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*Forum*

# Determining Causality and Controlling Disease is Based on Collaborative Research involving Multidisciplinary Approaches

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**Abstract:** Understanding the causes of infectious disease to facilitate better control requires observational and experimental studies. Often these must be conducted at many scales such as at the molecular, cellular, organism, and population level. Studies need to consider both intrinsic and extrinsic factors affecting the pathogen/host interaction. They also require a combination of study methods covered by disciplines such as pathology, epidemiology, microbiology, and ecology. Therefore, it is important that disciplines work together when designing and conducting studies. Finally, we need to integrate and interpret data across levels and disciplines to better formulate control strategies. This requires another group of specialists with broad cross-disciplinary training in epidemiology and an ability to readily work with others.

**Keywords:** wildlife epidemiology, causation, experiments, diagnostic tests, scientific method, amphibian pathogens, *Batrachochytrium*, chytridiomycosis, multidisciplinary

Infectious disease is a complex interaction between pathogen, host, and external determinants. It can lead to one of several possible outcomes for a host, such as death, reduction in fitness without death, or recovery without long-lasting impacts on health. We are only just beginning to appreciate and document the multiplicity of complex pathways and mechanisms that lead to these endpoints of a pathogen/host interaction. Determining the causes and controlling disease is based on understanding these processes that includes intrinsic and extrinsic factors affecting the interaction. Deductive experiments are certainly one approach to improve our understanding as rightly stated in the paper by Blaustein et al. (this issue). However, they are of limited value if used in isolation and if additional work is not undertaken to understand the causal pathway of disease. Plowright et al. (2008) suggest a framework using multiple lines of evidence for determining causal inference. A similar framework was used by Skerratt et al. (2007) to show that the spread of chytridiomycosis had caused the greatest loss of vertebrate biodiversity due to disease in recorded history. Blaustein et al. argue that experiments "can provide an unambiguous rejection of the null hypothesis that pathogen X is not harmful to its host." However, experimental conditions do not fully encapsulate conditions experienced by the host in the real world, and any experiment is by definition limited in explanatory

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power. Changes in abiotic parameters can have strong influences on both pathogen and host physiology and thus virulence and pathogenicity of a disease (Wolinska and King, 2009; Ostfeld, 2009), and pathogen dynamics can be regulated by host density- or frequency-dependent transmission processes, which are difficult to replicate experimentally (Monello and Gompper, 2009; Seppälä et al., 2008; Ryder et al., 2007). Thus the outcome of most host/pathogen interactions is context-dependent and it is impossible for individual experiments to capture the breadth of host and pathogen responses to the environmental spectrum they inhabit. This is certainly the case for the pathogen *Batrachochytrium dendrobatidis* (*Bd*)/amphibian host system, where the outcome of exposure to the pathogen is affected by host species, life stage, age, body condition and behavior, environmental temperature, pathogen strain and infective dose, and habitat (Berger et al., 1998, 2004, 2005, 2009; Berger, 2001; Johnson et al., 2003; Piotrowski et al., 2004; Bosch et al., 2007; Kriger and Hero, 2007; Skerratt et al., 2008; Woodhams et al., 2008; Fisher et al., 2009; Garner et al., 2009; Richards-Zawacki, 2009).

Blaustein et al. use the example of one of their own experiments (Blaustein et al., 2005) to demonstrate the value of experiments. Tadpoles of *Bufo boreas* were exposed to *Batrachochytrium dendrobatidis* (*Bd*) cultures and died at higher rates within 48 hr compared with controls. From this, they state that the results of this experiment provide an empirical foundation for the suggestion that mortality due to *Bd* may affect whole amphibian populations. This is extremely speculative, as the statement has been made without the appropriate pathological analyses to confirm the cause of death. Nor is the experiment placed in the real world context. It must be remembered that larval exposure to pure cultures of *Bd* does not occur in nature and the results cannot be extrapolated directly to wild amphibian populations. We would argue that it is necessary to describe patterns of tadpole mortality in nature before concluding tadpole mortality due to *Bd* does occur or has any relevance with regards to population effects. Published reports of declines due to *Bd* in *B. boreas* report evidence of infection and advanced chytridiomycosis in adults consistent with the cause of death (Muths et al., 2003; Patla and Peterson, 2004; Murphy et al., 2009) and surveys of tadpoles have failed to detect infection (Murphy et al., 2009). Survival analyses of adult toads at locations experiencing declines support the hypothesis that mortality was substantial and the most parsimonious explanation for the sharp interannual decline is a substantial amount of adult, not larval, death (Scherer et al., 2005). Experimental challenges of recent *B. boreas* metamorphs caused mortality, but for a far longer time span than exhibited by the tadpoles in the experiment reported by Blaustein et al. (2005), and in the one case where metamorph mortality did occur soon after exposure, molecular screening for infection did not reveal any evidence of *Bd* DNA (Carey et al., 2006; Murphy et al., 2009). Most studies of tadpoles suggest that mortality during larval stages is less prominent than in metamorphosed forms (Berger et al., 1998; Rachowicz et al., 2006; Garner et al., 2009). Taken together, these studies provide empirical support for adults and juveniles as being the key life history stages affected in disease-driven population regulation of this host species.

