Infectious diseases in wildlife: the community ecology context

Lisa K Belden[1*] and Reid N Harris[2]

Species diversity can have important effects on disease dynamics. While these effects are often considered with respect to alternate hosts and predators, the influence of diversity may also be seen at the level of the parasite or pathogen. Pathogenic microbes face an array of abiotic and biotic challenges, both within their host and, often, in the external environment. Here, we examine the role of microbial ecology in maintaining health and in contributing to disease. As suggested by some medical scientists and others, we argue that placing pathogens in an ecological context can contribute to our understanding of emerging infectious diseases in natural systems. In addition, we suggest that this view could provide important insights for the conservation of species, including many amphibians, that are threatened by disease outbreaks.

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Species diversity impacts a number of processes in ecological communities, including productivity, stability, and susceptibility to invasive species (Hooper *et al.* 2005). In addition, species diversity can alter disease dynamics (Keesing *et al.* 2006). For example, the risk to humans of contracting Lyme disease is reduced when there is a greater diversity of potential hosts for the disease-bearing tick (LoGiudice *et al.* 2003). However, while the role of alternate hosts and predators in altering disease dynamics has received increased attention in recent years, less work has been done on the role that species diversity at the parasite or pathogen trophic level plays in disease dynamics. Work on macroparasites, such as helminths, suggests that interspecific competitive interactions within the host can alter infection levels and reproduction of parasites (eg Lafferty *et al.* 1994).

We suggest that interspecific competitive interactions among bacteria and fungi are also determinants of disease dynamics. All microorganisms exist in ecological communities, within the bounds of ecological constraints, and are limited by abiotic conditions, both within their host and, often, within the external environment. When abiotic conditions change, as has recently been documented at upland sites in the neotropics, outbreaks of disease can occur (Pounds *et al.* 2006). In an increasing number of systems, it appears that biotic interactions, especially competitive interactions, of pathogens with non-pathogenic microbes in the environment or in the host can also influence disease outcomes. In particular, we suggest that the normal microbiota of metazoans can prevent colonization of microbial pathogens or keep pathogens at low population densities through competition.

In a nutshell:

- All species are host to a diverse array of microbes that constitute their natural microbiota
- Ecological interactions occur continuously between pathogenic invaders and non-pathogenic members of the microbiota
- Many environmental changes are occurring that could disrupt microbial communities, alter the composition of species' natural microbiota, and potentially lead to disease outbreaks
- We may be able to take advantage of these microbial interactions to prevent diseases in at-risk natural populations

[1]*Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061* [*]*(belden@vt.edu);* [2]*Department of Biology, James Madison University, Harrisonburg, VA 22807*

The ubiquity of microbes

All metazoans serve as habitat for numerous symbiotic microorganisms that constitute their natural microbiota (Figure 1). In adult humans, an estimated 1.5 kg of body mass is composed of microbes (Wilson 2005). While this has long been appreciated, the influx of modern molecular techniques into microbial ecology research in the past several decades has rapidly advanced our ability to characterize these microbial communities. Estimates suggest that up to 99% of microbes cannot be cultured using traditional plating methods (Mlot 2004). Culture-independent techniques, including sequencing of the 16S rRNA gene, have therefore greatly expanded our ability to detect and identify microbial species within complex communities. As an example, recent application of these techniques to the human intestinal flora, which is one of the best characterized of the natural microbial communities, resulted in the identification of 244 novel species, representing 62% of the samples analyzed (Eckburg *et al.* 2005).

