreasing the pathogen potential of landscapes (see WAIFW model in prior results). We will measure how the host and pathogen communities depart from the state measured in our pristine sites as we move along the gradient from low to high landscape modification, i.e. how the pathogen potential of landscapes changes due to land-use development. To do this, **we must develop an entirely new knowledge bank on the interrelationships between biodiversity and pathogen diversity, and the role that land-use changes play in perturbing the evolutionary stable state of the system.**

***Aim 1 Methods:*** We will leverage preliminary data from our Deep Forest Project and additional data collected here to characterize known, and estimate unknown, host and viral diversity, and describe in detail the relationship between pathogen diversity, host diversity and land-use change. We have already commenced data collection in three sites (pristine, intermediate and disturbed) in both study regions, and we will **add a fourth gradient level (completely transformed habitat e.g. plantation or urban extent)**. Three replicate sites are used for each gradient level, such that we will have 24 local field sites across our two study regions (2 regions x 4 gradient levels x 3 replicates in each level = 24), with 6 of those added here. We will conduct 2 wildlife surveys per year at each site trapping rodents and bats, using separate field teams for each region. Adding the fourth gradient level in two regions will require 12 surveys per year (2 regions x 1 gradient level x 3 replicates x 2 surveys per year = 12) over 2 years = 24 surveys. Each survey lasts for 5 days, making a total addition of 120 trap nights (each site is surveyed for a total of 20 trap nights). Given our preliminary data, this will take the total number of trap nights to achieve Aim 1 to 480 trap nights (at each site, [5 nights x 2 surveys per year x 2 years = 20 trap nights] x 3 replicates per gradient x 4 gradient levels x 2 regions = 480 trap nights in total). Our prior data shows that we will catch an average of 14 bats and 10 rodents per night of sampling, ~45% of which come from the two most common bat species and ~60% from the two most common rodent species. We have established that 20 nights of trapping per site will be sufficient to achieve three primary requirements: 1) sufficient sample size (~280 individuals, approximately 1120 samples) for the two most common species in each community in order to deeply probe their viral diversity[65](#_ENREF_65), compare between them (species turnover; from 8 communities in total), and compare between sites (geographic turnover; 24 pairwise comparisons per region), 2) capacity to adequately characterize host diversity and quantitatively compare between sites (using species-abundance distributions, diversity metrics and species richness estimators), and 3) ability to establish the slope of the viral community accumulation curve and hence compare relative pathogen potential between sites. We will use statistical models (see below and results from prior work - ‘virodiversity’) to estimate the undetected fraction of both the wildlife host and pathogen communities given incomplete sampling (see below). Together, this will allow us to construct a metric of pathogen potential for each study site. Sampling will follow the same protocol as described above (see results from prior work – Deep Forest). Each sampling site has an area of 1 ha (100 x 100 m). Bats will be captured with mistnets and harp traps (10 mistnets and 2 harp traps total per site). Rodents will be captured using a combination of Sherman and cage traps (100 total per site) laid out in a 100 x 100 m grid. Samples will be collected in duplicate and will be stored in liquid nitrogen in the field and shipped to our partner laboratory at the University of Sao Paulo (senior personnel Durigon) for PCR testing (see below).

***Animal handling and sampling procedure:*** All animals will be identified and non-invasively sampled to collect blood and oral, urine and rectal swabs using techniques we have employed in the field for over 10 years. Voucher specimens for each species will be collected as necessary and vouchered at the American Museum of Natural History; and Instituto Nacional de Pesquisas da Amazonia (INPA). All bats will be identified by local experts and will be based on field guides for Brazilian bats[78](#_ENREF_78) and will be confirmed by DNA barcoding using standardized methodologies[79](#_ENREF_79). Genus, species, age class and sex, and morphometrics (e.g. forearm length, weight will be recorded[80](#_ENREF_80)). Bats will be placed in cotton bags with drawstring mouths and kept in a cool dry place until sampling; holding time will not exceed six hours. Fine polyester swabs will be used to collect oral and rectal swabs, as well as urine if available. We will use the venipuncture system to collect blood in a ratio no greater than 10µl of blood for 1g of body weight equivalent to 1% of body weight, as described previously[81](#_ENREF_81). Swabs will be placed in 200µL of virus transport medium (VTM). Sealed, labeled vials with samples will be placed in a liquid nitrogen tank in the field to maintain cold-chain during transport to the laboratory for viral analysis.

***Vertebrate Animals*:** All vertebrate research methods proposed here are previously approved by IACUCs issued to EHA from Tufts Univ. (G2011-106) and UC Davis (16048) under a cooperative agreement with EcoHealth Alliance.

***Sample Preparation and Extraction*:** All samples (n=7680) will be subject to crude viral particle purification. Centrifugation and filtration will be used to remove bacterial and host cell debris, and nuclease (RNase/DNase) treatment used to remove naked nucleic acids. Viral RNA/DNA will then be extracted using the EasyMag® (bioMérieux, Inc) platform, and cDNA synthesis performed using SuperScript® III first strand synthesis supermix (Invitrogen). All cDNAs for each animal (maximum of four) will then be pooled and viral discovery performed using broadly reactive consensus PCR and/or high-throughput sequencing (HTS).

