

NEON Infectious Diseases Subcommittee
DESCRIPTION OF KEY SCIENTIFIC CHALLENGES
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INTRODUCTION

In recent years, newspaper articles have alarmed readers with descriptions of outbreaks of newly emerging infectious diseases, such as Avian Influenza, SARS, West Nile virus, and Sudden Oak Death, as well the reemergence of more familiar diseases such as malaria and cholera. Epidemiologists have long studied the transmission of disease agents in populations of plant and animal hosts. The dynamics of many infectious diseases, however, depend on complex interactions among humans as well as wild and domestic species, and may be closely linked to environmental drivers. Understanding the ecological factors that influence the abundance and distribution of pathogens, vectors, and hosts will be critical for predicting the spatial and temporal dynamics of infectious diseases. Our ability to understand patterns of disease occurrence and emergence in both natural and managed systems is currently insufficient to facilitate disease forecasting. In particular, we need to be able to forecast which organisms are likely to emerge as health risks, as well as where and when these organisms are likely to emerge. This will require a spatially distributed continental research platform to assay, track, model, and ultimately predict the spread of disease organisms over space and time.

FORECAST

Our goal is to forecast a) which organisms or types of organisms are most likely to become health risks or transmit pathogens to humans, other animals, and plants and b) to determine where and when infectious diseases of humans, other animals, and plants will emerge. The integrated NEON program will be uniquely suited to meet our national needs:

a) To forecast which organisms or types of organisms are most likely to become health risks or transmit pathogens to humans, non-human animals, and plants, we need to address:

- 1) What are the patterns of, and explanations for, variation in pathogenic effects of disease-causing organisms and their vectors?
- 2) What are the ecological and evolutionary properties of pathogenic organisms and vectors that influence health risks?

b) To forecast where and when infectious diseases of humans, non-human animals, and plants will emerge, we need to address:

- 1) What biotic and abiotic factors regulate distributions and control ecological and evolutionary dynamics of key vectors, hosts, reservoirs, and pathogens?

JUSTIFICATION

The questions posed here are of critical significance to the science of ecology and are highly relevant to public health and well-being. In particular, infectious disease outbreaks in humans, crops, and livestock often have severe impacts on human health, economics, and society. Many infectious diseases involve complex interactions between animal and plant populations and

communities, and the emergence of these diseases is often driven by environmental factors. Thus, our ability to make predictions about the emergence and spread of diseases depends critically on ecological science. Increasingly, ecological studies are aimed at characterizing the interactions involved in disease dynamics, but our understanding of disease ecology is constrained by the limited spatial and temporal scale of current investigations. Also, our inability to measure with precision critical variables -- such as disease prevalence, contact rates, and transmission -- limits our ability to parameterize models such that accurate forecasting is generally difficult. Diseases are typically characterized by emergence in specific locations and spread to other locations via contact with other susceptible hosts, resulting in transmission. These diseases can spread rapidly over large geographic areas (e.g., the transcontinental spread of West Nile Virus in less than five years), so a continental-scale research platform such as NEON is required to address these critically important questions. Moreover, studies of disease ecology are inherently multi-disciplinary, requiring the expertise of ecologists, evolutionary biologists, geneticists, veterinarians, physicians, social scientists, biomedical researchers, epidemiologists, and mathematical modelers.

DEFINITIONS

Box 1. Terminology used in infectious disease research. Infectious disease refers to the health consequences of infections in the host organism. Therefore, the disease is a manifestation of an infection, but infection can occur without causing disease. In this document, infectious agents include prions, viruses, bacteria, fungi, protozoans, and multicellular endoparasites. Although not all of these are organisms in the strict sense (i.e., prions), we use the terms “infectious agent” and “infectious organism” interchangeably. A pathogen is an infectious agent that causes disease. Various definitions of the term “parasite” exist. In an inclusive sense, a parasite is any organism that lives in or on another organism (the host) and reduces host fitness. This definition would lump all infectious organisms together into the “parasite” category. In a stricter sense, parasites are distinguished from infectious agents by virtue of being eukaryotic, single-celled or multicellular organisms that can infest hosts either externally (ectoparasites) or internally (endoparasites), rather than infect the host. In this document, we incorporate eukaryotic endoparasites capable of causing disease in the general category, “pathogens.” Ectoparasites such as fleas, ticks, and mosquitoes are considered to be “vectors,” when they are capable of transmitting infectious agents among hosts.

