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Causal inference in disease ecology: investigating ecological drivers of disease emergence

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Despite awareness that disease emergence may be related to ecological change, few studies have rigorously analyzed the underlying environmental drivers of the dynamics of disease emergence. This may be due to the fact that ecological change and disease emergence are often mediated through complex and large-scale processes that are not amenable to traditional reductionist approaches to causal inference. Here, we suggest strategies assembled from diverse disciplines, including ecology, epidemiology, and the social sciences, to analyze complex relationships, promote cooperation, increase efficiency, and minimize bias when investigating the ecological drivers of disease emergence. These techniques, which complement traditional hypothesis testing, include epidemiologic causal criteria, strong inference, causal diagrams, model selection, and triangulation. We also present several examples from recent emerging infectious disease investigations, including Hendra virus, Nipah virus, coral diseases, and avian influenza, where these techniques were successfully applied. Here, we outline some of the barriers to advancing our understanding of causation in disease ecology and offer some solutions for investigating large-scale ecological drivers, such as global warming, pollution, and land-use change.

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Emerging and re-emerging infectious diseases of humans and wildlife are increasing at an alarming rate, with detrimental consequences for public health, ecosystem health, and biodiversity (Cohen 2000; Dobson and Foufopoulos 2001). Emerging infectious diseases (EIDs) are those that have recently increased in incidence, geographic range, or host range (Daszak *et al.* 2000). The emergence of viruses such as Nipah, Hendra, SARS, and avian influenza A during the past decade has increased scientific and public awareness about the threat of infectious disease and the importance of the links among human, animal, and ecosystem health. Although less publicized, the influence of EIDs on ecosystem and wildlife health is equally important. EIDs such as chytridiomycosis in amphibians, avian malaria in Hawaiian avifauna, canine distemper in ferrets, and multiple disease syndromes among corals have been linked directly to declines in wildlife populations (Van Riper *et al.* 1986; Williams *et al.* 1988; Richardson and Voss 2005; Rachowicz *et al.* 2006). Diseases are also thought to contribute to species extinctions, especially in cases where they act synergistically with anthropogenic impacts such as harvesting or habitat destruction to reduce populations below a critical size threshold for survival (Cunningham and Daszak 1998; Daszak and Cunningham 1999; de Castro and Bolker 2005; Choisy and Rohani 2006; Lips *et al.* 2006; Schloegel *et al.* 2006; Smith *et al.* 2006). Many EIDs are inherently difficult to study for a variety of reasons, including the fact that their etiologic agents are poorly characterized (eg coral disease), disease risk is mediated by complex interactions among multiple hosts (eg Lyme disease, canine distemper), or they have very large-scale to pandemic distributions (eg avian flu).

Global and regional ecological change is often mediated through complex and large-scale processes, making the links between ecological change and disease emergence difficult to demonstrate scientifically. Human activities can result in rapid ecological change through factors such as climate variability, urbanization, invasive species spread, changes in biogeochemical cycling, pollution, habitat fragmentation, and biodiversity loss

In a nutshell:

- Researchers trying to identify environmental drivers of disease emergence are challenged by the complexity, scale, and natural variability of the systems involved
- Traditional approaches to studying cause-and-effect relationships (eg experiments and hypothesis testing) are often not possible when study units are large and complex (populations or ecosystems) and risk factors have non-linear, hierarchical effects
- We suggest techniques that complement hypothesis testing and outline a rigorous approach to causal investigation in complex systems

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Table 1. Recent emerging infectious diseases with proposed ecological drivers for which the associations between ecological factors and disease emergence are supported with empirical evidence (partial list)

EID	*Proposed ecological driver*	*Hypothesized mechanism(s)*	*References*
Hantavirus pulmonary syndrome in North America	Environmental change driven by El Niño Southern Oscillations	Increased rainfall facilitated an ecological cascade resulting in increased rodent reservoir populations and subsequent emergence into human populations.	Glass *et al.* 2000; Hjelle and Glass 2000; Yates *et al. 2002*
Nipah virus in Malaysia	Agricultural intensification	Orchard planting around pig farms increased fruit bat–pig interactions while agricultural intensification allowed Nipah virus persistence within piggeries.	Daszak *et al.* 2006; J Pulliam pers comm
Schistosomiasis in western Africa	Anthropogenic land-use change (dam building and increased land with standing water)	Dam building changed river flow regimes, salinity, and pH, leading to increased populations of the snail intermediate host	N'Goran *et al.* 1997; Southgate 1997
Human malaria in the Amazon basin	Deforestation	Deforestation changed mosquito breeding habitat, leading to an increase in abundance of a mosquito species highly efficient at maintaining and transmitting malaria. Biting rates were hundreds of times higher in deforested habitats than intact forest.	Vittor *et al.* 2006
Lyme disease in the eastern United States	Forest fragmentation	Forest fragmentation and urbanization led to increased numbers of competent rodent hosts.	LoGiudice *et al.* 2003
Avian malaria in Hawaii	Climate warming	Originally linked to the unintentional introduction of a novel mosquito vector to Hawaii, the range of avian malaria has recently expanded to higher altitudes due to warmer summertime air temperatures, which led to increased mosquito breeding at higher elevations.	Freed *et al.* 2005

