Efficacy of Emerging Infectious Disease Interventions in Wildlife

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**Abstract**

In addition to a variety of anthropogenic factors contributing to biodiversity loss, emerging infectious diseases have been linked to severe population declines and extinction events. Mammals have experienced numerous diseases such as rabies, chronic wasting disease, and canine distemper virus that lead to declines, and amphibian biodiversity have been particularly affected by the rapidly-spreading and highly fatal fungal disease chytridiomycosis. The adverse effects of these diseases may be mitigated by a variety of inoculations, including vaccinations and bioaugmentation, although the extent to which these programs are practical and effective is understudied. To examine the efficacy of inoculating infected mammalian and amphibian populations, I carried out a meta-analysis that examines of the success of experimental inoculations compared to control treatments via risk differences in a variety of mammalian and amphibian interventions. Across all studies, inoculations appear to be effective at reducing infection prevalence and/or mortality compared to control treatments. Efficacy of inoculations in mammals compared to amphibians does not appear to differ, though that effect may become clearer as more quantitative inoculation studies are conducted. These results suggest that inoculating populations of infected wild animals is a worthwhile pursuit, and could contribute to mitigating already extreme declines in biodiversity.

**Introduction**

As humans continue to destroy habitat, utilize natural resources, alter global climate regimes, hunt unsustainable populations, and introduce invasive species and pathogens, the rate of population declines and extinctions has increased so significantly that we are entering the sixth mass extinction (for review, see Barnosky et al. 2011). While threats such as loss of habitat and climate change are established drivers of biodiversity loss (Travis 2003), infectious diseases are increasingly becoming recognized as threats to wildlife biodiversity (Smith et al. 2006). Although human diseases have been well studied since the advent of modern medicine, comparatively little is known about the ecology and epidemiology of wildlife diseases (Daszak 2000).

Diseases in plants and animals are typically defined by the impairment of normal bodily or behavioral functions, particularly when the result is a specific set of deleterious symptoms (Delahay et al. 2008). Parasites and pathogens are the primary agents of chronic and acute disease, respectively, and both affect hosts by altering normal physiological processes and/or host behavior. These adverse effects frequently lead to declines in growth rates and reproductive success, as well as increases in mortality at the population level (Hudson et al. 2002). Epizootic and especially enzootic diseases are often limited to sub-lethal effects within infected populations (Delahay et al. 2008). However, these sub-lethal affects are highly influential in complex communities over extended time periods of time, for example by modifying the structure and demography of ecological communities (Wood et al. 2007) and driving evolutionary change within the communities (Clayton & Moore 1997). Of course, some emerging infectious diseases can cause wildlife population declines that occur rapidly, particularly when spreading into naïve populations (i.e., those that have not previously come into contact with the pathogen) (McCallum 2012). In some cases, declines are so severe that they lead to extirpation of local populations or actual extinctions (Wake & Vredenburg 2008).

In recent decades, a variety of emerging infectious diseases have definitively caused the declines of multiple animal taxa. Moreover, the number of emerging infectious diseases, as well as the number of extirpations and extinctions resulting from their spread, has increased significantly (Daszak et al. 2000; Fisher et al. 2012). Some of the more well-known examples include chronic wasting disease in North American deer (Gross & Miller 2001), sylvatic plague in North American rodents (Cully et al. 2010), West Nile virus in North American birds (LaDeau et al. 2007), canine distemper virus in African dogs (Alexander & Appel 1994) and lions (Roelke-Parker et al. 1996) and North American rodents (Williams et al. 1988), white-nose syndrome in North American bats (Frick et al. 2010), and chytridiomycosis in amphibians worldwide (Berger et al. 1998). While most of these severe outbreaks have occurred in North America, this bias may due to the fact that these diseases were enzootic and causing only sublethal effects in European and Asian populations, but caused higher mortality when introduced into naïve North American fauna. One important detail to note in all of these cases is that these disease outbreaks have occurred in nearly all animal taxa. Additionally, taxa that have not yet been as severely impacted by infectious diseases, reptiles for example, are clearly vulnerable to pathogens that have the potential to cause substantial declines in the future (e.g., snake fungal disease, Allender et al. 2015).