In some cases, host species may rely on close associations with specific microbes for critical life processes. For instance, in termites, microbes in the gut, along with symbiotic flagellates, may assist with the breakdown of cellulose (Wenzel *et al.* 2002). Some squid possess ventral

Figure 1. *All organisms serve as hosts to a diverse microbiota. (a) Redback salamanders live in forests of the eastern US and (b) have a diverse microbiota growing on their skin, many members of which have antimicrobial properties (reprinted with permission from Lauer* et al. *2007). (c) Human infants begin developing their microbiota during passage down the birth canal. By the time adulthood is reached, approximately 1.5 kg of body mass will be composed of (d) prokaryotic cells, like those shown here, from the human intestinal tract (reprinted with permission from Macfarlane and Macfarlane [2006]).*

light organs filled with luminescent bacteria, which eliminate any silhouette visible to predators (Jones and Nishiguchi 2004), and some legumes make use of symbiotic nitrogen-fixing bacteria when nitrogen is limiting in the environment (van Rhijn and Vanderleyden 1995). These examples represent a tiny fraction of the non-pathogenic microbes that have metazoans and plants as hosts. We know very little about how most members of the natural microbiota contribute to, or detract from, host life, but research in this area is growing, especially in terms of health and disease.

The natural microbiota in health and disease

Recent work on amphibians provides an important example of the role that the natural microbiota may have in preventing disease. Identifying amphibian pathogens has become important because of worldwide amphibian population declines. Most of the recent research has focused on three pathogens: *Batrachochytrium dendrobatidis* (chytrid fungus), *Saprolegnia* spp (water molds), and ranaviruses (reviewed in Daszak *et al.* 2003). Of these, *B dendrobatidis*, a fungal pathogen that attacks keratinized skin, has been associated with the greatest proportion of population declines in the past decade. Since this pathogen was originally described in 1998 from dead and dying frogs in both Central America and Australia (Berger *et al.* 1998), *B dendrobatidis* has been isolated from amphibians around the world, and appears to be continuing to cause population declines and extinctions in susceptible populations as it spreads (Lips *et al.* 2006).

There are key questions remaining, however, about why some individuals, populations, or species are resistant to *B dendrobatidis* infection or are carriers that never develop the disease state. For instance, there are resistant and susceptible populations of *Rana muscosa* in California (Briggs *et al.* 2005), remnant populations of *Taudactylus eungellensis* in Queensland, Australia that persist in spite of the presence of *B dendrobatidis* (Retallick *et al.* 2004), and some species, such as *Rana catesbeiana*, that appear to be carriers of *B dendrobatidis*, but do not develop disease (Daszak *et al.* 2004). The innate immune system, and in particular the production of antimicrobial peptides in amphibian skin, clearly has an important role in preventing infection (Rollins-Smith and Conlon 2005). However, recent evidence that some bacterial members of the natural microbiota of the salamanders *Plethodon cinereus* and *Hemidactylium scutatum* can inhibit growth of *B dendrobatidis* (Harris *et al.* 2006; Figure 2), suggests that amphibians have a well-developed skin microbiota and that some members appear to play a role in preventing colonization by pathogenic microbes. This idea is also supported by studies on the skin microbiota of the salamander *Plethodon ventralis*, where it was found that approximately 27% of bacterial isolates from the skin exhibited antimicrobial properties, although these were not tested specifically against *B dendrobatidis* (Austin 2000). Many bacterial species remain difficult or impossible to culture, and bacteria from amphibian skin are no exception. For example, by using culture-independent techniques, we recently discovered a ubiquitous, non-culturable bacterial species from the skin of *P cinereus*. Based on sequence similarity, this species is related to a culturable species that has antifungal properties (Lauer *et al.* 2007). Given this similarity, we are hopeful that the species will eventually be cultured from the salamander skin and its antifungal properties assessed. Using sequence similarity as a starting point for culturing new species identified by culture-independent techniques may be a useful approach in other systems, as well.

A number of examples from other systems further demonstrate the importance of natural microbiota in maintaining health and preventing infection. Medical scientists are becoming increasingly aware that changes

in the human microbiota, especially in the intestinal tract, can lead to an increased incidence of some diseases (Blaser 2006; Ley *et al.* 2006). Intestinal microbiota provide a barrier against infection by many potential microbial pathogens (Servin 2004). Disruption of the natural intestinal microbiota with antibiotic use can have considerable impacts on the microbial community in the gut and on the outcome of interactions between the commensal and opportunistic organisms that live there (Levy 2000). Similarly, it has long been recognized by plant pathologists that alteration of the soil microbial community is associated with pathogen outbreaks in some plant species (Klironomos 2002; Hamel *et al.* 2005). In these cases, the plants themselves are thought to alter the soil environment, eventually leading to an altered microbial composition and a subsequent increase in disease.