Consensus PCR: Broadly reactive primers targeting the following viral families/genera will be included: *Coronaviridae*[82](#_ENREF_82),[83](#_ENREF_83) (2 assays), *Paramyxoviridae* (1 assay)[84](#_ENREF_84), *Astroviridae* (2 assays)[85](#_ENREF_85),[86](#_ENREF_86), *Flavirivirus* (2 assays)[87](#_ENREF_87),[88](#_ENREF_88); *Bunyaviridae* (2 assays)[89](#_ENREF_89); *Hantavirus* (2 assays)[*90*](#_ENREF_90)*,*[*91*](#_ENREF_91); *Influenzavirus A* (1 assay)[92](#_ENREF_92); *Enterovirus* (1 assay); *Bocavirus* (1 assay)[93](#_ENREF_93); *Adenoviridae* (1 assay)[*94*](#_ENREF_94); *Polyomaviridae* (2 assays)[95](#_ENREF_95); and *Herpesviridae* (2 assays)[96](#_ENREF_96),[97](#_ENREF_97). These specific viral families were selected to account for potential differences in RNA and DNA genomes, prevalence, strain diversity in a population, and the potential for co-infection. When positive cDNA pools are identified, cDNA from the individual samples (throat, faecal, urine and blood) will be tested separately. Positive PCR products will then be cloned into Strataclone™ PCR cloning vector and 12 white colonies sequenced to: 1) generate sequence data for analysis, and 2) to identify any co-occurring viruses present in the sample. Based on our previous work[65](#_ENREF_65) we anticipate ~30% of samples to test positive for at least one virus.

***Quantifying Viral Richness and Diversity, Comparisons:*** We will analyze viral sequence data to delimit unique ‘operational taxonomic units’. Such delineations will be performed at multiple levels for comparison, including: i) the identification of monophyletic clades/clusters via Bayesian and Maximum Likelihood phylogenetics, and ii) the use of hierarchical clustering to group sequences based on genetic distance between established viral ‘species’ (where determined and generally accepted in the literature or by the ICTV). All viral sequence data together with individual level data (e.g. specimen ID, sex, age, species) will be entered into our central database (**see data management plan**). We will aggregate data at different hierarchical levels (e.g., individual, species, gradient level) using different resolutions (e.g. prevalence and binary data) relevant to each test below. We will measure viral richness using non-parametric species discovery curves, which are commonly used in biodiversity studies[98](#_ENREF_98). Viral assemblage indices (e.g. Shannon’s H and Simpson’s index, that account for relative abundance) will be calculated for each hierarchical level (e.g., bat species per tissue type sampled).From our samples, we will first construct virus accumulation and rarefaction curves for visualization. The asymptote of the rarefaction curve provides the estimate of the number of viruses that characterizes the assemblage (See Fig. 5 – “virodiversity”). However, sampling to reach this asymptote may be impractical as the number of samples required could be prohibitively large[98](#_ENREF_98). We will thus use statistical methods to estimate the asymptote from the data at hand. We will use the non-parametric estimator, Chao2[99](#_ENREF_99),[100](#_ENREF_100) and also calculate ICE and Jackknife statistics for comparison. Unlike conventional curve fitting procedures, the non-parametric estimators make no assumptions on an underlying abundance distribution, do not require *ad hoc* or *a priori* model fitting, are relatively robust to spatial autocorrelation and scale and frequently outperform other methods of richness estimation[98](#_ENREF_98). They rely on the principle that the frequencies of the rarest species in a set of samples can be used to estimate the frequencies of undetected species, and provide a minimum richness estimate. In addition, we fill follow Chao *et al.*[*99*](#_ENREF_99) to calculate how many additional samples would be required to detect any proportion (including 100%) of the estimated virus richness and help adjust our target sample sizes during the first two years of the project to maximize diversity estimates and comparisons (*see* Preliminary Data[99](#_ENREF_99),[101](#_ENREF_101)) and inform us about our sampling completeness. We will take a null model approach to test for non-random patterns when comparing similarity of viral community assemblages between species and sites[102-105](#_ENREF_102). This will involve calculating Jaccard’s index of similarity (*J*) for the viral assemblages between pairs of species/sites and testing for deviations from that expected by random chance. Deviation from expected similarity (*Jdev*) will be calculated by randomizing the observed viral occurrence matrix using Monte Carlo randomizations. Randomizations will be constrained to ensure fixed row and column totals, equating to maintaining observed viral richness in each host species and maintaining observed viral occupancy across host species (commonly referred to in the biodiversity literature as a fixed-fixed model)[106](#_ENREF_106). For each randomized matrix, we will calculate all pairwise *J* scores. Our null model will assume the mean *J* of these randomizations (*Jnull*). Deviation from the null model will be calculated as the difference between the mean *J* observed (*Jobs*) in the data and the mean *J* expected, such that *Jdev*= *Jobs* - *Jnull*. Positive values of *Jdev* will thus indicate that viral community assemblages between host species/sites are more similar than would be expected by random chance, while negative values would indicate greater dissimilarity in the viral assemblages than would be expected by chance. For a practical example of this method, see[107](#_ENREF_107).

***C. Aim 2:*** **Contact potential: Is human-reservoir contact *rate* predictable at the landscape level** under land use change?