To make the issue of wildlife disease more compelling to a public audience, between 1940 to 2004, about 60% of emerging infectious disease events in humans were zoonotic in nature, and 72% of those diseases originated in wildlife (Jones et al. 2008), including avian flu (Shinya et al. 2006), swine flu (WHO, 2009), Ebola virus (Leroy et al. 2004), and HIV (Gao et al. 1999). The ease with which pathogens can be transferred from wildlife to humans suggests the dangers that humans as a society face if we do not proactively seek solutions to infectious diseases in wildlife. The recent surge in frequency and severity of wildlife disease epidemics should be considered ample evidence for the need to implement methods of controlling outbreaks both before and after they occur. Fortunately, successful efforts have been made to control existing outbreaks of infectious diseases (e.g., successful vaccinations for rabies: Cleaveland et al. 2006; Freuling et al. 2013), research on disease interventions is being conducted (e.g., treating infected amphibians with probiotics: Harris et al. 2009), and laws are being passed to prevent the spread of infectious pathogens into naïve populations (USFWS, 2016). This review assesses the current state of the efficacy of emerging infectious disease interventions across taxa, and seeks to predict whether current strategies successfully mitigate population declines in wildlife due to infectious diseases.

**Methods**

**Literature search**

Quantitative studies in the literature that examined the effect of inoculations of wild populations of animals were gathered from the ISI Web of Knowledge (http://apps.isiknowledge.com) and and Google Scholar (http://scholar.google.com) from March 6 – 9, 2016 with no restriction on publication year, and using the following search term combinations: (wildlife OR wild\* OR population) AND (disease\* OR infect\*) AND (treat\* OR inoculat\* OR vaccin\*). Additionally, references from all retrieved articles were screened for relevant publications that may not have been identified in the database searches. Some of the most relevant articles, which were oftentimes review articles, tended to reference ‘grey literature’ from governmental, international, or veterinary reports and were therefore excluded from this analysis.

Each article was assessed for fulfilling the following criteria: assessed outcomes of inoculation (i.e., vaccination or other generalized treatment, including bioaugmentation) of infected animals, quantitative evaluation of a measure of treatment success or efficacy, and published in a peer-reviewed journal. Although these types of studies use numerous variables to determine the efficacy of treatments, including percent survival compared to uninoculated organisms after inoculation, antibody titer and seroconversion, or protection from challenge viruses, many such variables can be distilled into an aggregate measure of overall inoculation efficacy simply by analyzing effect sizes for the studied variables and weighting the effect sizes based on standard error and inherent variation among datasets. After examination and selection of relevant articles from lists of thousands of articles per database, an initial set of 14 articles was selected to more closely analyze for this analysis. Of these 14 articles, nine were selected that met the above criteria exactly. Of those nine articles, seven articles met the criterion of measuring a treatment variable that corresponds directly to efficacy of inoculations (i.e. survival or immune assessment).

**Data** **extraction and conversion**

A total of seven articles that assessed the efficacy of inoculations of wild populations of animals was used in the final analysis. Mammal and amphibian taxa were most commonly represented in the selected studies, although various assessments of aquaculture (i.e., captive fish experiencing extremely unnatural living conditions and disease dynamics) dominated the literature. The risk difference (RD) was calculated to assess the difference in occurrence rates between experimental (PT) and control (PC) inoculations. Datasets from each study were also weighted by the reciprocal of their variance. Using one-way analysis of variance, the outcomes of experimental and control treatments were compared across studies to evaluate the effect of inoculation on infection. Host taxon was included as a factor in a follow-up analysis of variance to assess differences in outcomes between mammal and amphibian taxa. All statistical analyses were conducted using R v3.2.4.

RD = PT – PC

Variance = [PT(1 – PT) / NT] + [PC(1 – PC) /NC]

**Results**

A total of seven studies were used in this meta-analysis, all of which examined variables that could be split into a proportion of unsuccessful and successful treatment within the study (e.g., proportion of inoculated organisms that did not clear infection and proportion that successfully cleared infection). Four of these studies examined inoculations of wild populations of amphibians, including salamanders and frogs. The other four studies examined wild populations of mammals, for example dogs infected with canine distemper virus.