Fish are similar to amphibians in that they have a well-developed skin microbiota, many members of which may play a role in the prevention of disease (eg Hatai and Willoughby 1988). The effective use of beneficial bacteria for the treatment or prevention of disease (probiotics) in fish aquaculture and even in humans (Irianto and Austin 2002; Rastall *et al.* 2005) also supports the idea that the composition of the microbiota could affect disease outcomes. Another well-studied system is the gut microbiota of insects (Gilliam 1997; Dillon and Dillon 2004). In honeybees, many members of the gut microflora show antimicrobial activity against the common bee pathogen, *Paenibacillus larvae* (Evans and Armstrong 2006).

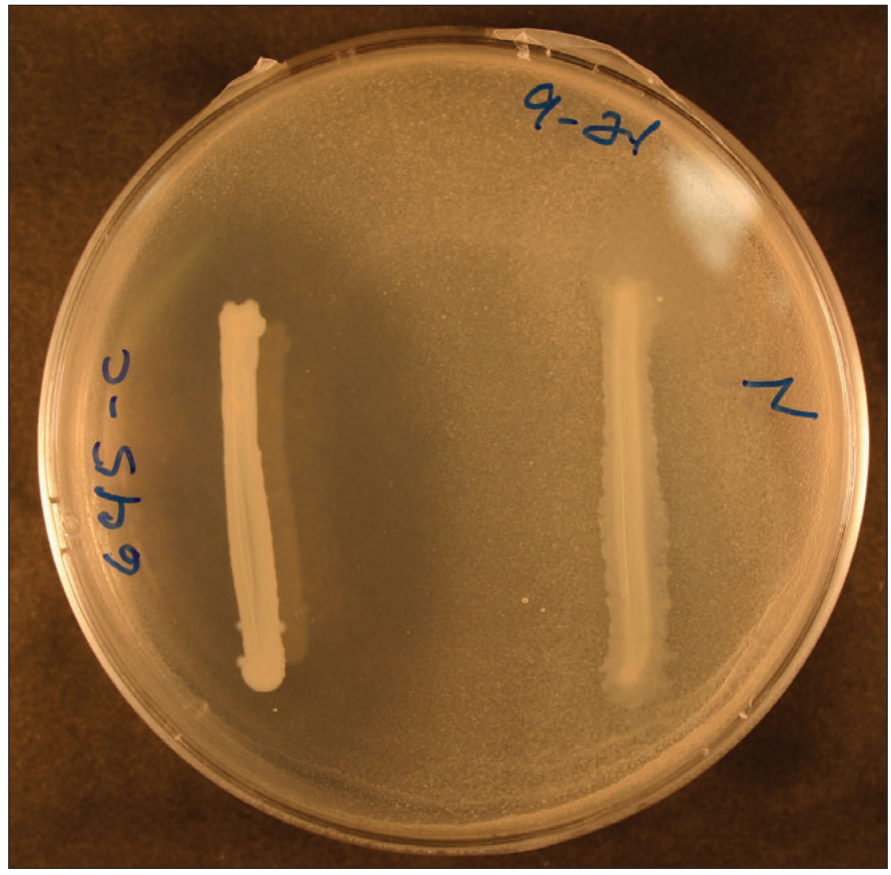

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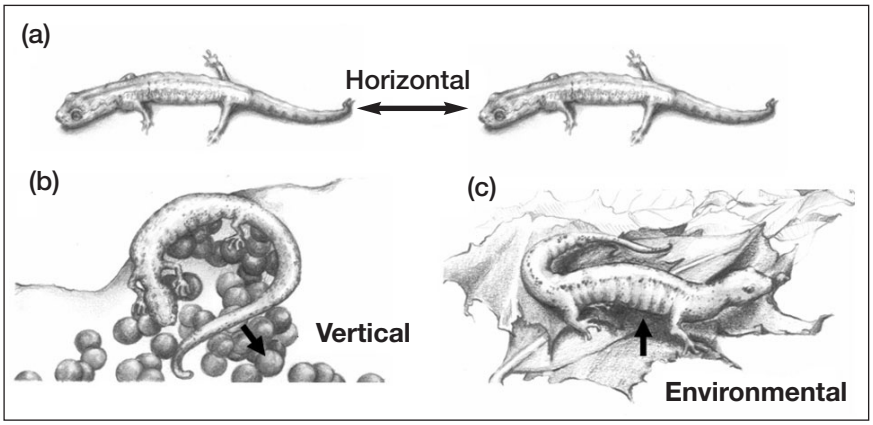
Figure 2. *Typical in vitro test of antimicrobial properties of the natural microbiota. Here,* Pedobacter *sp, a member of the natural microbiota of several species of amphibians, is shown to inhibit the growth of the amphibian pathogen,* Batrachochytrium dendrobatidis. *Reprinted with permission from Woodhams* et al. *(2007).*

Several studies have suggested that increased microbial species richness per se may prevent colonization by disease organisms. For wheat, increased microbial diversity in the rhizosphere results in decreased ability of a potential pathogen, *Pseudomonas aeruginosa*, to invade the system (Matos *et al.* 2005). Similarly, recent work in locusts suggests that higher species richness in gut microbiota is associated with decreased ability of pathogens to colonize the gut (Dillon *et al.* 2005). These novel experiments recreated the gut microbiota with one, two, or three species of bacteria and revealed that total microbial density in the gut increased with species diversity, even though the density of the initial gut inoculum was constant across treatments. These results suggest that the bacterial species facilitated each other's growth, perhaps due to more effective resource use when more species are present. This outcome would leave less niche space for invaders, including pathogens. However, other possible mechanisms exist in this and other systems; for example, the presence of more species may increase the likelihood that a single inhibitory species or functional group will be present or may increase the likelihood that the host's immune system will be stimulated.

