Characterizing Environmental Factors Influencing Zoonotic Disease Reservoirs Using Meta-Parasite Prevalence

By Cynthia L. Mills

A Thesis

Presented to the Department of Preventive Medicine and Public Health and the Oregon Health and Sciences University in partial fulfillment of the requirements for the degree of Master of Public Health
June 2007

School of Medicine
School of Public Health and Preventive Medicine
Oregon Health and Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Cynthia L Mills

has been approved

TABLE OF CONTENTS

Acknowledgements

Thanks are due to Nichole Carlson, Nancy Press and Luis Ruedas, advisors who were indispensable to the conception and execution of this project. Scott Gardner is another essential mentor who taught me how to find and preserve parasites, as well as helped with the design of the collection and analysis. Special thanks must go to Laurie Dizney, whose PhD project provided the inspiration and materials, and all of the members of Dr. Ruedas's lab: Barb Edmunds, Lorelei Patrick, Phil Jones and Zac Harlow who were all only too happy to look at roundworms in situ when I found them. Last, I must always thank Mike; he knows why.

Abstract

In five parks around Portland, Oregon, where there is a dense human population, investigators have found a 5.3% prevalence of the host of the Hantavirus Pulmonary Syndrome, Sin Nombre Virus in deer mice, *Peromyscus maniculatus*. Previous studies show the prevalence of SNV varies inversely with the level of biodiversity measured for each reserve. The current study uses a preserved subset of 257 specimens from the same populations to examine a second disease system: gastrointestinal meta-parasites, to compare the impact of the same environmental factors on a parasite with a different transmission strategy. The results were similar: biodiversity has an inverse effect on prevalence of meta-parasites, but population densities of P. maniculatus and of all small mammals have no significant effect. Analysis was performed using GEE modeling for correlated data. Models with more than one variable tested did not retain significance suggesting that some of the effect of biodiversity can be explained by changes in population dynamics. Mass was also a significant predictor, although not in a model stratified by weight or age. Biodiversity proves to be a persistent if not robust factor influencing wildlife disease dynamics.

Introduction

Historically, wildlife populations have been a 'black box' for epidemiologists studying disease behavior. In human populations, tracking disease and calibrating the forces impinging on pathogens has been straightforward, if not simple. But in wildlife the picture is muddied by our inability to know even population numbers with any real precision, much less measure incidence, the number of new cases of infection occurring in a given time. Therefore we should regard as salient any opportunity to study a natural population with a pathogen with the potential for emergence.

The population in question includes deer mice, *Peromyscus maniculatus*, in the Pacific Northwest that harbor the pathogen responsible for Hantavirus Pulmonary Syndrome (HPS). That pathogen, Sin Nombre Virus (SNV), a member of the genus *Hantavirus*, was first identified after causing an outbreak in the Four Corners region of the southwestern United States in 1993. After extensive study, this outbreak and a subsequent one were related to climate conditions (El Nino), and various social and environmental links in its causal chain were elucidated. [i]

As in all of North America, SNV is enzootic (endemic for animals) in the Portland, Oregon greater suburban area, where it infects *P. maniculatus* and a handful of other small mammals. The prevalence is low. HPS does not pose a significant public health threat. In all of Oregon, four human cases occurred in 2006, with one

death. One case occurred in 2007 in Multnomah County, a county that contains much of the greater Portland area. Studying the animals in this region provides an opportunity to evaluate a potentially emergent pathogen and its reservoir in their natural state, as influenced by factors of environments characterized as slightly to heavily impacted by the influx of human habitation.

Peromyscus Maniculatus in Portland, Oregon

That *Hanta* virus SNV has persisted locally in the northwestern states of the US (and all of North America) has been known since 1993.[ii] More recently, Dizney and Ruedas have found a prevalence of 5.3 % in *P. maniculatus* living in five parks and reserves in close contact with the human population of the highest density in Oregon, specifically the greater Portland area.[iii]

Dizney and Ruedas have measured some of the dynamics of this reservoir. Their findings support a role for biodiversity in inhibiting disease transmission and dampening viral prevalence in the reservoir.[iv] The relationship they found was not linear, but more closely approached a threshold effect, wherein above a certain level of biodiversity the SNV infection prevalence fell significantly.[v] In addition, they did not detect a significant relationship between SNV prevalence and population density of *P. maniculatis* or population density of all small mammals.

Previous work has shown that SNV is most likely transmitted by direct contact: for a mouse, being positive for SNV is strongly associated with scars and male gender, suggesting transmission is largely effected male-male agonistic

interactions when seeking mates as well as territorial fighting.[vi] It is not clear whether SNV does or does not cause significant pathology in mice[vii viii]; in fact some evidence suggests that SNV is associated with longer lifespan in infected mice (Ruedas, unpublished data). While individuals of other species may test positive for SNV, the numbers are low and there are no documented cases of SNV transmission from these other potential host species.

The ability to predict emergence of SNV from wildlife reservoirs would serve to protect the public as well as deepen understanding of disease emergence in general. This project furthers understanding of this SNV reservoir, by analyzing a parallel infection in a subset of the preserved specimens that had been surveyed for meta-parasites of the gastrointestinal tract, e.g.: nematodes. Determining the prevalence of meta-parasites offers a possible second approach to measuring the magnitude of impact of environmental and natural history factors on disease behavior. By comparing the same forces' effects on two parasites (SNV and nematodes) within the same host population we hope to test and calibrate, if possible, influences of some of the complex factors that drive disease emergence.

Infectious Disease Emergence

Recent decades have witnessed the emergence of new pathogens and the re-emergence of old ones. Of the emergent diseases, an estimated 75% had animals as their starting points, that is, they were zoonotic.[ix] This statistic takes on new meaning when compared to the estimated total proportion of human diseases that are zoonotic,

which has been estimated as low as 49%, but is more often reported at 61%. [x] In recent analysis, Taylor and colleagues[xi] found that pathogens causing zoonoses were at least twice as likely than non-zoonotic pathogens to be associated with emerging diseases. Consequently many researchers recommend increasing efforts to investigate disease dynamics at their source: wild animals in their natural settings.

Human health is not the only reason to investigate the dynamics of emergence. Improving our understanding should also benefit wildlife species and could be important in efforts to protect and conserve threatened or potentially endangered species as well as species of economic importance such as game animals. Several investigators have argued that diseases have been emerging in wildlife populations at a rate above some baseline. [xii] Recently disease has played a role in the local and global extinctions of species like the Hawaiian Raven, [xiii] a species of South Pacific land snail and many species of frogs.[xiv]

Considering disease as an extinction risk is a relatively new concept for wildlife biologists and managers. Traditionally disease has been considered as exerting a more or less natural, even beneficial, impact on wild populations.[xv] Over-exuberant populations would have their growth curbed by disease, and then disease would conveniently die out as population densities (and transmission events) dwindled.[xvi] Complete extinction of a population seemed impossible, since sub-populations would survive that were genetically resistant to the disease organism, creating a residual 'rescue' population 'selected' for its hardiness in the face of the pathogen.