Risk differences across all studies varied from 0.1708 to 0.9394. When populations of infected organisms are inoculated with a treatment, analysis of variance suggests that treatment is significantly effective at reducing infection prevalence (*F*1,5 = 34.658, *p* = 0.0020). Based on the weighting in the model used to analyze this dataset, though, it is evident that the study with a comparatively high sample size and weighting (by an order of magnitude, n = 198; weight = 3369 as opposed to 27 - 231) contributes to the observed level of significance. Considering that all studies except one had sample sizes between 6 and 26 individuals, the analysis was re-run with this study removed; the model still indicated that inoculation of infected wildlife is effective (*F*1,4 = 17.519, *p* = 0.0139).

In comparing studies that inoculated mammals with those that inoculated amphibians (Fig. 1), analysis of variance suggests that host taxon group is a marginally significant predictor of success (*F*1,5 = 6.48; *p* = 0.0515). When the disproportionately-weighted study was removed, the effect of taxon group was not significant in success of wildlife inoculations (*F*1,4 = 3.05; *p* = 0.156). Overall, it appears that inoculation of infected wildlife is successful in reducing the prevalence of infection in the treated organisms. Additionally, there is no difference in success rates of inoculations between mammals and amphibians, although this effect may become more apparent as more studies with higher sample sizes are conducted.

**Discussion**

This analysis suggests that inoculating infected populations of wild mammals and amphibians is an effective approach to reducing the prevalence of infection in treated animals. However, it is plausible that mammals and amphibians may experience differences in inoculation efficacy. For example, the marginally significant effect of host taxon when all originally-selected studies were included in analysis indicates that there is a difference in efficacy between mammals and amphibians. Overall, mammals may experience greater rates of success after inoculation than amphibians (Fig. 1), although the results herein provide tenuous support for this. Provided that quantitative inoculation studies continue to be conducted, and that larger sample sizes are favored such that treatment effects are more accurately assessed, the potential host taxon effect in inoculation efficacy may become considerably more apparent.

Given the low number of studies included in this analysis, the conclusion of such a result suggests that inoculation is an effective method of reducing infection in treated mammals and amphibians. While this analysis revealed a significant effect of inoculation, the results should be interpreted with caution due to the inherent bias in the literature against reporting negative results. In other words, it is possible that inoculations are not as effective as observed herein on that basis that those ineffective inoculations did not get published, and are not available to include in meta-analyses. Moreover, the bias in sample size clearly suggests that studies with higher sample sizes have greater power in detecting treatment effects (e.g., host taxon group in this analysis).

The vertebrate immune system is composed of both innate and adaptive components. The innate immune system is characterized as a non-specific set of responses that responds rapidly to invading pathogens, whereas the adaptive immune system involves specialized immune cells that produce highly-specific responses to a comparatively large diversity of pathogens (Janeway et al. 2005). In this sense, both mammals and amphibians are protected in some way by a broad-spectrum immune system, as well as a more specific and derived acquired immune system. However, amphibian skin is considerably thinner and more permeable than mammalian skin (Simmaco et al. 1998), and plays a major role in regulation of physiological processes (Fisher and Farrer 2011). Although amphibian skin is protected by a layer of mucosal secretions that contain antimicrobial bacteria and compounds in many cases (Toledo and Jared 1995), such permeable skin makes amphibians more vulnerable to pathogens in the environment. Moreover, because the skin performs such critical functions in amphibians, even comparatively minor infections may compromise their health. Despite the presence of both innate and adaptive immune responses in amphibians, immune responses in certain frogs are easily suppressed small quantities of hydrocortisone due to a variety of stressors (Tournefier 1982), and by the chytrid fungus *Batrachochytrium dendrobatidis*, the etiological agent of chytridiomycosis in amphibians (Ellison et al. 2014), and vaccination appears to be ineffective at preventing infection and mortality (Stice and Briggs 2010).

Mammals, on the other hand, seem to respond favorably to a variety of vaccinations and inoculations (Meeusen et al. 2007). A substantial amount of research has gone into the development of treatments for mammalian infectious diseases such as rabies and canine distemper virus, and the success of many inoculation programs has been promising despite logistic impracticalities (Visser et al. 1989; Haydon et al. 2002; Slate et al. 2005). Continued research into appropriate inoculation strategies and applied treatment of infected populations is critical if disease prevalence is to be mitigated, especially in urgent cases of endangered species like the Ethiopian wolf. For example, even though the goal of protecting all species, or individuals in a population, from an infectious disease is an impractical approach, models suggest that even protection of 20 – 40% of Ethiopian wolves is effective and worthwhile (Haydon et al. 2002). Furthermore, the spread of diseases from mammals to human (i.e. zoonotic diseases) has resulted in substantial human mortality and sickness (Daszak et al. 2000), and as growing human populations continue to expand into natural areas, contact rates of humans or their pets with infected wildlife will likely increase pathogen transmission rates in both directions (Woodroffe et al. 2012). Fortunately, planned preventative inoculations of both wild and domestic animal infected populations may reduce rates of transmission and thereby reduce rates of zoonotic disease transmission and outbreaks.