Other evidence suggests that the presence or absence of specific species can also be important for the health of the host. One potential example of this is the near extinction of *Helicobacter pylori* from the human intestinal microbiota in recent years, because of increased antibiotic use and better hygiene (Blaser 2006). While *H pylori* is considered by many to be a dangerous microbe, linked to stomach cancer and gastric ulcers, there has been some evidence that, depending on the strain, it may actually decrease the incidence of gastroesophageal reflux disease and cancer. The consequences of its loss from the human intestinal flora are still being debated (Blaser 2006).

Linking changing microbial communities with increasing disease

Disease emergence and re-emergence is a growing problem in human and wildlife populations (Daszak *et al.*



Courtesy of A Class

Figure 3. *Potential routes of inoculation with natural microbiota. Although these routes are shown in the salamander,* Hemidactylium scutatum*, they probably apply for most taxa. (a) Horizontal inoculation occurs via contact with conspecifics, especially in highly social species, or during mating or aggressive interactions. (b) Vertical inoculation occurs when the microbiota is passed down from parents to offspring. (c) Environmental inoculation occurs when members of the microbiota are obtained directly from the environment.*

2000). Emerging infectious diseases are defined as those that are increasing in prevalence within a population, have exploited new populations or species, and/or have increased in geographic range. For example, *Mycoplasma gallisepticum*, a bacterial pathogen, has been identified as the causative agent of an emerging infectious disease in house finch populations in the eastern US. This disease has been associated with population declines and has spread in an epidemic pattern over the past two decades (Hochachka and Dhondt 2000). Increased interaction between domestic animals and wildlife, the global transport of species and pathogens, habitat alteration, and global environmental changes have all contributed to the current increase in emerging infectious diseases in wildlife populations (Daszak *et al.* 2000).