(Midgley and Thuiller 2005; Hong and Lee 2006). Such rapid global ecological change is thought to reduce ecosystem functioning (Chapin *et al.* 2000), degrade ecosystem services (Foley *et al.* 2005), and threaten wildlife and public health (Daszak *et al.* 2001; Patz 2001; Patz *et al.* 2004). Despite growing awareness that disease emergence may be related, at least in part, to ecological change (Woolhouse and Gowtage-Sequeria 2005; Table 1), there is a notable paucity of studies to confirm or refute whether, and how, ecological change plays this role. Many factors contribute to our poor understanding of the causes and mechanisms of disease emergence. These include the inherent difficulties of studying ecosystems and wildlife populations, insufficient funding for ecological research, gaps in baseline data concerning prevalence of diseases in natural systems and, most importantly, the limitations of applying traditional approaches to studying causal inference in large-scale, complex systems (Allenby 2005).

Reductionist scientific methods separate problems into elements and then focus on the elements in isolation, ignoring the complex and often non-linear, hierarchical effects of changes at one level of organization on another level, a phenomenon widely recognized in ecology (Hilborn and Mangel 1997). Reductionist methods alone, such as individual hypothesis testing, may not be sufficient for analyzing complex relationships such as global disease emergence and ecological change. In this paper, we propose techniques that complement traditional hypothesis testing and outline a rigorous, interdisciplinary approach to causal investigation in disease ecology. These techniques include: epidemiologic causal criteria, strong inference, causal diagrams, model selection, and triangulation.

Epidemiologic criteria for establishing causation in ecological systems

The complex systems studied in epidemiology and ecology are often remarkably similar, and the methodologies used routinely in each discipline, although rarely integrated, are complementary (Anderson 1991). In epidemiologic theory, the ideal method of establishing causality, called the "counterfactual" definition (Lewis 1973; Rubin 1974), involves theoretical backward time-travel to remove a causal factor and demonstrate that, in its absence, the effect, in this case disease emergence, does not occur. Since this kind of control condition is impossible,

an imperfect proxy must be used. In traditional epidemiology, randomization in carefully designed experimental studies emulates the "counterfactual" method, by ensuring comparability of experimental units. These are selected so that control and treatment groups resemble each other in all conditions except exposure to a proposed causal factor. Uncontrolled and unmeasured differences among groups that alternatively explain the outcome, called "confounders" in epidemiologic terms, can lead to confusion when identifying causal factors (Pearl 2000).

In ecological investigations that attempt to establish causation, study units may be large and complex (such as entire communities or ecosystems), so that finding comparable replicates for experimentation, or challenging the system with a treatment, is nearly impossible (Hilborn and Mangel 1997). For example, dozens of newly-emerging coral diseases have been recorded in tropical waters (Richardson 1998) and hypothesized causes include such diverse factors as nutrient pollution (from runoff or sewage) and increased sea surface temperature (possibly linked to climate change; Sutherland *et al.* 2004; Bruno *et al.* 2007). In such a case, how does one find comparable replicates for manipulation, or carry out experiments that impose control over the risk factors of interest, when the scale of the question involves global change and disease emergence throughout entire oceans? One problem in trying to understand complex, tightly coupled human–natural systems is that the overall resilience of the system usually cannot be reduced to a linear relationship between a small set of variables. Furthermore, understanding key variables may require input from many different disciplines – anthropological risk factors, for example, may be just as important as ecological ones in the overall understanding of disease emergence. Because experimental manipulation and randomization are not sufficient to answer ecosystem-level or global-scale questions, ecologists have to complement experimental work with observational studies when examining the causal nature of large-scale processes. In observational studies of disease emergence, where a paucity of baseline data often leaves researchers with no basis on which to determine confounders a priori, there may be no way to ensure comparability between study units.

Similarly, in epidemiology, observational studies are necessary when it is not possible, either practically or ethically, to impose experimental or controlled challenges on the study subjects (eg for studying the relationship between toxin exposure and cancer rates in humans). To use observational evidence to pass "from [an] observed association to a verdict of causation", Sir Austin Bradford Hill published the following set of nine epidemiologic criteria, which expanded upon the 1964 US Surgeon General's report *Smoking and health* (US Public Health Service 1964): (1) strength of association, (2) consistency, (3) plausibility, (4) coherence, (5) experimental evidence, (6) analogy, (7) specificity, (8) temporality, and (9) biologic gradient (Hill 1965; Table 2). Hill's epidemiologic causal criteria offer a set of tools from epidemiology that can be adapted to benefit ecological investigations of disease emergence (Table 2).