Based on the results from this meta-analysis and available studies, mammals infected with bacteria, viruses, fungi, or parasites may be more responsive to inoculation than amphibians. Of course, the vast majority of immune studies have been conducted using mammals as opposed to other taxa, so there is still much to learn, or at least verify, in taxa like amphibians. For example, many crucial immune responses and components in mammals have been observed in amphibians when they were not previously recognized, such as antimicrobial peptides (Barra and Simmaco 1995), various lymphoid tissues (Ardavin et al. 1982; Colombo et al. 2015), and antibacterial lysozyme genes (Zhao et al. 2006). It is entirely plausible that amphibian biodiversity declines due to infectious diseases could be mitigated by inoculations, though more research must be conducted on the efficacy of various treatments (e.g., antifungal agents vs. bioaugmentation with probiotic bacteria), response of different strains of *Bd* to different treatments, and efficacy of treatments across amphibian families. There is still a vast amount of research and development to be done with inoculations intended to reduce infection rates and/or mortality in both mammals and amphibians, but these results substantiate the notion that the required time and funding is entirely worthwhile and necessary.

**Literature** **Cited**

Alexander KA, Appel MJG. 1994. African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. Journal of Wildlife Diseases **30**(4):481-485.

Allender MC, Raudabaugh DB, Gleason FH, Miller AN. 2015. The natural history, ecology, and epidemiology of *Ophidiomyces ophiodiicola* and its potential impact on free-ranging snake populations. Fungal Ecology **17**:187–196.

Ardavin CF, Zapata A, Villena A, Solas MT. 1982. Gut-associated lymphoid tissue (GALT) in the amphibian urodele *Pleurodeles waltl*. Journal of Morphology **173**(1):35-41.

Barnosky et al. 2011. Has the Earth’s sixth mass extinction already arrived? Nature **471**(7336):51-57.

Barra D, Simmaco M. 1995. Amphibian skin: a promising resource for antimicrobial peptides. Trends in Biotechnology **13**(6):205-209.

Berger L, et al. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. 1998. Proceedings of the National Academy of Sciences USA **95**:9031–9036.

Clayton DH, Moore J. 1997. Host-parasite evolution: General principles and avian models. Oxford University Press, Oxford.

Cleaveland S, Kaare M, Knobel D, Laurenson MK. 2006. Canine vaccination—providing broader benefits for disease control. Veterinary Microbiology **117**(1):43-50.

Colombo BM, Scalvenzi T, Benlamara S, Pollet N. 2015. Microbiota and mucosal immunity in amphibians. Frontiers in Immunology **6**, published online March 13.

Cully Jr. JF, Johnson TL, Collinge SK, Ray C. 2010. Disease limits populations: plague and black-tailed prairie dogs. Vector-Borne and Zoonotic Diseases **10**(1):7-15.

Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science **287**(5459):443–449.

Delahay R, Smith GC, Hutchings MR. 2008. The Science of Wildlife Disease Management. Pages 1-8 in Delahay R, Smith GC, Hutchings MR, editors. Management of Disease in Wild Mammals. Springer Science & Business Media, Tokyo.

Ellison AR, Savage AE, DiRenzo GV, Langhammer, PF, Lips KR, Zamudio, KR. (2014). Fighting a losing battle: Vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. G3: Genes Genomes Genetics **4**:1275–1289.

Fisher MC, Farrer RA. 2011. Outbreaks and the emergence of novel fungal infections: lessons from the panzootic of amphibian chytridiomycosis. The Journal of Invasive Fungal Infections **5**:73–81.

 Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature **484**:186-194.