***Quantifying contact potential -*** **mapping human-animal contact rate**: Previous studies have shown correlations between exposure variables and risk of zoonotic infection. For example, people highly exposed to non-human primates occupationally and those hunting non-human primates or keeping them as pets are at higher risk of infection by their viruses[17](#_ENREF_17),[108-110](#_ENREF_108). Other studies have shown that prolonged exposure to wildlife and arthropods in occupational settings results in higher incidence of zoonotic infections than working primarily in administration or management[111](#_ENREF_111). These studies imply that contact rate (or contact type – see below) with reservoirs/vectors correlates with risk of zoonotic infection. Our aim is to develop spatially explicit predictions of the component of spillover risk contributed by contact rate. In zoonotic disease models, the dynamics of infectious diseases are dependent on the rate of transmission from infectious wildlife hosts to susceptible human hosts, captured by the risk of transmission given contact (Aim 3). To determine this component of risk, we must estimate the rate at which humans are coming into contact with potential wildlife hosts and/or vectors – a fundamental, but difficult to measure, parameter in zoonotic disease models. Aim 2 will investigate this parameter, which is comprised of the coupled dynamics of two highly complex and interacting ecologies, that of reservoirs hosts (and in some cases vectors) and that of humans. With known diseases, what constitutes a contact event will depend on the specific infectious agent and its mode of transmission. However, for unknown pathogens this information is unknown. For this reason, our base unit of contact potential is any ‘contact event’ between humans and animals during which infection could conceivably occur. **The key element of understanding contact potential is thus to evaluate, quantify and characterize human ecology**[42](#_ENREF_42)via interview surveys and observational studies. Our aim is to quantify and map patterns of human-animal contact across our land-use gradient to elucidate the relationships between specific land-use activities and contact. Thequantification of human-animal contact rates using an integrated approach will provide more accurate estimations of these variables and improve parameterization for the modeling of this coupled natural-human system. Our working hypothesis is that **unique and measurable attributes of landscapes at varying stages of land-use change dynamically interact with and influence varied and quantifiable aspects of human ecology to modify the relative rate and characteristics of human-animal contact events** (Aim 2 and 3 Methods are described together below).

***D. Aim 3:*** **Transmission Potential: Is human-reservoir contact *type* predictable at the landscape level** under land use change?

***Quantifying transmission potential -*** **mapping human-animal contact type:** The risk of transmission given contact is the final parameter in our general spillover model. Clearly, spillover cannot occur if likelihood of transmission given contact is not a positive value. For a known pathogen, we could infer this likelihood by quantifying infection prevalence in the spillover host (humans) given data on the contact rate (Aim 2) with the infected fraction of its reservoir host(s) (Aim 1). However, for unknown pathogens this is problematic for several reasons. First, it is not possible to test for an unknown pathogen since we do not know what we are looking for. Second, even with our target viral families that have been predefined as pathogens of interest for their zoonotic potential (and our measure of them as contributing to the pathogen potential of landscapes), spillover itself is expected to be an extremely rare event and our target viral families may not be detected in the human host if spillover has not yet occurred. Third, unlike prevalence and contact rate, likelihood of transmission is a confounded parameter comprising traits attributable to 1) the contact event (e.g., type of contact), 2) the pathogen (e.g., mode of transmission, mechanism of infection) and 3) the receptive host (e.g., in this case human susceptibility). This means that we would need to know these additional parameters in order to link cases of human infection with the spillover risk of landscapes via the mediation effect of human-animal contact. Again, however, we cannot know these traits in advance for unknown pathogens. With respect to our model of generic spillover risk in changing landscapes, this is thus the most difficult parameter to quantify in practice. Nevertheless, **we hypothesize that some features of the transmission given contact parameter will be generalizable** (e.g., transmission likelihood will be relatively higher when butchering versus handling animals for blood-borne pathogens). From our human ecology study (also Aim 2, and see Methods below), we will thus be able to estimate relative risk of transmission given contact by quantifying the different types of human-animal contact reported in landscapes and by developing an index of risky behaviors for specific pathogen groups of interest (e.g., blood borne, airborne, vector borne etc.)[42](#_ENREF_42),[112](#_ENREF_112). We assume that the susceptibility factor is fixed, but in future work could explore varying receptivity or susceptibility of the spillover host as well. For example, HIV is of growing concern in Brazil and we could conceivably add the component of our model incorporating relative differences in susceptibility of human hosts and parameterize with regional HIV prevalence data, which would affect susceptibility.

***E. Aims 2 and 3 Methods - Quantifying human-animal contact rate and type:*** We will quantify the rate and different types of human-animal contact by undertaking a human ecology study across an urban-to-rural gradient in our two study regions of Brazil. We will collect data using an integrated approach that combines both qualitative and quantitative research methods, including: (1) an extensively pre-tested structured household survey, (2) focal subject follows, and (3) transect surveys.

***(1) Structured household surveys*:** Most structured surveys rely on self-reporting and may not accurately capture the type, frequency and duration of contact with specific wildlife hosts. This is largely due to the difficulty associated with identifying behaviors that might go unreported due to the limitations of procedural memory. Highly routine or unconscious behaviors are notoriously easy to miss during surveys. Further, there are some topics for which people cannot or will not accurately report their own behavior (hunting, trespassing in protected areas, etc.). For these reasons, we will use participant observation and ethnographic methods to minimize this form of self-report bias and obtain a more valid understanding of these behaviors[113](#_ENREF_113). These surveys will be used to quantify levels of human contact with the three key mammal groups (rodents, bats and primates), in addition to mosquito vectors (see Aim 4) as well as domestic animals that can serve as alternative reservoirs or intermediate hosts of zoonotic agents (e.g. pigs, chickens and pet primates). We have already compiled a list of relevant human communities through our Deep Forest research. Independent samples will be obtained for men and women in each community. Where it is not feasible to interview all households in a given community, the proposed sample size per one sex group will be based on estimating contact rates within +/-10% (α = 0.05; power = 80%, base proportion = 50%). The random sample size for this criterion is 97 men and 97 women from each community (total of 194)[114](#_ENREF_114). Independent samples of men and women in each village will be selected using systematic sampling with different random starts. These surveys will provide data on which sub-groups have the highest rates of contact with the key animal groups which will be used to focus human sampling efforts outlined in **Aim 4**, below. Example data that will emerge from the survey include (1) the frequency at which people encounter different animals and the type of contact; (2) perceptions and awareness of diseases that are transmitted from animals to people; (3) how contact may vary by ethnicity, subsistence strategies and other socio-economic factors; and (4) the identification of specific activities associated with high-levels of contact with key animals. As survey questions often include categorical responses, survey data will be analyzed using logistic regression and other multivariate techniques. Results will be incorporated into modeling of the coupled system of spillover risk (**see Aim 5**).