These analyses worked for healthy wildlife populations, but have been found wanting for understanding wildlife in a world experiencing the impact of a burgeoning human population. More modern models are being created to reconsider the threat of disease for dwindling populations; researchers are finding that parasites do not behave in the same way in distinct host populations. The old belief that pathogens tend to evolve to lesser virulence, thus preserving their hosts and own survival and reproduction has not proved to be the only successful strategy. Many pathogens cannot be separated from their virulence; they use their virulence as means of transmission. [xvii] In addition, some investigators now believe that not all pathogens depend on high population densities of their hosts for transmission. [xviii] The simple, intuitively attractive concept that the more hosts there are in close contact, the more chances for transmission, has not held true in wildlife populations. While a disease that is transmitted by direct contact between potential hosts should certainly increase transmission if there are a higher number of susceptible hosts in a given area, this same principle does not hold true for vector-borne diseases. A vector such as a mosquito will make up for fewer available hosts by working harder and flying farther to find potential hosts. Similarly, sexually transmitted diseases can and do depend for transmission on increased effort and energy expenditure by their hosts for mate-seeking. For pathogens exploiting their hosts' efforts for transmission, the more dependable predictor of disease propagation is frequency: the proportion of hosts and the proportion of vectors that carry the pathogen.

Additional complicating factors include the genetic characteristics and population dynamics of host populations. In dwindling, bottlenecked host

populations there may be very little genetic variability. This could mean there may be no resistant individuals available for rescuing the population. For some species, (i.e. cheetahs) the genetic variation of the entire population may be less than that seen in single families of humans.[xix] This has long been thought to be a threat to the species survival in case of introduction of an emergent disease.

Finally, environmental factors appear to be playing an expanding role in both transmission of and resistance to disease. In recent die-offs of grey seals in Northern Europe, many biologists suspect pollutants played a role by suppressing immune system function in the seals.[xx] Others cite over-fishing in the region, postulating that the seals were stressed by malnutrition.[xxi] Still others note that the intrusion of other species (harp seals) into the grey seal range meant the introduction of new pathogens into a putatively naïve population.[xxii], noting that the change in the range of the harp seals was probably a result of over-fishing.

Disease Emergence: Definition and Factors

Disease emergence is defined as a recent increase in geographical range, host range or prevalence.[xxiii] The geographical range can refer to a disease invading new continents, such as Rinderpest arriving on the African continent from Europe and Asia in the 1890s. The number of recent examples of pathogens expanding their host ranges is impressive: West Nile Virus, HIV and Avian flu to name just a few of the more infamous instances of the phenomenon. A simple increase in prevalence can come about through a change in virulence, such as the annual mutation of new

influenza strains, or through changes in pathogen resistance, such as recrudescence of tuberculosis with drug resistant strains, or a decrease in host resistance, such as tuberculosis (again) with the advent of AIDS creating a new population of 'super' susceptible hosts.

Investigations into the emergence of these diseases have identified some forces that promote disease emergence in humans, domestic animals and wildlife. Table 1 is a compilation of several lists, and follows most particularly by Daszak.[xxiv] .

Among the factors listed promoting disease emergence in human populations, cultural and social changes rank fairly high. These include such events as the construction of an East-West highway across Africa that led to changes in traditional social structures. The highway afforded new mobility and new employment opportunities. The subsequent emergence of long-distance trucking and emigration of people away from villages and out of traditional lifestyles, prostitution: all contributed to the initiation of the HIV pandemic. [xxv] Understanding these steps helps in the efforts to control disease emergence as well as to suggest measures to mitigate HIV transmission occurring even now.

The table gives no equivalent category for wildlife–there are no cultural and social changes listed for animals–yet I contend that analogous forces exist for wildlife species. Alterations do and have occurred in the natural history of some species, usually initiated by anthropogenic impact. For example, raccoons live at higher population densities in suburban and urban areas as compared to densities recorded historically or in rural areas. [xxvi] These increased densities are easily attributed to human impact: people provide generous food resources in concentrated areas in the

form of agriculture, garbage and feeding stations (either pet food or purposeful provisioning for wildlife.) This provides a direct attractant more or less forcing raccoons to accept coming into close contact with their conspecifics. This affects not only the present population of raccoons, but it also creates pressure for genetic selection as well. Raccoons that are genetically and behaviorally ill adapted to tolerate such densities or closeness to anthropogenic disturbance will move out and be less likely to flourish and reproduce. In this way the simple movement of people into an area inhabited by raccoons can, it can be argued, result in a form of 'accidental' domestication. The raccoons evolve into a subspecies that is more tolerant of humans and each other and, in essence, their natural history (social structure) is changed. In the case of raccoons, this increased density promoted the emergence of the epizootic of rabies that has raged through the eastern seaboard states over the latter part of the twentieth century. This epizootic has and continues to cost local public health entities millions of dollars.[xxvii]

Human encroachment can mean changes in wildlife populations both in absolute terms and in terms of density. Human impact constrains some species, but others benefit and flourish. Anthropogenic forces driving these population changes are various. Some of these forces act directly on wildlife numbers, such as hunting, extermination efforts, supplement feeding and protections. Others may have indirect effects, from the elimination of predators of some species to unintentional provisioning with agriculture, landscaping or providing man-made shelter or corridors. Some species deftly exploit new and abundant resources provided by human incursions−others find it hard to tolerate the stress of contact with humans and

human technologies. In some areas raccoon, white-tailed deer and black bear populations have rebounded in parallel with forest re-growth, sometimes to numbers far exceeding historical densities. These species benefit from the protection from hunting found in and around suburban regions. Add to that unintentional and intentional provisioning with food–such as garbage, feeding stations, agriculture and landscaping–and the reasons for their burgeoning populations are obvious.[xxviii] An ultimate result is that some species adapt well to living in close contact with humans, and they bring their parasites with them. The human desire to live in natural areas and alongside wild animals not only throws people into close contact with pathogens, it also creates wildlife populations more likely to carry pathogens. This dynamic has often been cited as a cause in the emergence of Lyme disease.[xxix]

So, although such a factor is hard to list per se, there is a reason to consider the force of anthropogenic impact on the social (or natural) history and population dynamics of wildlife. For that reason, it may be helpful to discuss the vectors of these forces and propose at least the outlines of a model that can be built to predict and measure these forces.

In order to design such a model, this thesis proposes to study simultaneous infections in local populations of *P. maniculatus,* SNV and meta-parasites such as nematodes and tapeworms. Since the two infections share the same population, indeed the same individuals, but do not share the same transmission strategies, we hope to use them as more or less simultaneous equations–akin to simple algebraic constructions–to assess the relative power of the factors on disease outcomes. This

method may help determine which environmental features are the most robust predictors of pathogen emergence.

Modeling Disease

A variety of models exist that attempt to predict if a disease will propagate or die out of a population. For the most part these models focus on human diseases and use variables as factors that are relatively easily measured, often by direct questioning of individuals of the population. In animal populations finding important factors is less tidy and depends more on inference. To some extent any models intended for understanding wildlife disease must be limited by factors that can be measured. Unlike human populations where such things as population can be fairly accurately assessed by direct questioning, wildlife statistics are not so accessible. Techniques used to measure wildlife population dynamics generally use inference; estimations of totals are calculated from samples taken by observation or trapping. While these techniques cannot claim to approach comparable accuracy, still the techniques are tested and reviewed and commonly accepted as valid. Given these caveats, it is not unreasonable to assume that factors affecting survival of parasites in both human and animal host populations are probably analogous and can be compared.

Factors Predicting Disease Emergence

The system this thesis describes, limits variables to measures of population dynamics and biodiversity, and what might be called the vital statistics of the individual mice: e.g. sex and mass. These factors were chosen on the basis of availability, having been measured by the previous investigators Dizney and Ruedas. These limitations notwithstanding, in the next few sections I will consider and discuss an assortment of factors that may be involved in disease emergence.