We postulate that a change in microbial communities and ecological dynamics among microbes within the environment is also leading to an increased rate of disease outbreaks in wildlife. If the natural microbial community is simply passed down from mother to offspring in each generation, then environmental changes in microbial communities are less likely to influence the natural microbiota (Figure 3). However, if inoculation with the natural microbiota occurs from the environment, or if members of the natural microbiota are constantly interacting with environmental microbes, then the composition of the environmental microbe assemblage could be important. Well-established differences in the intestinal microbiota between human infants that are breast and bottle fed suggest the possibility that the source of microbes available for colonization of the natural microbiota does impact final composition (Harmsen *et al.* 2000). For some of the host species studied, it seems that inoculation with microbiota from the environment occurs quite often. For example, many components of the skin flora of both humans and amphibians are species commonly found in the environment (Austin 2000; Wilson 2005; Harris *et al.* 2006; Culp *et al.* 2007). For intestinal colonization, vertical (mother–offspring) and environmental inoculation appear to dominate, although horizontal transmission (among conspecifics) is also possible, especially for social organisms (eg Gilliam 1997; Harmsen *et al.* 2000).

It therefore seems likely that, at some point during development, members of the natural microbiota are obtained from the environment or interact with microbes in the environment. Changes occurring in microbial communities in the environment are thus also likely to have direct and/or indirect impacts on members of the natural flora, and ultimately on pathogens. It is clear that many changes are occurring in microbial communities in the environment, although it is a technical challenge to accurately characterize change in these diverse and dynamic communities. We consider two categories of changes that can influence microbial communities. The first is incidental, whereby changes in the environment, often due to human activity, unintentionally alter microbial communities. Factors ranging from chemical and pharmaceutical contamination (eg Seghers *et al.* 2003; Schmitt *et al.* 2005) to global environmental changes, such as increased atmospheric CO_2 (eg Hu *et al.* 1999), can alter microbial communities.

A recently identified human activity that could influence microbial ecology and emerging disease is the widespread use of antibiotics in humans and livestock, and their subsequent contamination of our water systems (Wilkinson 1999). Many members of the microbiota produce antibiotics, and evolution of resistance to antibiotics that are naturally encountered during microbial interactions is expected. However, recent evidence suggests that antibiotics added to the environment by humans persist (Kolpin *et al.* 2002). These antibiotics could result in changes in microbial communities that (1) cause the extinction of ecologically important microbial species, (2) allow pathogenic organisms to outcompete other strains or species, or (3) encourage the evolution of new virulent strains or horizontal gene transfer of antibiotic-resistant genes (Martínez and Baquero 2002). Researchers are beginning to look at how antibiotics in the environment impact other organisms living within these systems (eg Richards *et al.* 2004). While this has not been examined in the context of disease dynamics, it is certain to have an impact in some systems.

The second type of change occurring in microbial communities is intentional change. This involves the deliberate addition of microbes to the environment for various

purposes, such as bioremediation (ie bioaugmentation when microbes are added) or biocontrol. There are currently 25 microbes (13 bacteria and 12 fungi) approved by the US Environmental Protection Agency for use as biocontrol agents in crops (Fravel 2005), and bioaugmentation as a remediation method for polluted soil and water is also increasingly being used (El Fantroussi and Agathos 2005). While historically it was thought that geographic variation in microbial communities was limited, it is now clear that, on a biogeographic scale, microbial assemblages show a pattern of historical divergence that is maintained by genetic isolation (Martiny *et al.* 2006). This suggests that human movement of microbes could have an impact on local microbial communities. The extent and effects of these introductions, beyond establishing whether the introduction was successful, does not generally seem to be considered in studies of microbial releases.

Whether current increases in emerging infectious diseases are linked to changes in hosts' natural microbiota can only be determined by further research. However, if we begin to think about pathogens in an ecological context, then these issues cannot be overlooked.

Probiotics and conservation: can friendly microbes save our frogs?