***(2) Focal subject follows:*** Recall error and biases represent a major concern to the validity of studies that rely on self-reported data[115](#_ENREF_115). To account for this, we will also use direct observation of human behavior through the use of focal subject follows to characterize the nature of human-animal contact. Focal subject follows involve observing a particular individual for up to a day at a time and recording the specific activity in which an individual is engaged[116](#_ENREF_116). This method is the gold standard for assessing behavioral data and has previously been used by human ecologists interested in assessing foraging patterns[117-119](#_ENREF_117) and human-animal interactions[119](#_ENREF_119),[120](#_ENREF_120). This method has also been used to assess potential pathogen transmission from humans to non-human primates[120](#_ENREF_120). From preliminary Deep Forest data on human-animal contact, we know that self-reported data on hunting and other forest interactions can be largely unreliable. For this reason, we will select 5-10 focal informants in each gradient to accompany during hunting and resource collection trips. During the follow, we will ask detailed questions in situ about the types of animals encountered, the frequency of encounters, and the uses of different animals. We will also record GPS data points to create a visual representation of activities in each of our sites.

**(*3) Transect surveys:*** We will also use transect surveys to generate spatialized human occupancy data, which may provide an unbiased estimate of the number of people occupying landscapes of varying characteristics (e.g. pristine forest, semi-degraded forest, fully transformed landscapes). This method will involve systematically counting the number of people along each randomly selected survey transect for a set duration and over time with a consistent number of replicate surveys to provide a rate of human presence (e.g. occupied x% per hour per grid cell). We will develop spatial models from these surveys that will overlay human occupancy with various environmental and geographic covariates to produce an overall human occupancy map. Previous methods have largely relied on fixed household data (e.g. censuses), which tend to correlate with maps of human density. This method will provide a more accurate picture of human occupancy by accounting for the dynamic nature of human mobility. Covariates of potential interest for their ability to predict human occupancy will include habitat characteristics, demographic data (e.g. habitation patterns), geographic relief (e.g. elevation), human constructions (e.g. dwellings, roads, plantations) and others. The model will then allow us to extrapolate via the use of predictive covariates to the unsurveyed regions between sampling points to provide a spatial map of relative human occupancy at moderate spatial scales. Combining this with the information derived from the other survey methods will allow us to generate **the first predictive models of human-animal contact rate and type at the landscape level.**

***F. Aim 4: Model validation:* Do patterns of human-reservoir contact mediate the likelihood of zoonotic infection** in humans from the available pool of pathogens in dynamic landscapes**?**

**Validating our general model:**Previous studies have been successful in broadly identifying risk factors for zoonotic infections (e.g.,[46](#_ENREF_46),[111](#_ENREF_111)), identifying changing prevalence of pathogens as a function of land use change in wildlife reservoirs as a proxy for risk (e.g.,[51](#_ENREF_51),[121](#_ENREF_121),[122](#_ENREF_122)), and mapping human movements in the context of contact likelihood with vectors (e.g.,[123](#_ENREF_123)). However, to the best of our knowledge, no previous studies have linked land-use change with the pathogen potential of landscapes and the risk of human infection via detailed mapping of human-animal contact potential (contact frequency) and transmission potential (contact type) for multiple reservoir species. Furthermore, we are aware of no studies that have adopted a similar framework to that proposed here to understand the risk of infection from as yet unknown pathogens (although conceptual models have been developed). To validate our general model, we must link Aims 1, 2 and 3 (see below, Aim 5) and test whether it has predictive value for describing zoonotic spillover. However, spillover of novel pathogens can only be detected after it occurs, using diagnostic development from cases of infection and typically clinical signs. Novel viruses could be screened for in people, but, as in our wildlife sampling study, we would have no way of knowing whether the detection of these viruses would actually represent spillover. We can, however, validate the general applicability of our model by testing human subjects for a surrogate (i.e., more detectable) pathogen. This would involve parameterizing the general model with 1) prevalence in reservoir data, 2) contact rate data, 3) contact type data (depending on mode of transmission), and 4) testing the ability of the model to predict actual infection data. Establishing the validity of our general model through such a test will allow us to extrapolate our findings to landscapes under land-use change with considerably increased confidence in the mechanistic underpinnings of our predictions.