One factor generally considered for most epizootic[1] analysis is population density. It makes intuitive sense that the more individuals living in a given area the more likely those individuals will contact each other, creating more opportunities for parasite transmission. In wildlife biology, population density is measured by inference, and is calculated from trapping results from specific trap-set configurations. These configurations have been established via experimentation and review and are considered to produce robust estimations of true population densities and biodiversity scores. Various configurations have been tested and compared; the configuration done by Ruedas and Dizney is accepted as an especially accurate representation of true population density. For the purposes of this project two population densities were calculated: one for *P. maniculatus* and another for all small mammals.[† 2]

[1] Epizootic is analogous to epidemic and refers to a disease outbreak in animals
[†] Range of size was determined by trap sizes and extended from shrews to raccoons.

While the potential to predict disease transmission from host population density seems intuitive, the predictive power from a multi-species population density does not. For if the small mammal population density is high, it could mean that contact between *P. maniculatus* would be diminished and so would transmission events. For example, if habitat resources could not support high densities of all small mammals and *P. maniculatus*, too, an absolute reduction in mice would reasonably be expected to result in less contact between mice. Conversely, high densities of all small mammals might not mean direct competition–if the other species of small mammals did not exploit the same food sources or shelter sites, there might not be any reduction in absolute numbers or density of *P. maniculatus*. This Darwinian rule of biodiversity suggests that these species would specialize and exploit different niches within the same area and all could flourish and no one species would necessarily face decline. In this case changes in population density or biodiversity levels might have no effect on parasite propagation in the reservoir, or, if any effects were there, they might be various and unpredictable.

The above cases describe parasites for which transmission is effected by direct contact. More complex transmission strategies result in more complex predictive models and may alter the dynamics of environmental forces dramatically. For example, if a vector is involved, the vector may make up for decreased host numbers by increasing its search effort. Thus, the incidences of diseases transmitted by mosquitoes are little affected by rural versus urban human populations. Likewise, sexually transmitted diseases depend on mate-seeking effort for transmission rates and both low host populations and low host densities may be mitigated by increased

effort. In the case of SNV, there is evidence that territorial fighting is positively related to transmission (there is a high correlation between male sex and scars and positive SNV status [xxx]). In that way SNV may act like a sexually transmitted disease if male mice (and females) actively seek to protect their territory or find better territories.

Gastrointestinal meta-parasites depend primarily on indirect contact for transmission. Hosts become infected after ingesting eggs or larvae deposited in the environment by previous hosts, usually in feces. There is possibly some direct contact transmission as infection could occur during mutual grooming, but this probably plays only a small role. It is unlikely that the larvae actively seek hosts. They are relative immobile in the environment–there is no evidence these larvae travel more than a few centimeters in the ground, and any motility seen in the laboratory appears non-progressive. A model might treat the contaminated environment as a sort of surrogate vector–although it would not put any 'effort' into transmission, it may still take an active role in transmission. While the environment doesn't move, it can spatially concentrate the foraging and social habits of the potential hosts, effectively mimicking an active vector.[xxxi]

This mimicking would come about through the role of habitat use. Habitat characteristics play an important role in determining whether animals interact with each other or not. Vegetation, microclimate and terrain work in concert to create a non-uniform, uneven distribution of individuals, thus affecting transmission opportunities. Animals will forage where there is food, travel where it is easiest and safest, and these places will tend to result in the overlap of territories pulling

individuals into contact. This has been demonstrated effectively by studies of the mouse SNV reservoir in the Four Corners Area. The desert environment provides a patchy habitat, with areas protected from heat and exposure being frequented, creating a population distribution that is functionally, if not absolutely, dense.[xxxii] This is analogous to the African highway forming a conduit for increased contact between humans, forcing a change in social habits and societal structure and changed epidemiology.

The environment may then act as an energetic vector if habitat use is non-uniform. This is even more likely for small mammals and for *P. maniculatus* in particular. *P. maniculatus* is a cryptic species, depending on hiding for safety. *P. Maniculatus* is also dependent on the environment for thermal regulation–mice are too small to maintain their own body temperature and must depend on ambient temperature for thermal support, moving to warm areas as needed. Both requirements suggest they do not distribute themselves uniformly across a landscape but instead use the habitat in a patchy way, concentrating their activities along corridors and in sheltered restricted areas. These areas could very likely be frequented by most of the mice living in a particular area. This relative concentration and focusing of mouse activity would then act with a concentrating effect, analogous to a vector species exerting increased effort to seek new and susceptible hosts.

Duration of infectiousness is also a factor considered in disease propagation modeling. This factor fades in importance in this system, since both SNV and nematodes are probably lifelong infections in *P. maniculatus* once they are established. Indeed, since there is evidence that SNV promotes longevity in the

species, this could affect duration because it would extend the period of possible transmission. But, because this increase in duration would be uniform across all populations it should not affect the current study and since the prevalence of SNV is low–5.3%--its effect would be small in any case.

Infection with meta-parasites is probably detrimental to the mice, but usually not severely except in cases where the number of worms, the parasite load (or intensity), is high. Laboratory studies suggest this is usually the result of individual mouse characteristics such as poor immune response. In this study it is likely that mice with such a handicap would be randomly distributed and relatively rare and thus unlikely to affect the results from the wild populations in this study.

For the case of nematodes, it is probable that *P. maniculatus* remains infected for its lifespan either because of re-infection (from the original infection or from outside) or the lifespan of the nematode. The infective larvae do have a limited lifespan in the environment, but this doesn't appear to be a strong limitation. Larvae of some nematode species known to be infectious to *Peromyscus spp* have a half-life of 17 weeks at temperate temperatures (or nearly five months). This duration probably closely approximates the adult lifespan of their host.

Biodiversity

The role of the biodiversity in the maintenance and emergence of pathogen reservoirs has come under study in recent years. Ostfeld and colleagues [xxxiii] coined the term 'dilution effect' in his studies of Lyme disease in *Peromyscus leucopus*. He

used the term to describe how having more than one species in an area could decrease the proportion of infectious hosts of any one species. 'Dilution' would occur if the number of contacts between individuals of one species would be decreased by the presence of individuals of other species. In essence, the effect predicts that a mouse would be less likely to become infected because it would be less likely to contact an infectious con-specific when it shared habitat with voles and gophers; the other species would act as physical buffers to transmission. This is an intuitively attractive idea, but, by itself, does not take into account every contingency.

For example, what if the population density of the target species does not decrease as the biodiversity increases? This could occur if the other species did not compete directly with the target species for resources, and more than one species could live comfortably in close contact and not 'crowd out' other individuals. Alternatively, if other species shared a pathogen (and were equally infectious when infected), then biodiversity might have an opposite effect, and might increase transmission events. Finally, a parasite that depended on vectors for transmission might present very different dynamics–and perhaps enhance or cancel the 'dilution effect.' Ostfeld himself later proposed that his dilution effect might only work for vector-transmitted species, and not for parasites that depended on direct contact for transmission.[xxxiv]

Analyses by Enzenwa demonstrate some of these factors.[xxxv] She examined the relationship between species richness and meta-parasite prevalence in ungulates in game reserves in central Kenya. Interestingly, she found that population density of ungulate species did not predict parasite load, and that biodiversity (number of

ungulate species in an area) had a promotional effect (the more species in an area, the higher the parasite load.) In another analysis, Ezenwa found that biodiversity, while having a promotional effect on prevalence of generalist parasite species, had an inverse effect on species specialists like coccidial Eimeria species, [xxxvi] as predicted by the 'dilution effect.'