Hill's criteria have been used extensively in medicine and epidemiology to establish causal links between risk factors and emerging diseases, for example, between smoking and lung cancer (Hill 1965), abnormal concentrations of cholesterol in the blood and coronary heart disease (Calvert 1994), and the use of post-menopausal hormone replacement and the development of breast cancer in women (Colditz 1998). Within ecology and related fields, Hill's causal criteria have been applied successfully in situations where it is difficult or impossible to assign treatments to experimental units (Beyers 1998), such as environmental impact studies, environmental toxicology investigations, and risk assessments (eg Woodman and Cowling 1987; Fox 1991; Beyers *et al.* 1995; Fabricius and De'Ath 2004). Fabricius and De'Ath (2004) applied a modified subset of Hill's criteria to assess the effects of agricultural runoff on coral reefs of the Australian Great Barrier Reef, an ecosystem for which the large spatial scale, lack of historical data, and high natural variability has traditionally prevented the early detection of ecological change and its causes. The authors demonstrated that water quality and ecological indicators such as macro-algal cover and community structure had an association which (1) was strong and ecologically important; (2) was detected independently in different coral reef communities; (3) agreed with established biological facts about how corals respond to pollutants; and (4) demonstrated a dose–response gradient (for most of the ecological attributes studied). It is important to note (as Hill emphasized is often the case) that not all of the criteria were fulfilled. For example, lack of historic data precluded study of a logical time sequence (although they note that the criterion had been met in other study systems). Nevertheless, the framework allowed the authors to synthesize and assess multiple and complex sources of data (their own field study along with results from other regions and laboratory experiments) and rigorously attribute a causal link between pollutants and some ecological indicators in a system where traditional experimental methods alone have failed to do so.

In the late 19th century, long before Hill's criteria were published, Koch's postulates (Table 3) were adopted by epidemiologists and medical professionals as a rigid, common set of guidelines by which to demonstrate that a particular microbe is the cause of a disease (Evans 1978). For decades, these guidelines were regarded as a "gold standard" for establishing causation in disease systems, and researchers often strive to fulfill them for modern, emerging infectious diseases. The postulates can be re-interpreted into a set of guidelines, applicable to the investigation of causes of disease emergence (Table 3), providing a simple, logical baseline which can be used to organize evidence to establish ecological cause-and-effect relationships. It is noteworthy that the first two postulates involve extensive *observational* evidence, even though it is typical for only the last postulate, which involves manipulative experimental evidence, to be emphasized. Although

Table 2. Hill's nine criteria (Hill 1965) and their application to investigating causation in disease emergence

Hill's criterion	*Epidemiologic explanation*	*Example recommendations for disease emergence studies*	*Caveats*
Strength of association	A causal factor must be correlated with disease and have explanatory power measured by a correlation coefficient, relative risk, odds ratio, or other statistical method.	Conduct studies to assess the biological associations between disease incidence, prevalence, and severity, and environmental, host, and pathogen risk factors.	Establishing association only in the laboratory, under a limited set of circumstances, or on a limited spatial scale may not allow generalization and thus may generate misleading conclusions. Demonstrating a strong association through multiple methods, in multiple scenarios, and at multiple scales is necessary.
Consistency	A causal factor must be associated with disease repeatedly in varying conditions across time and space.	Establish collaborations among disease researchers to replicate studies investigating risk factors using comparable methodologies.	Consistency can be observed with confounding effects as well as causal factors when correlation between causal factors and confounding variables is strong. Identification and control for confounders is imperative.
Plausibility	A causal explanation must make biological sense.	Limit inclusion of potential risk factors in regression and correlation analyses and disease models to those with plausible causal mechanisms, continually re-evaluating "plausibility" as new information is gathered.	Explanations that are plausible are not necessarily true; alternative hypotheses need to be considered carefully.
Coherence	A causal explanation should be consistent with the current body of knowledge.	Consider all current knowledge about disease as well as ecology and biology of environmental and host factors when posing new hypotheses about causation.	Current knowledge is a shifting baseline. This criterion needs continual reconsideration as information becomes available and our understanding changes. For example, Warren and Marshall's proposition that a gastric spiral bacteria caused gastric ulcers contradicted all that was known about the causal mechanisms for ulcers as of 1982. They were eventually awarded the 2005 Nobel Prize for their work, which led to new treatments for ulcers – but only after a long period of skepticism (Thagard 1999).
Experimental evidence	Manipulation of the causal variable through laboratory or natural experiment should change the outcome.	Establish specific pathogen-free hosts, model hosts, and/or cell lines in the laboratory or field and design challenge studies to investigate cause-and-effect relationships.	A laboratory system is always an imperfect proxy for the real ecological system. Hierarchical emergent properties and interaction effects in a system can be missed by studying overly simplified versions of the system.
Analogy	The causal hypothesis under consideration may be compared to an analogous relationship demonstrated in another system.	Look to other systems for analogues to each causal scenario in question.	In ecological systems, the complex relationships among organisms and the environment sometimes preclude comparability with other systems, even if the two systems appear superficially similar. For example, in the US, disease dynamics in east coast and west coast ecosystems (eg Lyme disease) often differ substantially, even if landscapes appear to be similar.
Specificity	A causal factor should be absent when disease is absent.	Use modeling techniques or manipulative experiments to remove a suspected causal factor from the system. If the factor is causal, its removal should result in measurable decrease in disease occurrence, spread, or impact. Identify and take advantage of natural experiments of this type.	The application of this criterion is dependent on the nature of interacting component causes: "necessary" components must always be present to result in disease but may not be causal alone, and "sufficient" causes are combinations of factors that are able to effect disease. It is possible for there to be multiple sufficient causes, and thus some truly causal components in this web may not adhere to the specificity criterion.
Temporality	A causal factor must precede the disease in time. (This is the only criterion that is a logical necessity.)	Use time series data to determine the time course of events – establish that the factor in question preceded the disease emergence outcome.	This criterion is often impossible to prove in ecological systems without good baseline data
Biologic gradient	A causal factor should show a dose–response relationship with the disease.	Collect disease data from areas with varying levels of a potential causal factor (comparable in all other ways) to establish a dose–response relationship (ie when the factor is present at lower levels, disease is less prevalent or severe, or vice-versa).	If interaction effects are present, dose response between a causal factor and an outcome may not be consistent across all levels of the interacting variable.