**Methods*:*** We will undertake a **human infection study** to evaluate how rate and type of human-animal contact interact with prevalence in reservoirs to translate into actual risk of zoonotic infection under land-use change. We will use 3 zoonotic pathogens previously reported in our study region as proxies for risk of novel zoonotic infection (spillover). We hypothesize that the relative risk of a known pathogen infecting humans from a wildlife reservoir will be proportional to the risk of contracting unknown pathogens of the same mode of transmission from the same wildlife reservoir group. We chose pathogens for which 1) serological assays are currently available, 2) human-animal/vector contact rate and type of contact with the reservoir/vector will be quantified (as in Aims 2 and 3), and 3) we will have prevalence and seroprevalence data in the reservoir species (as in Aim 1, and from previously available data). We have selected **Mayaro virus, Mucambo virus (both family: *Alphaviridae*), and Andes Virus (family*: Bunyaviridae,* genus*: Hantavirus*)** for this study. This selection represents agents that are both vector-borne (the alphaviruses Mayaro and Mucambo) and directly transmitted (the Hantavirus, Andes virus). We will use Andes virus as our primary validation model, and include Mayaro and Mucambo viruses as informative comparative models. **We hypothesize that, due to the more accurate mapping of human-animal contact with rodents relative to mosquito vectors, our model will be more effective at predicting relative risk of Hantavirus infection.** Nevertheless, we will also gain critical information about risk factors for both vector-borne and directly transmitted agents across the land-use gradient.

***Virus information****:* **Hantaviruses**are predominantly rodent-borne viruses, where infection in humans occurs after contact with rodents (family: *Sigmodontinae*) or their urine and faeces. The local species *Andes virus* includes both Araraquara virus and Juquitiba virus (in addition to other strains and genotypes), and both are found in the local area. In Brazil, these hantaviruses are reportedly becoming an emerging health problem due to land-use change, as regions in use and developed by humans may contain or attract wild rodent reservoirs[124](#_ENREF_124). **Mucambo** virus is primarily a mosquito-borne alphavirus that can cause encephalitis in humans. Mucambo virus (and related subtypes) is commonly isolated in specific ecological habitats (forests), where it circulates among rodents, birds and bats, and it's arthropod host *Culex* mosquitoes. Infections have been reported from handling infected rodents, and laboratory experiments suggest aerosolized virus is highly infectious. Risk from human-animal contact may thus be related to contact with either reservoirs and/or vector hosts[125](#_ENREF_125). **Mayaro** virus is another zoonotic mosquito-borne alphavirus, causing febrile exanthematous illness in Amazonia. Mayaro fever is considered a public health concern with increasing case rates in the Amazonian basin putatively linked to ecosystem disturbance. The main reservoir hosts include primates, rodents and birds although the role of these reservoirs in the epidemiology of Mayaro fever is unclear[126](#_ENREF_126).

***Human infection and risk factor surveys:*** In Brazil, ethical controversy has arisen over DNA collection from indigenous peoples for study by scientists largely resulting from past research where informed consent was not properly obtained. For example, Yanomami religious traditions prohibit the storage of any bodily matter after the death of an individual. Currently, several prominent Yanomami delegations have sent letters to researchers demanding return of their blood samples collected in studies over 40 years ago. Issues such as these make it difficult to obtain ethical approval for the collection of genetic material from indigenous peoples in Brazil. **Fiocruz-Amazonia** (PI Luz, current partner) is uniquely positioned to implement the human sampling component of the project, providing unprecedented access to human communities in both our study regions in Brazil. FioCruz is a science-based public health institution and an arm of the Brazilian Health Ministry. Building upon our existing collaboration, we will assess previously identified subgroups (Aims 2 & 3) for baseline seroprevalence to the three zoonotic pathogens described above. FioCruz labs have identified and can analyse all three pathogens using existing laboratory materials and serological assays. ***Survey:*** Public health workers from Fiocruz- Amazonia and Foundation of Sanitary Vigilance of the Amazon will collect whole blood and serum samples from volunteer study participants to determine the incidence of zoonotic infections. A questionnaire survey will be conducted concurrently to identify potential factors associated with increased risk of transmission, which will be used in conjunction with survey data from our broader human-animal contact surveys. In addition, we will specifically recruit individuals with high rates of contact (of a specific type, relevant to each pathogen’s mode of transmission), as identified by the human ecology program in Aims 2 and 3. Whole blood (in EDTA) and sera will be collected separately from consenting participants to test for antibodies and/or RNA to the three viruses described above. Study participants with exposures in natural settings along the land-use gradient will be administered a questionnaire on work practices and potential exposures to infections of animal origin. The questionnaire will take about 20 minutes to complete. After the screening has been completed, test results will be delivered to the Local Project Managers at each site. In addition, we will test a subset of human samples for the same viral families that we are testing for in our wildlife sampling program using consensus PCR to serve as a link between the pathogen potential component (Aim 1), the human-animal contact data (Aims 2&3) and human infection data (Aim 4). We anticipate very low prevalence of these pathogens in the human population, but no prior information at all is available and hence this step will serve as a critical baseline regarding unknown pathogen diversity in the local human populations across a gradient of land-use change and how that relates to the pathogen diversity identified in wildlife reservoirs in each of these regions. ***Sample size***: Our target sample size is to obtain blood from 100 people in each of the 4 gradient levels in the Manaus study region in each of two years of the study (total 800 samples). Although we cannot conduct accurate power analyses due to the lack of available prevalence data in the human population (target sample size would typically depend on expected prevalence of the pathogens being tested for) and because we do not yet know the total population size of the human communities (no finite population correction), if we assume a seroprevalence of 50% (which maximizes sample size requirements), a sample size of 200 per gradient will allow the quantification of pathogen prevalence at a precision level of +-7%. This would be adequate for then testing for differences in prevalence (relative risk of infection) among our gradient levels, in addition to conducting a risk factor analysis using multimodel inference[46](#_ENREF_46).