Biodiversity presumably would impact SNV prevalence inversely by exerting the so-called dilution effect. But any dilution effect would ostensibly depend on there being a decrease in population density of mice. Dizney and Ruedas' study did not entirely support this conclusion, as they did not find any significant relationship between the biodiversity of parks and population densities of parks (of either *P. maniculatus* or all small mammals.) Another mechanism of effect proposed is through alternative host species having different and lower transmission rates. If more than one species can be infected, the pathogen might exhibit differential transmission rates between and among infected species. Mathematical modeling shows that this differential transmission can result in a decrease in overall prevalence. In this case although the second species supports the virus, it still acts as a buffer (if not a diluent) and slows overall disease propagation by being a less efficient transmitter. Dizney and Ruedas found individuals of other species that were positive for SNV, but at a very low prevalence. This evidence suggests differential transmission rates could affect prevalence as biodiversity increases, but that the effect should be small.

It is unknown whether or not nematodes found in this system are likely to affect more than one species, i.e. we do not know if the species here are generalists or

specialists. Many nematode species are at least facultative generalists–they may 'prefer' one species but they are capable of surviving and even propagating in more than one species. The relative rates of transmission between versus among species may very well differ, thus promoting an inverse relationship between biodiversity and prevalence. (The next step in this project is to perform species identification on the isolated nematodes. This will be an important step in understanding parasite dynamics in this system.)

One other way to regard these variables is how they affect populations in a more traditionally ecological sense. For example, if increased population densities tax the area's carrying capacity, lifespans may fall and duration of infectiousness will likewise fall. This could lead to a lower prevalence. Likewise increased biodiversity could impact single species populations through interspecies competition, similarly lowering lifespan and duration of infectiousness, and, finally, prevalence.

Last, biodiversity may act as an index of human impact. It is clear that human impact typically causes regional biodiversity to decrease, and thus biodiversity could act as an indicator or quantifier of human impact. At the same time human impact could include increased exposure to environmental toxins, increased predation and pressure from people and domestic animals, and stress. All of these factors could affect disease prevalence by decreasing overall lifespan and the duration of infectiousness, or there may be a general inhibition of activity decreasing transmission events. In this way biodiversity would only appear to have an effect on prevalence, while other effects were the real actors.

In summary, the factors to consider in this system to assess risk of disease emergence are:

- Population density: with the caveat that it may depend more on habitat use and functional density may be higher than what is measured.
- Frequency, or the proportion of infected individuals. This is low for SNV but may be more significant for nematodes where it is likely to be higher. Because of habitat and social history it could be the more important factor for one of both of the parasites.
- Frequency in the form of the proportion of infected habitat. This has not been measured in this system, but is possible to measure. It could help to build a more accurate model for predicting meta-parasite infection, but probably wouldn't have influence over SNV, which is directly transmitted.
- Biodiversity, which could work in various ways:
    - As it affects population densities, which it does not appear to do in this system
    - As it affects frequency, which it may do in nematodes.
    - As it affects transmission between and within species populations.
- Host characteristics, and how these are affected by environmental changes
- Parasite characteristics: what are the species of parasites, and which are generalists and which are specialists. Does biodiversity have a different impact between the two?

In conclusion, these analyses indicate the difficulties in predicting disease behavior and emergence. While progress has been made in determining what forces

are at work, determining their exact effects is necessarily a complex task. It may be that there are no generalizable 'laws' of effect; instead there may only be complex models of indeterminate worth. Still, it is useful to attempt to measure and calibrate any potential effects.

Materials and Methods

The specimens were collected over three years from five parks or reserves in the Portland, Oregon, area. For this thesis the parks were often delineated by number and are:

1) Forest Park
2) Powell Butte Park
3) Tryon Creek Park
4) Tualatin River National Wilderness Area (TRNWR)
5) Oxbow Park

The specimens were collected over all seasons, with only brief time gaps. The trapping was done one park at a time on a regularly rotating weekly basis. Traps were set out on Mondays, checked every morning, re-baited as necessary and set again until Friday when the traps were collected and removed. This yielded four trap-nights per week and a total of 1,408 trap-nights/week. The traps were humane, live traps including box traps, wire traps and pit-fall traps. Bait was peanut butter and oatmeal with a 'pledgette' of polyester filling provided for warmth. For the first phase of collection, trapped animals were sacrificed if they were *P. maniculatus*, and released if not. (For a later phase *P. maniculatus* were ear-tagged and released and a re-capture formulas initiated for further population studies.) At time of trapping all rodents were weighed and sexed, and their reproductive status determined by visual inspection and a blood sample was collected to test for SNV, usually by peri-ocular

bleeding. Animals that were sacrificed, or otherwise found dead or moribund were collected, tagged and frozen.

Testing for SNV was done using ELISA technique. Tissues were taken from selected mice for DNA sequencing including PCR testing for presence of SNV in tissues. DNA sequencing was also used to compare phylogenetic trees for analysis for co-evolution of *P. maniculatus* and distinct strains of SNV in separate studies. (Mice removed for tissue collection or for museum preparation were often not dissected for meta-parasites. Because the project investigators were primarily interested in SNV infection and co-evolution, it is probable they removed SNV positive mice preferentially. For that reason the SNV prevalence on the subset examined in this study is not an accurate representation of the SNV prevalence of the entire collected population.)

Traps were set in a trap-web configuration[xxxvii] designed to provide a framework for calculating population densities of *P. maniculatus* and all small mammals and biodiversity scoring. The sites chosen for the trap webs did not change over the three-year period of trapping. The configuration consisted of approximately 352 traps and pit-falls, set in a tight circle at the apex and radiating outward in circles with traps initially set at 5m intervals and at the 20m point at 10m intervals. In this configuration, theoretically the innermost circle is tight enough that all animals in that area are trapped, and as the circles get larger a progressively and predictably smaller proportion of the total population is trapped, forming the basis for extrapolation and calculation of the true population density.

The parks were chosen in order to theoretically sample the spectrum of biodiversity based on island biogeographic theory which predicts a linear relationship between area and biodiversity. Because the parks were in fairly close proximity to Portland, climate, vegetation and terrain was fairly similar for all parks. All could be characterized as being in foothill regions with temperate rain-forest evergreen riparian habitats predominating. Sites were selected that were moderately remote, i.e. not directly adjacent to permanent habitation, roads or campgrounds. Some sites were crossed by paths that appeared to be only lightly used.

Historically the parks differed considerably. Forest Park, although large, has been logged repeatedly and close to habitation and is currently heavily used by people. In contrast, Oxbow Park was old growth forest and public use is tightly controlled. Powell Butte, TRNWR and Tryon Creek Parks are smaller and surrounded by suburban habitation. All can be characterized as mixed habitats with meadows, shrub and forestlands. Forests are typically mixed deciduous coniferous evergreen forests and all contain riparian habitat.

Population densities were calculated by Dizney using the program "DISTANCE" [xxxviii] and the results graciously provided for analysis. Population densities were calculated over four month intervals. Biodiversity scores were also provided using calculations based on the trap-web configuration over yearlong intervals. The intervals varied for parks; i.e., the intervals were not exactly concomitant, but did overlap for the most part.

Meta-Parasite Collection

Parasites were collected by direct dissection. Incisions were made into the abdomen and the gastrointestinal tract removed from stomach to colon. Each portion of the tract was then opened under dissecting microscope and parasites isolated, counted, removed and placed in ethanol. (Counting at the time of collection could not be completely accurate, as breakage of worms could occur and was often undetectable. Since this was a random event, the error introduced should be a random error. Worms were tentatively identified as either roundworm or tapeworm at time of dissection; since only roundworms were found only roundworms have been counted.) Vials containing parasites were labeled with the number assigned to the mice. The operator was blinded to the source of the trapped specimens−tags were numbered but the park the animals were collected from was not indicated .