Koch's postulates have endured through time, it is important to recognize the drawbacks inherent in the postulates which, many argue, preclude their application in most complex, causal investigations where both infectious and non-infectious factors may work in concert (Cochran *et al.* 2000). These drawbacks include (1) that the postulates assume a single-factor/single-outcome relationship and thus do not account for multifactorial causation, interaction effects, or multiple possible outcomes of a causal factor, and (2) that the third postulate necessarily requires control conditions, which may be impossible to achieve in complex systems. Lastly, as Hill's criterion of consistency implies, when investigating complex systems, even Koch's postulates should be repeated in a variety of settings before a generalized causal inference can be reached.

Causal criteria provide a useful framework for evaluat-

Table 3. Koch's postulates (Evans 1978) and their application in disease ecological investigations

Henle-Koch postulate (paraphrased)	*Application to disease ecological investigations*
(i) The organism must be present when the disease is present.	(i) There must be observational evidence that the causal factor is present where disease emerges, persists, and/or spreads.
(ii) The organism must not be present in other diseases or normal tissues.	(ii) There must be observational evidence of reduced risk of disease emergence, persistence, and/or spread when the causal factor is not present.
(iii) The organism must be isolated from tissues in pure culture and be capable of inducing disease under controlled experimental conditions.	(iii) Controlled manipulation of the causal factor must be used to demonstrate that application of the causal factor in isolation (ie while controlling for all other differences among treatment groups) leads to a measurable increase in disease emergence or spread.

ing data collected by different researchers with diverse disciplinary and methodological approaches. They enforce a logical, consistent, and comprehensive reflection of multiple lines of evidence. Although it is tempting to view the epidemiologic causal criteria as a stand-alone checklist for establishing causation, the criteria must be used on a case-by-case basis, while being evaluated in conjunction with other rigorous and standardized scientific methods. However, situations will arise where multiple competing and plausible hypotheses emerge from this process. In the following sections, we outline several scientific methods that can be used in conjunction with causal criteria to evaluate multiple working hypotheses, as well as to promote cooperation, increase efficiency, and minimize bias in causal inference.

Strong inference, causal diagrams, and model selection

In the late 1800s, Chamberlain suggested that hypotheses which propose and test a single explanation for a phenomenon are not suitable for investigating complex systems, but rather that determining "the measure of participation" of each of a multitude of component causes is a more appropriate goal. He also suggested that, in this case, the "simultaneous use of a full staff of working hypotheses is demanded" (Chamberlain 1897). This idea was later expanded by Platt (1964) and others, and when combined with the traditional methodological principles outlined by Popper (1959), evolved into the method of strong inference. In contrast to traditional hypothesis testing, strong inference involves devising and testing multiple working alternative hypotheses that can plausibly account for an observed pattern, and then using inferential methods to decide whether each hypothesis is rejected or explored further. For example, Kilpatrick *et al.* (2006) explored multiple hypotheses for how highly pathogenic H5N1 avian influenza spreads between countries, including trade in poultry and wild birds and the movements of migratory birds. Their analyses demonstrated that current American surveillance plans – based on the hypothesis that H5N1 would enter the US with migratory birds from Siberia (US Department of the Interior 2006) – would probably fail to detect the introduction of H5N1 into the country in time to prevent its spread to domestic poultry. Kilpatrick *et al.* (2006) suggested, using strong inference, that the highest risk of H5N1 introduction to the western hemisphere would be through the trade in poultry, with subsequent spread to the US through migratory birds from countries such as Canada, Mexico, and Brazil.