If, indeed, exposure to *Bd* was the cause of death in the experiment reported by Blaustein et al. (2005), then the mechanism is unclear: was the pathogen viable at the time of exposure; if so was it the direct effect of infection, the effect of a toxin produced during infection or the effect of a toxic metabolite produced by the fungus while in culture that caused death? Understanding the mechanism is essential if we are to interpret these findings and treat them as any other than an experimental artifact and use them to guide future research or disease control. Blaustein et al. argue that "it is not necessary to conduct detailed post-mortem examinations or diagnostic assays to quantify pathogen load, or even to determine whether the hosts were infected." This advice contradicts that of medical, veterinary, and wildlife health professionals. It relies on all of the assumptions of an unbiased experiment having been met, that the pathogen is viable at the time of exposure, and that understanding of the causal pathway of disease is not important. We refer to one of our own experiments to counter their argument. Common toad (*Bufo bufo*) tadpoles exposed to relatively low doses of *Bd* usually, but not always, die at or soon after metamorphosis often without detectable infections, whereas high doses resulted in comprehensive infection and mortality through metamorphosis: both categories exhibited growth costs attributable to exposure and possible infection with *Bd* (Garner et al., 2009). Even with the benefit of histological and molecular screens for infection, we cannot determine the mechanism leading to death from this experiment; however, we could postulate that there may be more than one, where pathogen proliferation after high doses leads to rapid mortality and host responses to exposure and possibly infection without the additional effects of extensive pathogen proliferation also incur costs that increase the probability of mortality. In

either case, toxins were not supported as a mechanism. These conclusions could not have been reached without the benefit of some form of post mortem examination for infection with *Bd*.

Contradicting their own advice, Blaustein et al. suggest a *Bd* toxin caused their tadpole mortality based on the work of other researchers which relied on postmortem examinations, diagnostic assays, and understanding of the life cycle of the pathogen (Berger et al., 1998). Further contradiction occurs in their example of an experiment that showed high temperatures cured frogs (Woodhams et al., 2003). This work was designed and based on previous work by Lee Berger and colleagues, which examined the causal pathway that showed that mortality due to chytridiomycosis increased in winter and could be experimentally explained by temperature (all frogs died at 17°C and 23°C, but 50% survived and were cured at 27°C and high temperatures were detrimental to *Bd* cultures (Berger, 2001; Johnson et al., 2003; Berger et al., 2004)). Both infection experiments, Berger's and Woodham's, also used histological diagnostic methods to demonstrate that frogs had been cured.

We sympathize with the frustration of Blaustein et al. regarding the lack of replicated, quantifiable experiments in *Bd* research using individually housed animals. Some current published experiments of amphibian hosts and *Bd* reflect a mindset that does not take into account the complexity of host/parasite relationships or established methods for investigating costs of infectious disease. Examples include, along with Blaustein et al.'s (2005) publication, Parris and Cornelius (2004) and Parris (2004). Typically, these experiments show that when tadpoles are reared in mesocosms or individually with or without the presence of *Bd*, tadpoles exposed to *Bd* may die or may exhibit growth-related costs reflected at metamorphosis. This information alone is inadequate for addressing questions relating to the causal pathways to morbidity and mortality in the wild. We do not disagree with Blaustein et al. that a well-designed experiment is critical for investigation of the pathogenesis of *Bd*. We acknowledge that experiments could benefit from greater statistical rigour. However experimental design alone will not answer questions about the physiological mechanisms/pathways leading to morbidity and mortality. An integrative approach is required to unravel the pathogenesis of *Bd* and this includes both "well-designed experiments" and investigative tools in various specialist disciplines, including molecular biology, clinical pathology, histopathology, and epidemiology (see Voyles et al., 2009). This can be done, as suggested by Blaustein et al., via collaboration involving multiple disciplines. This is the means whereby other complex host-pathogen investigations significantly increased understanding (see recent work on emerging diseases, such as henipaviruses, SARS coronavirus, West Nile virus, and avian influenza) and indeed was the approach used for the original work involving the discovery and characterization of *Bd* (Berger et al., 1998; Longcore et al., 1999). If this approach was taken by Blaustein et al. (2005), data would have been available to confirm the presence or absence of *Bd* infecting their tadpoles; with this information they could have rapidly advanced to other questions relating to the pathogenesis of *Bd*.

Wildlife health is rapidly growing in importance as significant diseases continue to emerge in and from wildlife (Jones et al., 2008). The dynamic nature of the field will result in new ideas and ways of doing things. Therefore, it is important to have these discussions to determine which ideas will lead to scientific advancement.

We recommend that researchers conducting infectious disease experiments of wildlife seek collaborations with biological scientists (including molecular biologists, microscopists, microbiologists, and ecologists) and veterinary pathologists and epidemiologists to enrich the design of their experiments, which will result in the generation of more comprehensive datasets from which definitive analyses and interpretations may be made. This recommendation has been made before and no doubt will be made again as long as it continues to demonstrate clear benefits (Shieh et al., 2000; Daszak et al., 2004). Finally, understanding disease is not all about reliance on deductive experiments to determine the effect of a pathogen; rather it involves multi-disciplined collaboration using a myriad of approaches focused on investigating causal pathways and mechanisms of disease.

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