***Laboratory methods:*** Hemagglutination inhibition (HI) will be used to screen for antibodies against alphavirus. Antigens (Ag) for MAYV and MUCV will be compared with EEV and WEEV, and a positive identification recorded when the observed titre is x4 higher for any one species. Additional screening for IgM antibodies (using ELISA) will be performed on positive samples. Detection of hantavirus antibodies will be performed using a serologic assay developed by FIOCRUZ Paraná, which is commonly used by the surveillance laboratories to make the diagnosis of hantavirus infection. In all cases, consensus PCR for both alphavirus and hantavirus will also be performed to look for the presence of viral RNA in whole blood***.*** Finally*,* we will screen our wildlife samples (Aim 1) for the same pathogens using the same methods.

***E. Aim 5:*** ***Model Application*** - Can we integrate these relationships to **derive mechanistic predictive models of zoonotic disease emergence** under land use change?

**Integration through modeling:** We will bring the data from Aims 1-3 together into a model of general spillover risk, that we will be able to apply to our landscapes of varying land-use development intensities given knowledge of their pathogen potential, contact potential and transmission potential across this gradient. We consider that the probability of a single spillover event from one animal reservoir host species (i) of a zoonotic pathogen (j) to a human is defined as . This probability of spillover to a single person will be proportional (equal) to the probability of contact between the human and the animal reservoir multiplied by the probability that reservoir (i) is infected by pathogen (j) , multiplied by the probability of transmission given contact between an infected reservoir and a human : . We can rewrite this such that the contact process is separated from the prevalence and transmission probabilities: , where is the sum of the risk over all the animal reservoir’s pathogens, and can be dealt with separately for each animal species. If we consider the per person spillover risk then represents the overall risk of at least one spillover event, given a local human population size of **N:** . We will validate this general model from empirical data derived in Aims 1-3 and in Aim 4 with human infection data. We will also separate the human population into empirically derived natural groupings based on relative risk categories for both contact and transmission parameters, in addition to having separate categories for pathogens of broadly differing transmission modes. We will then use this model to extrapolate spillover risk to the broader landscape using spatial modeling tools as a function of land-use development intensity. We aim to develop our general model to run in each pixel of our quantified landscape to provide a landscape view of relative spillover risk. This requires having land-use development integrated directly into each parameter. For each study site, we will quantify a metric of land-use change using an index of landscape development intensity (LDI), which is based on the energy used to develop a given land use type (i.e. emergy)[127](#_ENREF_127). This index takes in to account the land use types surrounding each sampling site and we have developed a method to calculate LDI across landscapes and at different spatial scales to account for the uncertainty as to what scale will be the most relevant for each parameter[128](#_ENREF_128). We will use LDI values as covariates in our models for each Aim (quantifying pathogen, contact and transmission potentials, see Methods Aims 1-3) to isolate the effect of land-use for each component of the spillover model. On the basis of the relevant covariates, our model will thus be generalizable to other, un-surveyed regions of the landscape.



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| **Results from Prior NSF Support** |

**Peter Daszak:** **NSF/NIH EEID R01-TW05869 (PI)**; $1,780,000, 2002-2007; “Anthropogenic Change & Emerging Zoonotic Paramyxoviruses”. Tested hypotheses on the underlying drivers of Henipavirus emergence. Produced >40 papers on the ecology of viral diseases, including papers in *J. Roy Soc. Interface*[52](#_ENREF_52) and *Proc Roy Soc. B.*[50](#_ENREF_50),[51](#_ENREF_51) that used modeling/field data to explain emergence of Hendra and Nipah viruses, a *Science* paperon the origin of SARS[48](#_ENREF_48), and a *Nature paper* on EID hotspots[16](#_ENREF_16). Trained 7 Ph.D. students and 11 undergraduates. Data used by Malaysian Dept of Veterinary Services, Queensland Dept of Primary Industries and the Australian Biosecurity CRC to set guidelines and policy on risk of Nipah and Hendra virus.

**James Holland Jones: NICHD 1K01HD051494 (PI),** 2005-2010; “Demographic Change and Dependent Social Structures.” Career grant to support the development of a research program integrating social networks and formal demography. 18 publications, including papers in *Nature*, PNAS, *PLoS Pathogens* and *PLoS Computational Biology*; 7 submitted; 1 accepted book proposal. NSF BCS-0947132 “RAPID: Structure of Contact Networks and the Spread of Flu-like Infectious Diseases: Implications for Dynamics and Control.” 3 publications (2 PNAS), 5 in preparation and submitted; 1 post-doc (now faculty at Penn State), 2 grad students. NSF BCS-1062879 (PI), 2010-2014; “Individual Decisions and Emergent Aggregate Patterns: Kin Co-residence among Hadza Hunter-Gatherers.” 1 post-doc (now faculty at Yale), ongoing fieldwork, 1 publication, 3 submitted.

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| **Education Plan** |

The education plan for this project includes education and outreach to local people, including indigenous groups, local schools, government and non-governmental agencies, and other stakeholders, as well as undergraduate, graduate and postdoctoral students. Initiatives will include educational outreach at schools, engagement with the public on the topics of land-use change and public health, and meetings with private sector and governmental agencies to build awareness of our research and development achievements.