DATA REQUIREMENTS

Table 1. List of the data needs to forecast disease emergence, including the frequency of measurements, the type of data (*in-situ*, remote sensing, genetic), where the data will be collected, and how the data will be collected, including infrastructure needs.

Parameter	Frequency	Type	Where	How
Host Abundance	Seasonally	In situ Remote sensing (RS) Population. Genetics	Terrestrial Aquatic Aerial	Longitudinal/Cross-sectional Sampling Traps, Satellite images, IR/Video, Airplanes, High-throughput genetic analyses
Vector Abundance	Daily to Monthly	In situ RS (Audio, Pheromone)	Terrestrial Aquatic Aerial	Satellite Longitudinal/Cross-sectional Sampling Traps High-Throughput Population Genetics
Pathogen Abundance/Prevalence	Daily to Seasonally; Event-Based	In situ; Genetics; Immunologic	Terrestrial Aquatic Aerial	PCR ELISA Microarrays Sentinels Longitudinal/Cross-sectional Sampling
Host Movement	Real-Time to Seasonally; Event-Based	In situ Genetics RS	Terrestrial Aquatic Aerial	GPS tracking Chips, Mots Stable Isotopes
Vector Movement	Real-Time to Seasonally; Event-Based	In situ Genetics RS	Terrestrial Aquatic Aerial	Molecular Labeling; GPS tracking devices; Stable Isotopes Smart Dust
Pathogen Movement	Real-Time to Seasonally; Event-Based	In situ Genetics RS	Terrestrial Aquatic Aerial	Molecular Labeling Population Genetics Environ. Sampling Surrogates (microspheres) Spore Sampling Stable Isotopes?

Patterns of Host and Vector Morbidity/Mortality	Daily to Seasonally; Event-Based	In situ RS	Terrestrial Aquatic Aerial	Biosensing Clinical and Pathological examination; Epidemiologic tracking tools; Longitudinal/Cross-sectional Sampling
Genetic Diversity and Gene Expression (hosts, vectors, pathogens)	Generational Event-Based	In situ RS ?	Terrestrial Aquatic Aerial	Genomics Proteomics Population Genetics Microarray and PCR
Human behavior and demography	Daily to annually (continuous?); Event-based	In situ	Terrestrial	Administrative data Epidemiological tracking tools
Wind patterns/particle transport	Daily to Annually	Distributed Grid	Aerial	Towers Smart Dust
Climate	Daily to Annually	RS (using existing federal, state and private weather monitoring sources)	Terrestrial Aquatic Aerial	Refer to Climate Change committee's data definitions, needs, and requirements
Land Use and Land Cover Change	Seasonally to Annually	RS (using existing assessment sources) Community Reporting	Terrestrial	Refer to Land Use committee's data definitions, needs, and requirements
Watershed Sources, Hydroecological properties	Daily to seasonally (continuous?); Event-based	In situ (using existing hydrological monitoring sources)	Aquatic	refer to Hydroecology committee's data definitions, needs and requirements
Plant Productivity (continental)	Seasonally	In situ RS (leveraging existing inventory and monitoring sources)	Terrestrial Aquatic	Refer to Biodiversity committee's data definitions, needs, and requirements

Community Composition	Monthly to Annually	In situ RS (leveraging existing survey & inventory sources [e.g., USDA FIA])	Terrestrial Aquatic Aerial	Refer to Biodiversity committee's data definitions, needs, and requirements
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INFRASTRUCTURE

Field

Primary sampling network - Spatial and temporal patterns of sampling

To fully capture patterns and processes critical to understanding disease dynamics, we recommend a continental scale, hierarchical sampling scheme. Sampling systematically at a broad scale (e.g., in a 50-km grid) throughout the country, combined with systematic finer scale sampling (e.g., in a 1-km grid) at representative locations within each biome, will allow cross-scale comparisons. Sampling particular defined gradients, such as rural/urban interfaces and productivity gradients, may be a less appropriate approach to infectious disease, but could be usefully incorporated in individual subprojects or regions with environmental heterogeneity at irregular small scales. For example, changes in land use may alter rates of flow and transport of pathogenic organisms through river and stream systems, which would justify a watershed-based sampling scheme.

Detailed sampling of host abundance and mortality will be conducted at specific sampling grid points. Use of satellite data to detect mass mortality or morbidity events of organisms will help to interpolate data between the primary sampling points and determine thresholds when disease outbreaks are a problem. This approach will be strengthened by the combined evaluation of satellite data (e.g., changes in heat signatures or net primary productivity) and climatic variables, such that changes in abundance of organisms not sufficiently explained by climatic variables will stand out as suggestive of an epidemic in real-time analyses.