Freuling CM, Hampson K, Selhorst T, Schröder R, Meslin FX, Mettenleiter TC, Müller T. 2013. The elimination of fox rabies from Europe: Determinants of success and lessons for the future. Philosophical Transactions of the Royal Society of London B **368**(1623):20120142

Frick WF, Pollock JF., Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH. 2010. An emerging disease causes regional population collapse of a common North American bat species. Science **329**(5992):679-682.

Gao F, et al. 1999. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. Nature **397**(6718):436-441.

Gross, J. E. and M. W. Miller. 2001. Chronic wasting disease in mule deer: Disease dynamics and control. Journal of Wildlife Management **65**:205-215.

Harris RN, et al. 2009. Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. The ISME Journal **3**(7):818-824.

Haydon DT, Laurenson MK, Sillero-Zubiri C. 2002. Integrating epidemiology into population viability analysis: Managing the risk posed by rabies and canine distemper to the Ethiopian wolf. Conservation Biology **16**(5);1372-1385.

Hudson PJ, Rizzoli AP, Grenfell BT, Heesterbeek JAP, Dobson AP. 2002. Ecology of Wildlife Diseases. Pages 1-5 in Grenfell BT, editor. The Ecology of Wildlife Diseases. Oxford University Press, Oxford.

Janeway CA, Travers P, Walport M. 2005. The Development and Survival of Lymphocytes. Chapter 7 in Janeway CA, editor. Immunobiology: The immune system in health and disease. Garland Science Publishing, New York.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. 2008. Global trends in emerging infectious diseases. Nature **451**(7181):990-993.

LaDeau SL, Kilpatrick AM, Marra PP. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature **447**:710-713.

Leroy EM, et al. 2004. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science **303**(5656):387-390.

McCallum H. 2012. Disease and the dynamics of extinction. Philosophical Transactions of the Royal Society B **367**(1604):2828-2839.

Roelke-Parker ME et al. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). Nature **379**:441-445.

Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. 2006. Avian flu: Influenza virus receptors in the human airway. Nature **440**(7083):435-436.

Simmaco M, Mignogna G, Barra D. 1998. Antimicrobial peptides from amphibian skin: what do they tell us? Peptide Science **47**(6), 435-450.

Smith KF, Sax DF, Lafferty KD. 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology **20**:1349–1357.

Stice MJ, Briggs CJ. 2010. Immunization is ineffective at preventing infection and mortality due to the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. Journal of Wildlife Diseases **46**(10):70-77.

Toledo, R. C., & Jared, C. 1995. Cutaneous granular glands and amphibian venoms. Comparative Biochemistry and Physiology **111A**(1):1–29.

Tournefier A. 1982. Corticosteroid action on lymphocyte subpopulations and humoral immune response of axolotl (urodele amphibian). Immunology **46**(1):155-162.

Travis JMJ. 2003. Climate change and habitat destruction: A deadly anthropogenic cocktail. Proceedings of the Royal Society B **270**(1514):467-473.

Visser EKG et al. 1989. Vaccination of harbour seals (*Phoca vitulina*) against phocid distemper with two different inactivated canine distemper virus (CDV) vaccines. Vaccine 7(6):521-526.

Wake DB, Vredenburg, VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences **105**(Supplement 1):11466-11473.

Williams ES, Thome ET, Appel MJG, Belitsky DW. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases **24**(3):385-398.

Wood CL, Byers JE, Cottingham KL, Altman I, Donahue MJ, Blakeslee AMH. 2007. Parasites  
alter community structure. Proceedings of the National Academy of Sciences USA **104**:9335–9339.

Woodroffe R, Prager KC, Munson L, Conrad PA, Dubovi EJ, Mazet JAK. Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (*Lycaon pictus*). PLoS One **7**(1):e30099.

World Health Organization. Swine flu illness in the United States and Mexico - Update 2. Available from http://www.who.int/csr/don/2009\_04\_26/en/ (accessed March 2016).

United States Fish and Wildlife Service. 2016. Injurious wildlife species; Listing salamanders due to risk of salamander chytrid fungus. Federal Register **81**(8):1535-1556.

Zhao Y, Jin Y, Lee WH, Zhang Y. 2006. Purification of a lysozyme from skin secretions of *Bufo andrewsi*. Comparative Biochemistry and Physiology C: Toxicology and Pharmacology **142**(1-2):46-52.

**Figure Legends**

Figure 1. The proportion of populations of each host taxon group (herp or mammal) that recovered from infection following inoculation.

**Figures**



(RD)