Land-use change and conservation issues are of growing interest to a broad range of local people and other stakeholders in Brazil. Brazil has made significant advances in slowing deforestation and better managing land-use changes in recent years, partly in response to recent initiatives by numerous local and international NGOs to develop in-country programs focused on better protection for the environment through awareness and education campaigns. Our project will play a large role in evaluating the links and impacts of land-use change on public health and we will leverage our links with other environmental NGOs to influence land-use dialogue in the future on the basis of our results. We also have contacts with numerous local organizations dedicated to these causes. In our Atlantic Forest site, we have active partnerships with the Director and park staff of Morro do Diabo State Park (MDSP), the Chief of the Health Department of Teodoro Sampaio, and representatives from the Department of the Environment. Further, during the past decade, we have carried out several successful educational programs in the settler communities located around MDSP directly relevant to health and conservation and in partnership with the local NGO Instituto de Pesquisas Ecologicas (IPE). In Manaus, we have active collaborations with the Universidade Federal do Amazonas (UFAM), the Instituto Nacional de Pesquisas da Amazonia (INPA) and the Health Division Center of Manaus (DISA east). We will endeavor to gain further exposure for the project through these channels. Success will be evaluated by the number of presentations and exchanges we hold with local and international stakeholders including local community members, the number of local people trained, the number of educational institutions engaged, and the level of media coverage for the project.

On the public health side, land-use change has been implicated in the rise of numerous diseases in Brazil, including those targeted in our human infection study. FioCruz-Amazonia, our in-country partner on this project, specifically focuses on improving the living conditions and health of Amazonian populations through 1) strengthening the regional capacity for the diagnosis of infectious and parasitic diseases; and 2) promoting the dissemination of information about science and health technology aimed at disease prevention and health promotion. Outreach and education is thus an integrated component of our project. EHA and FioCruz are uniquely positioned to disseminate the findings of this study through our ongoing education and training campaigns. This outreach will be interactive, as the project seeks to understand the risk factors of zoonotic infections under land-use change and we will share our scientific findings with local people, managers, researchers, and public health officers.

During the study, as part of the data collection process and the standard operating procedure for FioCruz, stakeholder meetings will be held to educate, sensitize and inform people about which infections animals may carry that can be transmitted to humans. All discussions will be held in a town-meeting format where questions may be readily posed and answered. In addition, if in the process of conducting the study, we learn clinically relevant information that would be helpful if reported to the community or individuals, we will return to the community to disseminate this information. Once initial group meetings have been completed, and the type of research to be performed introduced, individual sessions with trained counselors will be set up for interested persons; consent forms will be presented during this time. After the screening has been conducted, test results will be delivered to the Local Project Managers at each site. Trained counselors will go to each site and set up individual sessions to deliver test results of diseases reportable by law. During these individual sessions, the counselors will answer any questions that participants may have; this may take between 15 minutes to 1 hour depending on the participant.

From the US partners, project status and current results will be presented at local events, at conferences, in popular media articles, in scientific publications, organization newsletters, and in newsletters that we will distribute in Brazil annually. Success of this outreach will be evaluated by the number of people our team consults with about health issues, the impact our results have on community attitudes towards infectious disease risks as evaluated over the two years of the study, and the level of support and participation we receive from local community members.

The project will also provide support for two US-based postdoctoral researchers (one jointly mentored by EHA and Columbia University, and one from Stanford) (see postdoctoral mentoring plan). In addition, as Brazil is a developing country[129](#_ENREF_129) we have allocated limited funds to contract a researcher as our Social Science Coordinator on the human-animal contact survey component specifically to strengthen the education impact of our proposal. Our project will also support 2 REUs (Research Experience for Undergraduates) during Yrs 2 and 3 from Columbia University who will be mentored by project staff. PI Daszak directs an NSF RCN grant (EcoHealthNET) which will be leveraged to train US and international graduate students in multi-disciplinary, field- and lab-based projects directly related to this proposal over the next 3 years. EHA will also use core funding to host 2 graduate scholars per year (Yrs 1, 2, 3) to undertake small research projects related to the project in our offices in New York as part of their research programs. We have a longstanding relationship with Columbia mentoring students in this way. This proposal will be able to support small projects on specific and varying topics in ecology, biodiversity, disease ecology, mathematical modeling, biodiversity science, virology, and anthropology. Success of our student outreach and education plan will be evaluated by the number of students we train, the number of independent research projects we supervise to completion, feedback from the students themselves, and the number of scientific studies published or conference presentations given arising as a result of these collaborations. We will also engage our media contacts and outreach offices to ensure adequate coverage and dissemination of these education opportunities. All PIs will give regular or invited lectures on topics related to this proposal for universities and other organizations to further the efficacy of the education plan.