If we accept that microbial interactions are important for determining disease outcomes, we come to the conclusion that, in addition to some of the possible negative outcomes, we may also be able to use microbes in our fight against disease in at-risk natural populations. Probiotics, which incorporate beneficial bacteria and their natural products for the prevention or treatment of disease, have been used successfully in aquaculture (Irianto and Austin 2002), livestock and poultry production (Patterson and Burkholder 2003), and, increasingly, in humans (Rastall *et al.* 2005). However, whether probiotics could be used effectively for wildlife conservation remains to be seen. For amphibians, there is evidence that maintenance of antifungal skin bacteria is important for the prevention of chytridiomycosis (Harris *et al.* 2006). If we find at-risk amphibian populations in which antifungal skin bacteria are absent or rare when we expect them to be common, a "probiotic approach" could potentially be applied. Ultimately, the hope is that this approach would help contain the ongoing worldwide epidemic of *B dendrobatidis* that appears to be responsible for many amphibian population declines.

We envision a scenario in which one or more species or combinations of species of cutaneous microbes are shown to inhibit disease in vivo, and are then used to inoculate free-living amphibians that are at risk of infection with *B dendrobatidis*. There are several possible approaches, including direct application of naturally occurring antifungal skin bacteria to individual amphibians or applying such bacteria to the soil and water where threatened amphibians congregate. This approach could also be used in captive (survival assurance) colonies, as a way of protecting them from inadvertent infection with pathogens. Alternatively, individuals could be inoculated with beneficial bacteria before they are reintroduced into natural populations.

This approach may not work for several reasons. For instance, the inoculation of beneficial skin bacteria may produce a short-lived protective effect. Amphibians in nature may rely on obtaining their skin microbiota from sources in the soil or water continually over time. If the soil or water microbial community has changed because of anthropogenic effects, then the possibilities for re-inoculation change. In addition, amphibians in certain areas may experience some chronic sublethal stress associated with drought, warming temperatures, or food shortages (eg Pounds *et al.* 2006). If stressed amphibians cannot maintain a natural protective skin microbiota, simply adding beneficial bacteria will not be a general solution for disease control. Research is urgently needed to examine the possibility of inoculating one or a few critical microbes onto amphibians to stop the spread of emerging pathogens, such as *B dendrobatidis*. If it is possible to increase the proportion of individuals in at-risk populations with antifungal skin bacteria, herd immunity may result, thereby preventing an epidemic. Of course, considerable testing would be necessary before such a plan is implemented by government agencies. For some amphibian populations on the brink of extinction, however, this may be the last hope.

Remaining questions

Despite the rapid increase, in recent years, of research investigating the role of the natural microbiota in health and disease, many questions still remain. We highlight two areas where we think research efforts should be focused. First, much more background information is needed for essentially all free-living species on what constitutes the natural microbiota and on how much variation exists between individuals in a population and within each individual over time. Once background information on the composition and variation of the microbiota is obtained, research into the role of the various members in disease and disease resistance can be initiated. Second, while, in some systems, in vitro pathogen inhibition tests seem to show a role for some microbiota members in disease prevention, these tests must be followed up with in vivo testing. In vivo experiments are currently lacking for most systems, and previous work has demonstrated that in vitro inhibition does not always translate into in vivo disease prevention (Gram *et al.* 2001).

Conclusions

We suggest that placing pathogens into an ecological context can be important for understanding disease emergence and should complement more traditional

approaches of examining host–pathogen interactions, modes of transmission, and mechanisms of pathogenicity. This will require interdisciplinary teams of scientists, including ecologists, working at multiple scales to successfully address the complex issues involved in disease emergence in wildlife and humans. We have identified key recent research that provides the first steps toward integrating microbial ecology and community ecological theory into disease research programs. Examining pathogens in an ecological context also provides a conceptual framework for developing a mechanistic understanding of how anthropogenic environmental changes can impact emerging infectious diseases. There is increasing evidence that anthropogenic effects, such as pollution and land-use changes, are altering microbial communities, and it seems likely that, at some level, these changes will alter disease dynamics in natural systems. Finally, we believe that the integration of these research programs could provide important insights for the conservation of species, including many amphibians, that are threatened by disease outbreaks. Probiotics have shown potential benefits for aquaculture and human health, and their use in conservation should be explored.

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