The strong inference approach has not been widely used in disease-related ecological or epidemiological research, due to both the conventions of traditional hypothesis testing and to "confirmation bias" (the bias brought about by focusing only on one's favorite hypothesis; Chamberlain 1897; Wynder *et al.* 1990; Gorman 1992). In practice, looking for positive evidence for a favored theory can be a useful first strategy, but can harden into a bias that prevents the evaluation of alternate explanations for patterns that surface as more data become available (Tweney *et al.* 1981). Strong inference can correct for this bias by encouraging the simultaneous exploration of multiple alternative hypotheses.

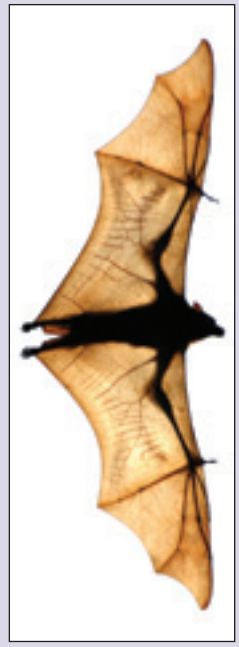
The first step in a strong inference approach is to outline the entire plausible "hypothesis space" for the system. This process is dynamic and iterative as data become available. One rigorous method to outline a hypothesis space is the generation of a complex causal diagram, a visual representation of the plausible mechanistic pathways, potential interactions, and confounders involved in a single outcome of interest (Greenland *et al.* 1999). Fields as diverse as epidemiology (Joffe and Mindell 2006), environmental health (Patz *et al.* 2004), and crime intelligence (Schroeder *et al.* 2003), have used causal diagrams to bring a logical and comprehensive approach to causal analysis. The use of causal diagrams not only facilitates communication and coordination among scientists and managers, but also often clarifies assumptions, lays a foundation for analysis, generates testable hypotheses, facilitates exploration of the effects of various management strategies, and identifies gaps in existing data (Hjorth and Bagheri 2006). An example of a causal diagram for the recent emergence of Hendra virus in Australia is presented in Panel 1.

Once a hypothesis space is comprehensively outlined in

Panel 1. A causal diagram approach to examining Hendra virus emergence in Australia

Hendra virus (HeV), along with several related viruses, has emerged in humans and domestic animals in the Australasian region in the past decade from fruit bats (*Pteropus* spp; Mackenzie *et al.* 2001; Field *et al.* 2001; Figure 1). Since these viruses probably have a long history of association with their host, the question arises: what caused these viruses to emerge into human populations when they did?

After generation of the entire hypothesis space for an emergence event, evidence must be gathered to support or refute each plausible causal pathway (Figure 2). Many plausible hypotheses from the causal diagram for HeV have been determined to be unlikely. For example, genetic analyses revealed that recent pathogen evolution is not likely to account for the switch to new hosts, because HeV has a stable, highly conserved genome, and all bat isolates from disparate geographic regions were shown to be genetically identical (Halpin 2000). Surveillance bias (or an increased detection of disease due to increased surveillance) was shown to be unlikely, because historical samples of undiagnosed encephalidites in humans yielded no anti-HeV antibodies (P Ketterer unpublished data; P Hooper unpublished data), and serosurveys of more than 4000 horses, the amplification host of HeV, failed to reveal past infection with HeV (Ward *et al.* 1996). Even though most of the hypotheses in the causal diagram have not been ruled out completely, this systematic approach has narrowed many of the plausible causes to those associated with the fruit bat hosts.



Courtesy of G Tabor

Figure 1. *Little red flying fox (*Pteropus scapulatus*). This species is one of a number of fruit bats that act as a reservoir for Hendra virus, an EID that has emerged repeatedly in Australia since 1994.*

Recent studies of fruit bat viral ecology have shown that multiple causal pathways could act in concert to produce HeV emergence. For example, fruit bat populations have become more urbanized in response to declining habitat and increased abundance of flowering trees in urban areas (Markus and Hall 2004; McDonald-Madden *et al.* 2005), increasing contact, and therefore potential disease transmission, between fruit bats and horses (Plowright 2007). Simulation models suggest that resulting changes in population dynamics may produce more intense HeV epidemics in bats (Plowright 2007). Additionally, nutritional stress has been linked with increased prevalence of HeV in fruit bats, suggesting causal links between environmental stressors that alter food resources, such as habitat loss and climate change, and disease emergence (Plowright *et al.* in press).