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| **Management Plan** |

This project is a collaboration between an expert in zoonoses, an ecologist and conservation biologist, a social scientist/anthropologist, a virologist and an in-country expert in public health related to zoonoses. Together, we will develop an interdisciplinary understanding of microbial spillover as a coupled natural-human system driven by anthropogenic land-use change. Each principal investigator and senior personnel brings unique perspectives and skills to this project. The project will be managed by a PI (Daszak, PhD), President of EcoHealth Alliance, with two decades experience managing international, multidisciplinary disease ecology research, including successful multi-year projects funded by NSF, NIH and USAID. Co-PI Murray (ecologist, EHA, PhD) will be the ecological project coordinator and lead the analysis of host and pathogen community ecological data, drawing on over a decade of experience designing and managing ecological field studies, synthesizing information and publishing high impact research. Murray and Daszak will jointly supervise a postdoctoral scientist who will be part of the ecological analysis team, which will also include senior personnel Hosseini (mathematical modeler, EHA, PhD) and Zambrana-Torrelio (biodiversity analyst, EHA, MSc), each recognized experts in their disciplines with strong publication records. Senior personnel Rostal (veterinarian, EHA) will oversee pathogen sampling in wildlife and livestock, drawing on her extensive experience as Latin American field coordinator for the 5 year USAID-funded PREDICT program. Co-PIs Luz (Director FioCruz Amazonas, PhD) and Jones (human ecologist and Associate Professor of Anthropology, Stanford) will oversee the human ecology program with senior personnel and human ecology coordinator Loh (social scientist, EHA, MSc). They will supervise a postdoctoral anthropologist who will analyze and interpret the human behavioral data. Jones draws on an impressive history in human ecological research with a focus on infectious disease. Luz will also manage the human sampling study and supervise staff and students at his institution who will participate in the field studies, undertake laboratory testing of human samples, and analyze the human infection data. Luz has demonstrated success in synthesizing human ecological and human testing data in the study area and is critical for the cooperation of local communities. Co-PI Anthony (virologist, Columbia University’s Centre for Infection and Immunity, PhD) in addition to providing expert virological knowledge to the project will oversee data quality control, ensure consistency of laboratory methods and provide training to technical staff for all laboratory work, visiting together with a lab technician each of the two laboratories involved in the study (FioCruz and University of Sao Paulo). Anthony directed similar virology capacity-building in Brazil and other countries for the USAID funded PREDICT pathogen discovery project. He will ensure that methodological rigor preserves our ability to cross-pollinate these complementary projects and maintain consistency with our prior studies that we build off here. Senior personnel Durigon (Professor of Microbiology, University of Sao Paulo) is Director of a the premier viral testing laboratory in South America at which our animal samples will be analyzed. EHA, USP and CII have a decade long partnership, shared staff, and extremely close collaboration. To ensure efficient progress among the five institutions, monthly video-conference calls will be set up with all project staff to review progress, using EHA’s video-conferencing facility purchased with US Federal Stimulus (ARRA) funds as part of an NSF/NIH EEID award to Daszak. In addition, an annual, in-person management meeting will be held between the PIs at EHA’s offices in New York for which budget has been allocated to each partner.

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| **Project Timeline** | **Year 1** | **Year 2** | **Year 3** |
| **Task 1: Field work - wildlife and pathogen sampling** |  |  |  |
| Field work/ Sample collection Manaus (animal) | X | X |  |
| Field work/ Sample collection Atlantic Forest (animal) | X | X |  |
| **Task 2: Field work - human sampling** | X | X |  |
| Field work/ Sample collection Manaus (human) and risk factor study | X | X |  |
| **Task 3 - Human ecology program** |  |  |  |
| Field work, human behavioral surveys, Manaus and Atlantic Forest | X | X |  |
| **Task 4: Laboratory work** |  |  |  |
| Wildlife pathogen analysis |  | X | X |
| Human pathogen analysis |  | X | X |
| **Task 5: Analysis** |  |  |  |
| Host-pathogen community ecology data analysis (pathogen potential, Aim 1) |  | X | X |
| Human behavioral data analysis (contact / transmission potentials, Aims 2, 3) |  | X | X |
| Human pathogen data analysis (validation study, Aim 4) |  | X | X |
| **Task 6: Analytical synthesis** |  |  |  |
| Integrate and link across tasks, refine coupled natural human system model (Aim 5) |  | X | X |
| Validate general model (human infection survey data) (Aim 4) |  | X | X |
| Develop location specific general spillover model (Aim 5) |  | X | X |
| Generate predictive spillover model and mapping, forecast analysis (Aim 5) |  |  | X |
| **Task 7: Collaboration and project management** |  |  |  |
| Annual PI meeting | X | X | X |
| Domestic and International Conferences |  | X | X |
| Monthly team video calls | X | X | X |
| **Task 8: Publish results** |  | X | X |

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| **Expected Project Significance** |

**Intellectual Merit:** Our proposed work provides a new strategy to examine the mechanisms that promote previously unknown pathogens to emerge into new hosts, and how land-use change influences this complex coupled natural-human system. We hypothesize that: 1) land-use change influences the community ecology of pathogens via impacts on the distribution and abundance of wildlife hosts and/or vectors, modifying the likelihood of cross-species transmission, and 2) human ecology in altered landscapes influences the contact rate between humans, livestock and wildlife hosts and/or vectors and promotes pathogen sharing (spillover). Our proposed work will test the relative contributions of these hypotheses via a detailed exploration into the three components of spillover: 1) pathogen prevalence; 2) contact; and 3) transmission. We will use a combination of wildlife, pathogen and human field studies, informed by, but also informing a mathematical model that describes spillover. This work is the first large-scale field study to examine the complexity of disease emergence across a large-scale urban-to-rural gradient in the Tropics. It will provide fundamental **new information on the relationship between host and pathogen biodiversity**, on the **rules that govern how land use change alters these relationships**, and will help **elucidate the full dimension of the impacts of global environmental change** on a dynamic coupled natural-human system.

**Broader Impacts:** This will contribute to the professional development of postdoctoral scholars, graduate students, field assistants in-country, and undergraduates. PI Daszak directs a current NSF RCN grant (EcoHealthNET) and all staff on this proposal are involved in this RCN. If funded, we will leverage our RCN to train US and international graduate students in multi-disciplinary, field- and lab-based projects directly related to this proposal over the next 3 years. Results from the proposed work will be published in high-impact journals, presented at conferences in the USA and internationally, and to the public as part of EcoHealth Alliance’s non-profit outreach programs, to the ICSU Future Earth program which EHA is a partner in, to the >25 conservation and health institutional partners of EcoHealth Alliance, to the IOM Forum on Microbial Threats, and to congress via the regular briefings EHA holds. Our data will also be made available to public health agencies, conservation and land-use planning bodies for the ongoing effort to reduce the risk and global burden of infectious diseases, protect ecosystems and influence land-use development decisions.

**End of Project Description section (limit page 20 – check page number above)**

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