Primary sampling network - Sensor types

Improved technologies are needed for detecting pathogens in water, soil, and air, in combination with measures of water, soil, and air quality (such as oxygen concentration, temperature, pH, nitrates). Sentinel host organisms would be deployed at selected sensor grid sites for measures of ambient pathogen loads (and would function as a common garden experiment within a region). Innovative technologies would include real-time infrared videos of animals to record abundance of various animal species, miniature satellite/GPS devices to detect movement of animals in time and space, and advances in remote sensing to better discriminate between plant species and types of plant stress. Methods for tracking host, vector, and pathogen movement are needed (radar, fluorescent labeling of individuals). Microarray technology will allow sampling for a broad diversity of pathogens and host responses to infection.

Mobile eco-investigation laboratories

When an outbreak of disease is noted, or a change in host demographics is observed suggesting the possibility of an outbreak, mobile eco-investigation laboratories would be available to collect

additional data. Roughly eight mobile eco-investigation laboratories would be deployed throughout the country for road access to any site within 8-12 hours, and one lab helicopter would be available. The work of the mobile labs would be distinct from response teams such as those from CDC or USDA-APHIS that have a responsibility to manage the pathogen, in that NEON eco-investigation groups would actively supply information and predicted responses to different management scenarios. These mobile laboratories would support the investigation of infectious diseases and collect critical ecological data at finer spatiotemporal scales than the main NEON, such as prevalence and transmission rates and abiotic parameters of particular relevance, to better parameterize and validate predictive models.

Manipulative large-scale experiments

Advances in containment technology will facilitate construction of new experimental field enclosures. However, particular attention is needed to address ethical questions surrounding the risk of increasing local disease levels if pathogens escape the enclosure. More advanced enclosures that include realistic wind and climate patterns will allow experiments in which labeled hosts, vectors, and pathogens are studied as they move through an ecosystem. Experiments in which pathogen transmission rates and prevalence are manipulated and tracked will be particularly useful to understand the ecology of disease. The construction of a minimum of approximately four replicate enclosures across eight representative ecosystem types (minimum of one hectare) will allow detailed study of existing and new pathogens. Manipulative experiments developed for other NEON themes will also offer useful frameworks for the study of infectious disease. Disease forecasting models will be more accurately parameterized with manipulative experiments.

Integration of primary sampling network, mobile eco-investigation laboratories, and epidemiological modeling and adaptations over the life of NEON

The primary sampling network would be maintained throughout the life of NEON, with calibrated updates to accommodate rapidly-changing sampling techniques, such as use of microarrays. When particularly important epidemics arise suddenly, the mobile eco-investigation laboratories can be used to provide more detailed information. As important and/or newly recognized pathogens emerge, new, replicated experiments targeted at rapid discovery of ecological dynamics can quickly be devised and undertaken within existing regional enclosure facilities. The best existing epidemiologic models for each study system would be assembled and developed at the outset of NEON. These models would be validated and updated every two years as part of a regular network-wide disease ecology workshop with additional modifications when disease outbreaks occur.

Archival

Disease forecasting via the NEON system will require proper archiving of both data and specimens. It is absolutely critical that data documentation and protocols be standardized across the network. In most cases it will not be necessary for NEON to build repositories, but rather to establish agreements with existing collections for archiving of specimens for verification of data and future research efforts. Archival methods will vary somewhat for different groups of organisms. Forming agreements with the Natural Science Collections Alliance (NSCA), as well as other existing archival facilities early in the process would be advisable.

In cases where whole host organisms are collected intentionally or salvaged from accidental death the following protocols should be followed by integrating specimen collection and archiving in a relational database:

- A primary voucher specimen should be prepared following standard accepted protocols. This specimen should be archived in an accredited collection and all associated data (collector, date, sex, specific locality including latitude/longitude, measurements, weight, reproductive condition, and accession number) captured electronically and added to a publicly (NEON) accessible database.
- Prior to archival, external and internal parasites and vectors should be removed and preserved using standard accepted methods and blood and tissue samples removed and frozen in liquid nitrogen, all containing the voucher specimens unique number. The specimens should also be properly archived in an appropriate collection. The Manter Laboratory of Parasitology in Lincoln, Nebraska, and the USDA collection in Beltsville, Maryland, are examples of facilities where parasites can be sent. Cryogenic archives such as the Museum of Southwestern Biology's Division of Genomic Materials and the American Museum Cryogenic collections are examples for frozen materials.
- Prior to archiving of the frozen materials, blood or other appropriate samples should be analyzed by a NEON facility for the presence of potentially infectious organisms. If some are found, most can be isolated and characterized from frozen tissues. All of these materials, including any resulting DNA or RNA sequences, must be linked to the material from which they were isolated and to their primary voucher specimens.
- Microbial pathogens should be preserved in facilities such as the American Type Culture Collection (ATCC) with detailed information about source host, etc., as described above.