Analysis

Trapping information, measurements and dissection results on 257 mice were entered into a database using the software Intercooled STATA 9.2. For each specimen the variables recorded included: park of origin, mass, sex, presence or absence of parasites, number parasites found, population density for *P. maniculatus* at date of trapping, population density for all small mammals at date of trapping and biodiversity score at date of trapping.

Formal analysis was performed using GEE (generalized estimating equations) with binomial longitudinal model with log linkage function. The working correlation structure was considered exchangeable. Outcome for this model was the presence or absence of worms in each mouse, testing the variables as predictors. This model was constructed in a forward fashion, testing all variables as crude estimates first, and then selectively adding variables. All models were tested, even when variables were not significant, to test the impact of all variables and to explore confounding.

The above analyses were also performed with specimens stratified for maturity. Maturity was assigned by mass, with a cut off at 14 g, selected because reported growth curves show most *Peromyscus* spp are 14 g when 75% of their growth had been achieved. [xxxix] Stratification was done to help avoid confounding by age, since age is a risk factor for parasitism simply because the longer a mouse lives, the greater the exposure to infection by ingestion of eggs or infective larvae in the environment. The increased risk is supported by the data (prevalence for immature mice was approximately half of that for mature mice. The decision to divide on the basis of 75% growth was done without regard to differences in prevalence. The greatest difference between groups would have resulted in groups stratified using a lower mass breakpoint.)

To verify results from the GEE model, the data were subjected to linear regression as well. For linear models, the data were collapsed into variables calculated as park-years. All variables (prevalence, mass, biodiversity and population densities) were averaged for each park for each year, resulting in a set of 15 data points for each variable.

Prevalence of parasites was measured as a constrained variable (a proportion from 0 to 1) and so, for the purposes of linear analysis, these data were converted to their natural log. In doing so two data points were lost because for two park-years: for Forest Park in year 3, and for TRNWR for year 3, since none of the dissected mice were parasitized, yielding a prevalence of 0, which could not be converted to log form. (Losing these two data points was regrettable. The two groups were unusual in that one, Forest Park in year 3, contained mice from a single trap night that had a low average mass. From that it can be surmised that the mice were a young cohort and not representative. The other group, TRNWR in year 3, was a very small group containing only 3 mice. These were the only available mice, as other specimens had been removed for museum preservation.) The associations between biodiversity, population density and average maturity and gender at each time point were assessed using linear regression.

Linear regression was also used to test for any relationships between the independent variables, biodiversity and the average mass of mice, the population densities of *P. maniculatus* and all small mammals.

The relationship between SNV prevalence and parasite prevalence was explored using two by two tables and calculating odds ratios. Since SNV prevalence is low and the disease is rare in the populations, the odds ratios should closely approximate risk ratios.

Intensity (or parasite load) was analyzed as the number of parasites found per individual mouse. Clumping of parasites or inordinately high parasite intensity for individual mice, indicate difference in intensity is more likely due to individual

differences rather than an effect of environmental factors. Average intensities for overall populations (all mice and mature mice) were analyzed for clumping using aggregation constants. Aggregation constants were calculated from the ratio of the variance of parasite numbers over the mean parasite load for overall populations and for each park.

Confounding was considered as possible, even likely mixing of effects of the variables, as was the possible interaction between variables. Population densities, biodiversity and mass were all parameters that may have had complex mixed effects and interactions. In order to test for confounding multivariate models were applied and coefficients were assessed for any change with change of any variable associated with adding variables was assessed. Any change in excess of 10% in the previous ratio was considered as evidence for confounding. Composite interaction variables were created and tested for significant interactions. Interaction was ruled out if the interaction variables did not reach a significance of $P < 0.1$.

Results

Tables 2 and presents the summary statistics for the all mice overall and by park and mature mice overall and by park. The subset of all dissected mice (from the larger collection of all mice trapped) had an overall average mass of 16.5 g. Using ANOVA, mass did not differ significantly between parks over the three years of observation ($p = 0.0688$). Oxbow had the smallest average mass at 15.6 g, Powell Butte had the largest at 17.5 g.

For the subset of all dissected mice there was an average of 42.7 small mammals per hectare with a range from 6 – 176 small mammals per hectare and there was an average of 19.4 *P. maniculatus* per hectare with a range of 1.7 to 86.3 *P. maniculatus* per hectare. Again, using ANOVA, differences between park parameters were assessed. Between parks the population densities for both *P. maniculatus* and for all small mammals per park differed significantly over the three years of observation (for both mouse and all small mammal densities $p < 0.001$). Highest density for all small mammals occurred in Forest Park at 55.2, and the lowest in Tryon Creek at 22 per hectare. Highest density of *P. maniculatus* occurred in Tryon Creek at 45 per hectare with lowest in Powell Butte at 10.9 per hectare.

Overall mean biodiversity score for the all parks was 0.5. ANOVA showed biodiversity scores also differed significantly between parks over the three years of observation ($P<0.001$), with highest scores in Oxbow at 0.74 ranging from 0.7 to .78

over the three years of observation; lowest scores occurred in Forest Park with a mean of 0.34 and range of 0.28 to 0.6.

Stratification of the population into mature and immature mice yielded two sub-subset populations: 74 immature and 183 mature mice. Prevalence of parasites in immature mice was 0.2432; prevalence for mature mice was 0.4372. The difference in prevalence between the two groups was significant ($p = 0.0038$). Dividing the population into male versus female mice (two specimens dropped due to lack of identification–some specimens were submitted as GI tracts only), yielded prevalence for 145 male mice of 0.4 and for 110 female mice of 0.364. There was no significant difference between these prevalence's ($p = 0.554$).

There were no significant relationships between biodiversity, average mass, population density of P. maniculatus, or population density of all small mammals. This was consistent across the data for all mice and for the subset of mature mice.

The results from the GEE model are included in Tables 4-7. In crude models using a data set of all mice, both mass and biodiversity proved to be significant predictors of prevalence of parasites. For a 4.15 g increase in mass (one standard deviation difference), the prevalence of parasitism in mice increased by 28 % (95% CI 11%, 49%) Mass retained its predictive power when controlled separately for biodiversity, population density of *P. maniculatis* and population density of all small mammals, and when adjusted for all variables simultaneously. Biodiversity also had a significant effect in a crude model; a 0.15 unit increase in biodiversity score (difference of one standard deviation) decreases the prevalence of parasites in mice by 9.3% (95% CI 17.4%, 4%). Neither the population density of all small mammals

nor of *P. maniculatus* proved to be significant predictors of the prevalence of parasitism.

After controlling for mass, biodiversity lost significance as a predictor, suggesting that some of its effects could be related to differences in mouse population average masses within parks. Similarly, biodiversity lost significance when adjusted for either population density of *P. maniculatus* and for all small mammals. Biodiversity on its own appears important, but part of its importance is explained by its relationship to population density of all small mammals and of *P. maniculatus*.

The relationship of biodiversity to risk of parasitism was shown to be more significant when limited to the stratified population of mature mice only. In a crude model, biodiversity was a significant predictor ($p = 0.007$) and marginally significant in a model including mass ($p = 0.059$). For a 0.145 unit increase in biodiversity score (difference of one standard deviation), the prevalence of parasites in mice decreased by 10% (95% CI 16%, 2.9%). Mass was not a significant predictor in any model containing only mature mice. Mass did appear to be a significant confounder of the association between biodiversity and the prevalence of parasites as the coefficient for biodiversity when adjusted for mass changed from 10% to 8.2%, a difference of 18%. In models containing both population densities (*P. maniculatus* and small mammal), biodiversity retained significance as a predictor ($p = 0.048$ and $p = 0.044$, respectively). Confounding by population density of mice was the lowest changing the coefficient for biodiversity by 11%. Models with more than two variables yielded no significant predictors.