These investigations help to identify and validate potential causal pathways, but many questions remain unanswered. Clarifying the role of ecological factors in driving HeV emergence will require a continual process of acquiring information to assess hypotheses, and eliminating them whenever possible. The strong inference approach used by moving from the bottom up on the causal diagram, will eventually examine "parent" variables (such as land use and climate change), and ultimately lead to a better understanding of how ecological change drives disease emergence.

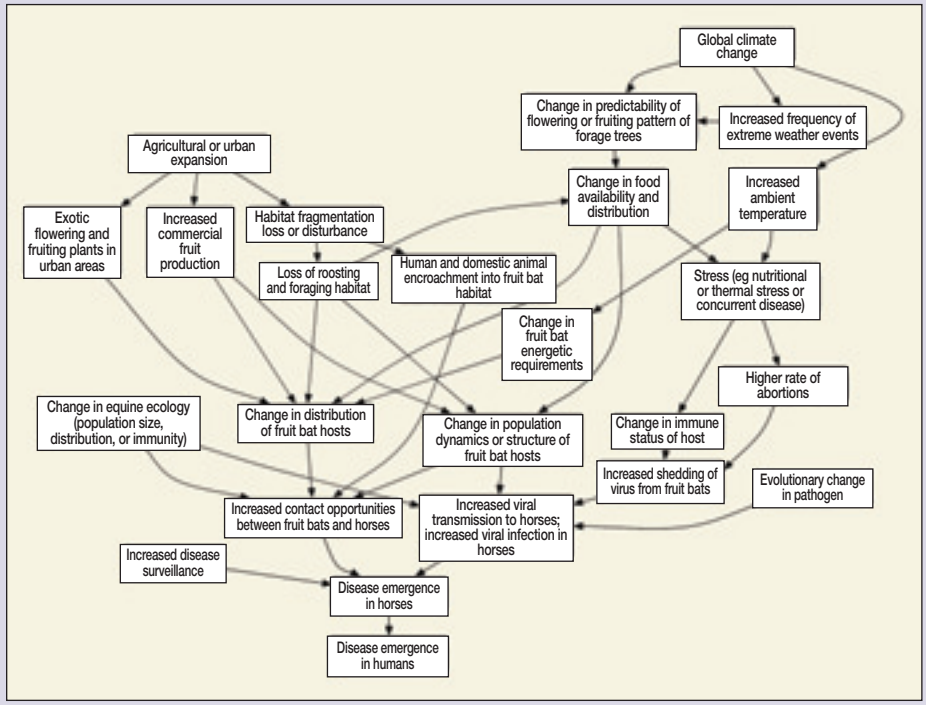


Figure 2. *A causal diagram using a specific disease emergence event – that of Hendra virus in Australia – to illustrate a strong inference approach to examining causation of an EID. The figure illustrates the hierarchy of host and environmental factors that must be considered when investigating disease emergence, ranging from the level of the pathogen through individual hosts, populations, communities, landscape variables, and even global factors.*

a causal diagram, the process of exploring and testing each of the potential causal links in the system requires evidence, in the form of empirical or simulated data and statistical analyses. For example, a strong inference approach using a statistical framework such as model selection, via such methods as the information–theoretic approach (Burnham and Anderson 2002) or structural equation modeling (Grace 2006), allow multiple causal models to be compared rigorously and concurrently. For instance, Ostfeld *et al.* (2006) used a model comparison approach to simultaneously test the importance of multiple variables in predicting Lyme disease risk. Using a 13-year

ecological dataset from Dutchess County, New York, the authors demonstrated that the prior year's abundance of mice and chipmunks, as well as the abundance of acorns 2 years previously, were highly correlated with cycles of Lyme disease emergence.

Cumming and Guégan (2006) used structural equation modeling, along with empirical data, to investigate trophic cascades affecting tick-borne disease in Africa. Their results suggest that the abiotic environment influences tick-borne pathogen diversity primarily through the effects of the environment on ticks (with changes in tick diversity influencing changes in tick-borne pathogen diversity), and secondarily through the direct effects of abiotic variables on pathogens. Their investigation of multiple hypotheses simultaneously helped to elucidate some of the complex potential effects of climate change on disease emergence, and had useful management implications for tick-borne diseases in Africa.