Analytical

The infrastructure for infectious disease investigation requires at least six laboratory centers that have capabilities to process and test samples collected from hosts, vectors, and abiotic sources for pathogen detection, quantification, and characterization. These centers must also have the ability to analyze, record, store, integrate, and disseminate data. One center will include a particular emphasis on modeling and data synthesis, and at least three of the centers should be designated as Biosafety Level 3 so as to permit the safe handling and analysis of BSL3 agents. High-level security systems will be required in the BSL3 laboratory centers due to the possible use of "select agents" in these facilities. The CDC defines select agents as those "biological agents and toxins that have the potential to pose a severe threat to public health and safety" and their possession is carefully regulated. Facilities, equipment, and trained personnel are required to detect distinguishing phenotypic, genotypic, immunological, and protein characteristics of hosts, vectors, and pathogens. This will require:

- Pathogen (eg., virus, bacteria, protozoa, fungi) and host cell in vitro cultivation for pathogen isolation and immunological analysis.
- Cryopreservation of pathogen, vector, and host samples/specimens which will be stored on-site or distributed to designated archival sites.

- Digital photographic and photomicroscopic capabilities with computerized systems for recording and integrating data.
- Rapid, high-throughput systems for the detection of pathogen-specific antibodies and/or antigens [e.g., ELISA (enzyme linked immunoabsorbent assays)], as well as facilities to simultaneously detect DNA/mRNA (e.g., quantitative PCR, RT-PCR, microarray analysis) from multiple pathogens in the same sample. Robotic sample processing and testing are required to ensure continuous, rapid, and large-scale sample analysis.
- Ability to fluorescently (or otherwise) label pathogens or vectors to track their movement and spread in the environment.
- Analysis of host cellular immune responses to infection (e.g., lymphocyte proliferation, cytokine gene expression and production).
- Analysis and interpretation of gene sequences and expressed proteins by high-throughput robotic systems.
- Proteomic capabilities to measure responses (e.g., metabolic enzymes/hormones and cytokines) of hosts to environmental stressors and pathogen infection as well as analysis of prions.
- Stable isotope analysis to detect origins and long-distance movements of hosts, vectors, and/or pathogens.
- A database management software system that will allow for highly distributed, relational, and transactional data warehouses to capture, organize, analyze, and integrate data relating to hosts, vectors, pathogens, and the environment.
- Broad-band computer networks such as the national LambdaRail to connect diagnostic laboratories, archival museums, public and animal health agencies, and universities within the NEON system so that data can be distributed and analyzed.

For greatest efficiency, these centers may be partnered with existing veterinary, human, and plant diagnostic laboratories. However, due to the demand for large-scale sample processing, rapid testing, and extensive data integration and analysis, they must be independently funded, constructed, and staffed.

Data analysis and integration

The NEON research platform should include establishment of detailed, long-term, longitudinal studies of representative disease systems at a fixed number of sites. NEON researchers would use the emerging understanding of these disease transmission processes and outcomes to forecast the occurrence and intensity of disease outbreaks, initially within the meso-scale sites and subsequently nationally. These studies would use empirically parameterized ecological and epidemiological models to link component population dynamics to leading environmental conditions that permit disease emergence. These models would be validated by predicting

pathogen prevalence/incidence at other locations and times, and subsequently testing those predictions by monitoring the timing and locations of epidemics. Once calibrated, researchers would use the models to forecast the disease outcomes of changing environmental conditions at regional levels. NEON can enable detailed biological studies of focal disease systems, which will be used to parameterize key character states of population members. Additionally, NEON would facilitate the integration of existing databases to enable higher-level simulation modeling techniques, extending current agent-based modeling approaches.