GEE analysis run on immature mice population yielded no significant predicting variables.

Linear regression on park-year parameters found no significant predictors in either the all mice subset or the mature mice only subset, which was likely due to small sample size. However, both linear regression models supported the GEE findings in that the magnitude and direction of the coefficients for mass and biodiversity were consistent. Mass had a positive relationship with prevalence (as mass increased so did prevalence) and biodiversity consistently affected prevalence inversely (as biodiversity increased, prevalence trended downward) and the coefficients were of similar magnitude. Population densities effects were variable, mostly positive but occasionally inverse as the models were expanded to include more variables.

Table 8 presents a summary of the prevalence of SNV for this subset of mice. Overall SNV prevalence was 3.1 % for all mice, and 3.7% for mature mice. Prevalence in all parks was higher in mature mice than all mice except in Oxbow Park. For all mice, the odds that an SNV positive mouse was also parasitized were greater than 2, which was consistent through all parks but Tryon Creek, where there were no SNV positive mice in this subset, and for Powell Butte, where the odds for being positive for SNV and parasitized were only 1.36. For mature mice the odds of being both positive for SNV and parasitized were slightly less, 1.75 overall for all mature mice (not classified by parks).

Table 9 present summaries of parasite load or intensity. Overall intensity was not high, being 1.33 for all mice and 1.67 for mature mice. Calculations for

aggregation suggested a high degree of clumping, being greater than 1 in all cases and as high as 6.28 in Forest Park.

Discussion

Findings of the Study

Only two variables had significant impact on parasitism in individual mice: mass and biodiversity. The role of mass is not surprising, but mass is not a direct measure of the role of the environment in predicting disease emergence. Biodiversity is an environmental factor, and of all the environmental influences it was the one with significant effect. This confirms the parent study of SNV[xl] prevalence and echoes earlier studies mentioned in the introduction.[xli]

That mass would be a predictor of risk is not a surprise because mass is a reasonable proxy for age, and a longer life results in more opportunities for infection. While this is obvious, age might also act against infection if the animals developed resistance to infection. This would be expected in longer-lived animals that can develop immune responses that can eliminate some parasitic infections. The data on aggregation support this to some extent, as there is considerable variability in parasite intensity, suggesting individual variation in immuno-competence plays a large part in resistance to parasites. The largest mice (i.e.: oldest mice) with masses above 19 g exhibit the highest prevalence (0.544) supporting a conclusion that they either continue to maintain parasites or continue to become infected. In P. maniculatus in the Northwest immune competency appears consistent with chronic, low level

infections and not elimination. For our disease model, age related persistence of parasitism means that duration of infection approximates the mature lifespan of *P. maniculatus*, and any modulation of duration from environmental factors would come from effects on the time of initiation of infection.

Notwithstanding the above, mass might reflect environmental factors if it were an indicator of the general health of a population. If so, mass could confound the model if differences were subject to the same environmental impacts that affected biodiversity. This is not unrealistic; pollution or anthropogenic stress, for example, could result in less healthy, less resilient and subsequently smaller and less resistant mice, at the same time it could eliminate other species with a resulting decline of biodiversity. Our study does not show this effect, since although the five parks differed significantly in biodiversity (and in population densities), they did not differ significantly in average mass of mice. Additionally, the study analysis was stratified on the basis of mass, which should eliminate confounding of mass.

The importance of mass in this study is supported by previous findings in a larger study of prevalence of another parasite, *Taenia taeniaeformis* in *P. maniculatus* in Northern California.[xlii] *T. taeniaeformis* is a liver fluke also transmitted by the fecal oral route via infectious eggs and larvae deposited in the environment. This study also found that only age and not sex, reproductive status or population fluctuations had significant effects on prevalence (1.2% in young mice and 4.2% in mature mice.) This study did not assess biodiversity's impact.

While mass does not represent an environmental effect, biodiversity does, and biodiversity proved to have some significant effect on parasite prevalence. This

relationship does not, in this study, present itself as particularly robust or independent. There is significant confounding of the association of biodiversity and parasitism by all other factors. Mass and population densities all changed the magnitude of impact significantly and often eliminated the significance of biodiversity's effect. The conclusion may be that all these factors are hard to parse, that mixed effects are the rule. Nonetheless, in comparison with the effects of other factors, also hard to study in this system, biodiversity did exhibit a significant effect in models using only one or two factors and the inverse effect remained the same, even if the magnitude lost statistical significance. As this parallels the effect of SNV on the same population, it bears examination.

Also, it is possible that the relationship of biodiversity to parasite prevalence will prove to be more robust that it appears here. This study does not present the natural history of the parasites themselves. If we presume the parasite population is diverse (that more than one species of roundworm comprise our collection, which is likely) then we may have a collection of generalist parasites mixed with species specialists. If that is the case, as described by Ezenwa[xliii], the generalists may be masking the true effect of biodiversity on risk of infection by specialist parasites. This would be a masking of the potential effect of biodiversity on the risk of zoonotic disease emergence from wildlife populations.

While this study demonstrates an effect of biodiversity with the same direction as the effect found in the parent study of SNV prevalence, the mechanism may not be the same. That study showed that biodiversity exerted a threshold effect on SNV prevalence: below a certain biodiversity level there was no demonstrated

influence. It is too early to conclude, but there does not appear to be this threshold type of effect for meta-parasites.

This may be a reflection of the different transmission strategies of the two parasites, and if this is so, it may be possible in the future to discern transmission characteristics from differences in response to environmental factors such as biodiversity.

The lack of a significant role for population density could be attributed to habitat use or to the characteristics of transmission (or both). As mentioned, other theorists believe frequency to be a more important factor in indirect transmission, as increased effort by vectors or by individuals driven by social factors such as mate seeking cancel out absolute population effects. Probably equally important and not unrelated is the likelihood that *P. maniculatus* does not use habitat in a uniform fashion.

Again, lack of direct influence notwithstanding, population densities did act as confounders for the effect of biodiversity on parasite prevalence. This suggests that differences in population densities explain some of the effects of biodiversity. Certainly it is easy to imagine that biodiversity would impact population densities of mice and other creatures. What is harder to explain is that there did not appear to be any significant predictable effect when relations between these factors were subjected to linear regression. Given the widely ranging nature of the population fluctuations and no means of knowing an appropriate lag time, failure to detect a relationship may be the fault of the investigator.

Limitations of the Study

Any study of wildlife is limited by the inherent difficulties to observing and measuring the lives of shy, covert and tiny subjects. *P. maniculatus* is a cryptic species, unseen unless trapped, and, just as an electron that inspired Heisenberg's Uncertainty principle, a mouse when trapped is no longer a normal reflection of itself. The best we can do is to study them in cross section–a mouse was trapped in one particular spot on this particular night and this infers a range and traveling habit that can be used in understanding their behavior. Likewise, we can assess age, reproductive status and sex by visual examination, but other social factors remain a mystery. Laboratory studies can give us a semblance of what may be their social structures, but only a semblance. Even typical causes of death are hard to determine as wherever scavengers and predators abound, carcasses are nearly impossible to find.

The natural history of parasitism in this population is also unknown. While there are reports in literature of parasites in *Peromyscus* spp., there is a dearth of such reports for this region. This is a common failing in all wildlife research. While diseases that affect humans and domestic species, and diseases that affect economically important species like deer and salmon generate volumes of literature, other wildlife diseases are next to unknown.