While a comprehensive review of model selection techniques is beyond the scope of this article, it is noteworthy that some of these statistical techniques, such as the information theoretic approach (devising a set of biologically plausible hypotheses and then using Akaike's Information Criterion [AIC], or any number of other established criteria, for selection), are increasingly being used, suggesting a paradigm shift from single-hypothesis testing to multi-model inference. Such a paradigm shift changes the goals of statistical modeling from the unrealistic goal of discovering "truth" or "full reality" to a more realistic goal of identifying which among a finite set of plausible (but admittedly imperfect) models offers a good *approximation* of the available data and thus a good basis for inference about the system (Burnham and Anderson 2002). As the philosopher David Hume noted, the full suite of causal factors can never be conclusively proven (Hume 2000). Thus, the question arises: how does one decide when to accept that the evidence is good enough to acknowledge that an ecological factor, or set of factors, is causal?

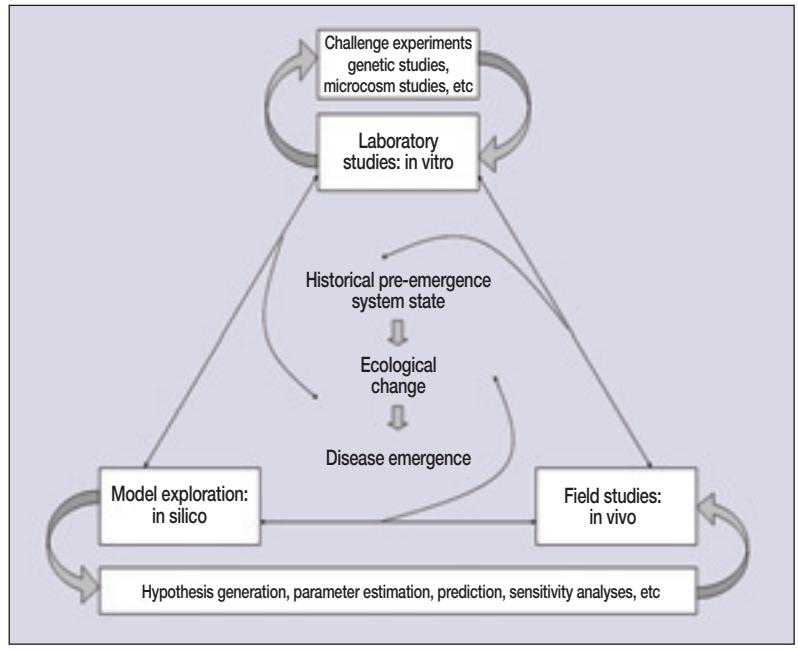


Figure 3. *Triangulation is the process of gathering scientific evidence about a system through a combination of laboratory, field, modeling, and historical investigations, facilitated by iterative and cross-disciplinary collaboration among research groups. Field studies suggest which components of the system should be manipulated in the laboratory and provide data to parameterize computational or analytical models. Models produce predictions, which can be compared to data and therefore drive further field and laboratory investigations. To isolate a change in an ecological system, new information is continually compared with historical information (in EID studies, special attention is paid to the pre-emergence state). We propose that the ongoing interaction of these methodologies will improve our understanding of causal processes in complex systems.*

Triangulation: multiple methodologies and cross-disciplinary collaborations

A hypothesis supported by multiple kinds of evidence is stronger than one that is demonstrable only if a particular technique is used. Therefore, research investigating the ecological drivers of disease emergence should draw upon many relevant and current methodologies to investigate causation. A well-established concept in the social sciences is a process analogous to triangulation in surveying, where multiple lines of evidence are assembled and compared to continually narrow uncertainty about a cause-and-effect relationship (Sharp and Frechtling 1997). This process clearly requires cooperation among diverse groups. For studies of disease emergence, combining multiple methods, such as in vivo studies (field research), in vitro studies (laboratory investigations), and modeling, may be particularly powerful in causal inference (see Figure 3). These methodological approaches can be combined in an interactive, iterative process, where data from one methodology feeds other methodologies. Such "triangulation" can help to remove a bias that stems from the belief that one method is superior to others (Dunbar and Fugelsang 2005).

The process of triangulation uses feedback between various methods to increase efficiency in the acquisition of data, generation of hypotheses, and establishment of causal inference. Field studies enable the detection of associations between causal factors and disease emergence within the system of interest. However, without control of complex interactions and confounders, one is often left with only correlative interpretation, and causal inference remains beyond reach. Therefore, field research can and should be complemented by laboratory investigation, where conditions can be controlled and experimental groups randomized to minimize the effects of confounding. Likewise, field research can suggest factors to explore in the laboratory, but the sum of the effects in