To apply these modeling approaches to dynamic real-world conditions will require a substantial extension of computational capabilities (both computational speed and storage). Separately, it also requires advances in spatially explicit statistical modeling methods of environmental conditions that define the conditions in which pathogen populations interact with reservoir, vector, and other populations. The spatially explicit nature of infectious disease processes should make use of many current technologies to both store and represent the data. In addition, NEON would facilitate advances in statistical methodologies, such as empirical Bayesian estimation approaches, to identify the strength of associations between the environment and the outcome of disease processes. These analytical methods would be integrated with data mining tools that can search for patterns in near-real time and backtrack to identify antecedent conditions that might provide lead-time indicators of disease outbreaks.

Currently existing and planned small scale (≥ 100 m resolution), extensive, in situ and remotely sensed environmental surveillance systems by NEON subgroups, and other agencies and programs, need to be integrated for regional scale environmental monitoring that will be used as predictors of disease outcomes. These coarse scale environmental data need to be calibrated with higher resolution (meter and sub-meter) microclimatic monitoring at a fixed number of meso-scale, regional locations. Small-scale data acquisition should be minimally at daily time scales while meso-scale monitoring should be monitored continuously. The meso-scale location environmental surveillance system would be the focus of the NEON group and coupled to the embedded disease studies. Methods and resources for rapid data acquisition and transfer are critical for event-based studies. The data are likely to be of terabyte and larger sizes. Links to existing agencies' databases at agencies and programs such as CDC, LTER, NIH, USDA [including the National Plant Diagnostic Network (NPDN)], NOAA, USFWS, USGS, NGIA, WHO, FAO, NASA, Consultative Group for International Agricultural Research (CGIAR), FIA, DoD, National Diagnostic Veterinary Laboratory (NDVL), as well as universities, zoos, botanical gardens, and state diagnostic laboratories, will need to be established. Interagency cooperation is also extremely important, as NEON is not meant to duplicate or supplant responsibilities or mandates of existing state, local, and federal agencies.

Education

The infectious disease component of NEON provides unique opportunities, at all educational levels, to take advantage of the public's fascination and concern with disease. Educational programs should demonstrate the important interrelationship between the health of humans, other animals (e.g., companion, food-producing animals, and wildlife), crop and wild plant,s and their environment. NEON is uniquely suited to integrate the ecology of infectious diseases with the

other component parts of the NEON platform, for example climate variability and biodiversity (impact on vector-borne diseases), land use and hydroecology (impact on water-borne diseases), and invasive species (impact on emerging and foreign animal and plant diseases).

Educational programs could range from TV or video dramatizations of disease scene investigation (DSI), like popular CSI programs, to multi-media presentations of animal, human, and plant disease cases and outbreak investigations geared to appropriate educational levels from K-12 to the general public. Innovative, interactive multi-media educational programs developed by NEON could be made available nationally through the NEON web portals for use in classrooms, as auto-tutorials for students or as current information to the public. The web-based programs should use an interactive storytelling approach to explain NEON research projects and give compelling insights of the NEON researchers. Press releases and articles in science or popular magazines could promote links to the NEON website. Field-based research opportunities for “citizen scientists” involvement in NEON projects could be incorporated for the collection of data about hosts, vectors, and environmental factors that relate to infectious diseases of humans, other animals, and plants. State and county extension personnel could also partner with NEON to help coordinate public education and research participation programs. Higher education opportunities would be available in NEON projects and center laboratories to train prospective researchers, animal/public and plant health professionals, and educators in the ecosystem health approach to infectious disease. NEON would be a valuable resource for medical, veterinary, and plant science institutions that currently use or are developing an ecosystem health curriculum for professional training. A real benefit is interdisciplinary training of undergraduate and graduate students, as well as postdoctoral researchers, in a true interdisciplinary approach to complex disease problems.

CONCLUSION

Infectious diseases are the outcomes of highly complex interactions among pathogens, vectors, hosts, their local and regional environments, and the global ecological system. Biomedical agencies at local, state, federal, and international levels are charged with responding to emerging and ongoing health threats from infectious agents, but no entity is charged with developing a holistic understanding of the potentially threatening agents in the environment, their patterns of abundance and distribution, and the environmental factors that can elicit outbreaks. We envision a NEON platform that will provide the baseline data, experimental and diagnostic facilities, cyberinfrastructure, modeling infrastructure, and archival material to facilitate the development of a predictive understanding of the disease-environment interface. The Infectious Disease component of NEON provides strong linkages to all other scientific components (Biodiversity, Biogeochemical Cycles, Climate Variability, Hydroecology, Invasive Species, Land Use, and Emerging Issues), as well as to all educational components. Finally, owing to citizen concern with threats to health, we envision the Infectious Disease component as motivating strong public support for NEON.