This study used a collection established for the study of another disease entirely and was thus a secondary study. With the use of specimens for phylogenetic analysis and preservation for museum collections, the subset studied here was more or less leftovers. If there was a bias that resulted from the previous use of specimens,

it was most likely a random bias that would not affect parasite numbers. Although it is not clear whether or not the methods of preservation (freezing) resulted in adequate preservation of all parasites, the entire collection was subject to the same treatment so again, any bias would not be related to biodiversity.

There were limitations in the parameters provided. Habitat characteristics such as vegetation type, were in process at the time of this study and are not included. These parameters could be invaluable in future analysis.

Collection of parasites by direct dissection requires time and patience and is a technical challenge. The time and effort involved are one reason for the relatively small sample, which in itself is another limitation of the study. Only one operator (after training) performed the dissections, so search effort and skill levels should be consistent, again, any bias should be random and unlikely to affect the outcome.

Finally, the parasites have yet to be identified and characterized. Since their nature (as generalists or specialists) is not known, the true magnitude of the environmental factor effects cannot be known for certain.

Strengths of the study

This study does not merely repeat, but instead tests and complements previously performed studies. Using different populations (*Peromyscus* spp instead of ungulates) it tests the results of Ezenwa. Similarly, while it uses the same host population as Ostfeld, it verifies those results using different parasites. It verifies the results of its parent study by Dizney and Ruedas. The combined studies complement

the extensive research into the characteristics of the *Peromyscus* reservoir for SNV in the southwest. While those studies linked disease emergence to climate phenomena, they did not evaluate the potential effects of biodiversity. The result is a more complete and comprehensive understanding of disease reservoirs in general, as well as this reservoir in particular.

Combined with its parent study by Dizney and Ruedas, this study provides a holistic perspective to wildlife disease reservoirs. It adds to the understanding of disease emergence by integrating some fairly disparate disciplines. As it does so it combines some fairly basic techniques with sophisticated modeling, from distance sampling of wildlife populations to advanced statistical modeling for correlated data. The result tracks a simple principle through a complex system and finds that biodiversity can decrease the transmission of some diseases.

Further Study

The next step of this study is to identify the parasites and characterize their natural histories and then re-doing the analysis to test the disease transmission dynamics between generalist species and specialist species. Testing soil in the trap sites for infective larvae could produce a better picture of habitat use and contribute to an understanding of the role of environment in indirect transmission of meta-parasites. With information on the comparative effects of biodiversity on the transmission dynamics of generalist versus specialist parasites a next step would be to try to use this information to predict transmission in other reservoir systems. This is

one study of one reservoir species and much can be learned from this one model species, but there are many other systems as yet unstudied. Wildlife disease reservoirs remain poorly understood; studying parasite transmission mechanics offers one key to shedding light on the contents of this black box.

Conclusion

The environmental factor biodiversity affects disease prevalence in natural reservoir populations. Historically biologists have shown that biodiversity is exquisitely sensitive to anthropogenic impact. The result can be a reverberating risk: human incursions cause environmental degradation and decrease biodiversity. Declining biodiversity threatens natural infectious disease buffering systems, resulting in increased risk of disease emergence into human populations.

Public health implications

While it could hardly be called surprising, the implications are that environmental health, as in the ecological health of the natural environment, has impact on public health. Just as the existence of healthy wetlands provides for the removal of pollutants from water and the existence of mangrove forests mitigates the impact of ocean storms, the emergence of infectious disease can be prevented by supporting biodiversity. In the past, biologists have defended biodiversity as a potential source for medications or other, as yet unknown, helpful commodities. This study combined with others indicates that human society should consider promoting species biodiversity for another reason: because it may protect us from infectious disease pathogens.

References

[1] [1]Yates, T. L., J. N. Mills, et al. (2002). "The ecology and evolutionary history of an emergent disease: Hantavirus Pulmonary Syndrome." BioScience **52**(11): 10.

[1]Yates, T. L., J. N. Mills, et al. (2002). "The ecology and evolutionary history of an emergent disease: Hantavirus Pulmonary Syndrome." BioScience **52**(11): 10.

[1] Personal communication

[1] personal communication

[1] personal communication

[1] Mills, J. N., T. G. Ksiazek, et al. (1999). "Long-Term Studies of Hantavirus Reservoir Populations in the Southwestern United States: A Synthesis." Emerging Infectious Diseases **5**(1): 8.

[1] Netski, D., B. H. Thran, S. C. St Jeor. (1999) Sin Nombre Virus pathogenesis in Peromyscus maniculatus. J of Virology 73(1): 585

[1] O'Connor, C. S., J. P. Hayes and S. C. St Jeor. (1997) Sin Nombre virus does not impair respiratory function of wild deer mice. J of Mammology 78(2): 661

[1] Taylor, L. H., S. M. Latham, et al. (2001). "Risk factors for human disease emergence." Phil. Trans. R. Soc. Lond. B **356**: 7.

[1] Ibid

[1] ibid

[1] Daszak, P., A. Cunningham, et al. "Emerging Infectious Diseases of Wildlife--Threats to Biodiversity and Human Health." Science's Compass.

[1] Walters, M. J. (2006). "Do No Harm." Conservation in Practice **7**(4): 6.

[1] Daszak, P., A. A. Cunningham, A. D. Hyatt. (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78: 103

[1] Anderson, R. M. and R. M. May (1982). "Coevolution of hosts and parasites." Parasitology **85**: 16.

[1] ibid

[1] May, R. M., S. Gupta, et al. (2001). "Infectious disease dynamics: what characterizes a successful invader?" Phil. Trans. R. Soc. Lond. B **356**: 10.

[1] Rudolf, V. H. W. and J. Antonovics (2005). "Species Coexistence and Pathogens with Frequency-Dependent Transmissions." The American Naturalist **166**(1): 6.

[1] Menotti-Raymond, M. and S. J. O'Brien (1993). "Dating the Genetic Bottleneck of the African Cheetah." Proceedings of the National Academy of Sciences **90**: 5.

[1] Daszak, P., A. A. Cunningham, et al. (2001). "Anthropogenic environmental change and the emergence of infectious diseases in wildlife." Acta Tropica **78**: 14.

[1] Ibid.

[1] Ibid.

[1] Dobson and Foufopoulos, 2001

[1] Daszak, P., A. A. Cunningham, et al. (2001). "Anthropogenic environmental change and the emergence of infectious diseases in wildlife." Acta Tropica **78**: 14.

[1] Hahn, B. H., G. M. Shaw, et al. (2000). "AIDS as a Zoonosis: Scientific and Public Health Implications." Science **287**: 9.

[1] Riley, S. P. D., J. Hadidian, et al. (1998). "Population density, survival, and rabies in raccoons in an urban national park." Canadian Journal of Zoology **76**: 11.

[1] Rupprecht, C. E., J. S. Smith, et al. (1995). "The Ascension of Wildlife Rabies: A cause for Public Health Concern or Intervention?" Emerging Infectious Diseases **1**(4).

[1]

[1] Ostfeld, R. S. and F. Keesing (2000). "Biodiversity and disease risk: the case of Lyme disease." Conservation Biology **14**: 6.

[1] Yates, T. L., J. N. Mills, et al. (2002). "The ecology and evolutionary history of an emergent disease: Hantavirus Pulmonary Syndrome." BioScience **52**(11): 10.

[1] Altiser, S., C.L. Nunn, et al. (2003) "Social Organization and Parasite Risk in Mammals: Integrating Theory and Empirical Studies." Annu. Rev. Ecol. Evol. Syst. 34:517

[1] Engelthaler, D. M., D. G. Mosley, et al. (1999). "Climatic and Environmental Patterns Associated with Hantavirus Pulmonary Syndrome, Four Corners Region, United States." Emerging Infectious Diseases **5**(1): 8.