real-life ecosystems is often far too complex for laboratory simulation. Therefore, modeling is an essential tool for exploring the complex interactions of multiple potentially causal factors. Feedback among these methods is continual: field and laboratory data enable accurate model parameterization, and sensitivity analyses performed using models, in turn, suggest parameters that are critical to measure with precision (and thus require more field and laboratory investigation). By offering specific predictions and generating new hypotheses, models can efficiently direct new lines of study that can be tested in field or laboratory settings. For example, a triangulation approach helped to identify chytridiomycosis, an emerging infectious disease of amphibians, as a cause of mountain yellow-legged frog (*Rana muscosa*) declines in the Sierra Nevada of California. After field studies by Rachowicz *et al.* (2006) demonstrated higher population declines and extirpation at infected sites compared to non-infected sites, and controlled laboratory experiments confirmed that the pathogen *Batrachochytrium dendrobatidis* could be a necessary and sufficient cause of mortality in infected *R muscosa* in a laboratory setting, further field experiments showed that the progression of chytridiomycosis observed in laboratory and field settings were comparable. Briggs *et al.* (2005) combined data from these and other studies to parameterize a model that examined the population level consequences of chytridiomycosis. Their model suggested that mortality rates measured in field and laboratory studies could explain the observed population declines and extinctions. The combination of methodologies applied in an iterative process – laboratory, field, and modeling – suggested a causal link more strongly than could any one method alone.

The goal of triangulation is not to conclusively "prove" cause-and-effect relationships, but rather to collect sufficient evidence, using all of the available tools and data, to reach a "verdict" of causation, which can then justify (and direct) management action. With respect to the ecological drivers of disease emergence, this means identifying where there is sufficient evidence to suggest that the regulation of an ecological factor will mitigate or reduce disease emergence risk. After such ecological factors are identified and implicated, adaptive management can be used with continual monitoring and evaluation using the triangulation methods, to hone the best strategy for maintaining or altering complex systems. In adaptive management, "policies become hypotheses, and management actions become the experiments to test those hypotheses" (Folke *et al.* 2005).

Because the process of triangulation proceeds well when factors that changed prior to disease emergence are identified, it is helpful to investigate the current state of a system in its historical context, and thus all results of field, laboratory, and modeling research should be evaluated in the context of historical data. One caveat is that, since the need to understand EIDs is usually only appreciated *after* a disease has emerged, quantitative data about the *prior* status of the system are often lacking. This highlights the need for more surveillance and baseline data in ecological systems.

For emerging disease ecology, the use of multiple methods for triangulation will probably increase efficiency and effectiveness in causal investigations, but will require substantial effort to create cohesive, interdisciplinary collaborations where data (both rigorously acquired and anecdotal), hypotheses, and insights are actively shared. The importance of this cross-disciplinary cooperation was recently demonstrated through investigations into Nipah virus emergence in Malaysia. Despite the complexities of Nipah virus ecology, its emergence was elucidated using a triangulation approach, incorporating field, laboratory, and modeling methods. Field interviews and historical data analysis demonstrated that an increased planting of fruit trees on pig farms resulted in closer fruit bat–pig interaction and subsequent spillover of virus from bats to pigs. Models incorporating data collected from disease outbreaks, experimental challenge studies, and field investigations, predicted that changing production practices in Malaysian piggeries created the conditions necessary for Nipah virus persistence in pigs, and therefore a longer window of opportunity for the virus to spread between farms, eventually infecting many humans. Assessing model fit to data and sensitivity analyses inspired an iterative process involving further field data collection, laboratory studies, and model building to evaluate and re-evaluate model predictions (Daszak *et al.* 2006; Pulliam *et al.* 2007; J Pulliam pers comm). This process required the coordinated efforts of virologists, veterinarians, ecologists, and mathematical modelers, utilizing many methodologies to understand the multiple components of emergence: viral shedding and transmission dynamics within and between host species, the ecology of reservoir and amplifier hosts, and the factors involved in the transmission event from bats to pigs, which eventually led to a novel outbreak in humans.

Conclusions

Investigations of disease outbreaks are often impeded by time and funding constraints. As such, an imperfect trade-off exists between the immediacy of the health implications of an emerging disease and application of the best methodological assessments of causation. The goal to conclusively "prove" causation may therefore be impractical. A more realistic objective may be to amass sufficient evidence to implicate ecological (or other behavioral, sociological, or economic) causes of disease emergence, in order to inform EID prevention and management. Determining whether or not ecological drivers such as climate change, pollution, and ecosystem destruction underlie emerging infectious diseases can only be achieved through systematic, interdisciplinary cooperation. By analogy, in a jury trial, multiple lines of evidence that connect the suspect to the crime scene, the weapon, and the motive are often required before a verdict can be reached. This paper offers several methodologies, includ-

ing epidemiologic criteria, strong inference, model selection, and triangulation, for enforcing a rigorous approach to establishing causation in disease ecology. Ultimately, cooperative investigations which assemble evidence efficiently and with minimal bias are required to determine the importance of ecological drivers in disease emergence, so that appropriate management strategies can be devised before public health suffers and ecosystems are irreversibly damaged.

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