[1] Ostfeld, R. S. and F. Keesing (2000). "Biodiversity and disease risk: the case of Lyme disease." Conservation Biology **14**: 6.

[1] Buskirk, J. V. and R. S. Ostfeld (1998). "Habitat heterogeneity, dispersal, and local risk of exposure to Lyme disease." Ecological Applications **8**(2): 14.

, Dobson, A. and J. Foulopoulos (2001). "Emerging Infectious pathogens of wildlife." Phil. Trans. R. Soc. Lond. B **356**: 1001-1012.

[1] Ezenwa, V. O. (2004). "Parasite infection rates of impala (Aepyceros melampus) in fence game reserves in relation to reserve characteristics." Biological Conservation **118**: 5.

[1] Ezenwa, V. O. (2003). "Habitat Overlap and gastrointestinal parasitism in sympatric Afrcan bovids." Parasitology **126**: 10.

[1] Anderson, D. R., K. P. Burnham, et al. (1983). "Density Estimation of Small-mammal Populations using a trapping web and distance sampling methods." Ecology and Genetics of Host-Parasite Interactions **64**(4): 7.

[1] Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers and L. Thomas. (2001) Introduction to Distance Sampling. New York: Oxford University Press xv+432 pp

[1] King, J. A. (1968). "Biology of Peromyscus (Rodentia)." Society of Mammologists Special Publication **special publication #2**.

[1] King, J. A. (1968). "Biology of Peromyscus (Rodentia)." Society of Mammologists Special Publication **special publication #2**.

[1] Personal communication

[1]Ostfeld, R. S. and F. Keesing (2000). "Biodiversity and disease risk: the case of Lyme disease." Conservation Biology **14**: 6.

Ezenwa, V. O. (2003). "Habitat Overlap and gastrointestinal parasitism in sympatric Afrcan bovids." Parasitology **126**: 10.

[1] J. H. Theis and R. G. Schwab. (1992) Seasonal prevalence of Taenia Taeniaeformis: relationship to age, sex, reproduction and abundance of an intermediate host (Peromyscus maniculatus). Journal of Wildlife Disease. 28(1): 42

[1] Ezenwa, V. O. (2003). "Habitat Overlap and gastrointestinal parasitism in sympatric Afrcan bovids." Parasitology **126**: 10.

Tables

Table 1)
A Summary of Factors Affecting the Emergence of Disease in Humans, Domestic Animals and Wildlife

HUMAN EIDS	EID'S IN DOMESTIC ANIMALS	EID'S IN WILDLIFE
o Worldwide travel and commerce o Expansion of human habitation into natural areas o Climate (El Nino and Global warming) o Technological advancements (transfusions, injections) o Microbial adaptation (drug resistance, opportunistic invasion of new hosts) o Breakdown in Public Health infrastructure o Immuno-suppression (aging populations, HIV, cancer and transplant patients) o Vector range expansion o Change in husbandry practices (feeds, factory farming) o Increased surveillance	o Global expansion domestic animal ranges o Intensive farming, expansion into new rangelands o Climate (El Nino and Global warming) o Technological advancements (veterinary) o Microbial adapation (drug resistance, opportunistic invasion of new hosts) o Breakdown in Veterinary infrastructure o Increased susceptibility in intensive farming systems o Change in husbandry practices o Increased surveillance	o Introduction (accidental and planned) into new habitats o Poor or no control of international trafficking of plants and animals o Encroachment of domestic animals and people o Climate (El Nino and Global warming) o o Microbial adaptation (drug resistance, opportunistic invasion of new hosts) o Failure of Regulatory efforts to enforce hunting and fishing restrictions o Hypothesized immuno-suppression by environmental factors o Vector range expansion via domestic animal and human hosts o Crowding with habitat disruption and semi domestication o Increased surveillance

Table 2. ALL MICE. Population Characteristics overall and by park

	OVERALL			FOREST PARK			POWELL BUTTE PARK			TRYON CREEK PARK			TRNWR			OXBOW PARK		
Variables	mean	StDev.	Range	mean	StDev.	Range	mean	StDev.	Range	mean	StDev	Range	Mean	StDev	Range	mean	StDev	Range
Mass	16.5	4.15	5, 27	16.9	4.4	7, 27	17.5	3.4	7, 24.5	15	5.3	5, 26	16.7	3.6	10.5, 25	15.6	3.4	7, 22.5
Biodiversity score	0.5	.15	.28, .78	.34	.12	.28, .6	.51	.02	.45, .53	.47	.07	.35, .55	.54	.04	.48, .63	.74	.04	.7, .78
Population Density P. maniculatis	19.4	16.6	1.7, 86.3	27	13	3.8, 37	11	7.3	1.7, 22.7	22	12	10, 52	12	7	4.3, 24.5	21	28.8	4, 86
Population Density of all small mammals	42.7	33.8	6, 176	55	35	3.8, 37	50	39.8	6, 115	39	38	13, 176	26	20	7.8, 68.5	33	17	10, 64
Parasite Prevalence	.39	.11	0, .58	.46	.14	0, .58	.42	.06	.25, .48	.42	.05	.33, .46	.28	.09	0, .32	.32	.03	.27, .33
Numbers dissected	257			72			53			45			43			44		

Table 3. MATURE MICE. Population characteristics overall and by park

	OVERALL			FOREST PARK			POWELL BUTTE PARK			TRYON CREEK PARK			TRNWR			OXBOW PARK		
Variables	mean	st. dev.	range	mean	st. dev.	range	mean	st. dev.	range	mean	st. dev.	range	mean	st. dev.	range	mean	st. dev,	range
Mass	18.44	2.81	14.5, 27	19.06	2.95	14.5, 27	18.33	2.76	14.5, 24.5	18.66	3.09	15, 26	18.29	2.88	14.5, 25	17.467	2.05	15, 22.5
Biodiversity	.497	.145	.284, .777	.341	.119	.284, .598	.51	.021	.454, .531	.469	.063	.349, .55	.542	.045	.48, .633	.729	.036	.703, .777
Population Density P. Maniculatis	19.94	18.05	1.73, 86.26	27.03	12.85	3.75, 37.39	9.975	7.025	1.73, 20.45	25.62	12.48	10.39, 52.06	10.45	6.38	4.33, 24.52	27.23	33.31	4.04, 86.26
Population Density all Small Mammals	44.74	35.22	6.64. 176.25	59.81	37.63	8.08, 111.34	49.79	39.51	6.64, 115.28	18.66	37.95	19.29, 176.25	24.38	19	7.8, 68.5	34.25	17.63	18.94, 63.8
Parasite Prevalence	.401	.111	0, .583	.47	.124	0, .583	.414	.059	.25, .476	.428	.047	.333, .458	.265	.099	0, .323	.375	.067	.25, .421
Numbers dissected	188			52			46			29			31			30		

Table 4. ALL MICE Models: Crude estimates and two variable GEE models. Prevalence Ratio of parasitism in GEE models containing one or two variables tested on all dissected mice.

BASE VARIABLES	CRUDE ESTIMATES		MODELS CONTAINING BASE VARIABLE PLUS MASS		MODELS CONTAINING BASE VARIABLE PLUS BIODIVERSITY		MODELS CONTAINING BASE VARIABLE PLUS POPDENS MICE		MODELS CONTAINING BASE VARIABLE PLUS POPDENS ALL SMALL MAMMALS	
	Prevalence Ratio*	95% confidence interval	Prevalence Ratio*	95% confidence interval	Prevalence Ratio*	95% confidence interval	Prevalence Ratio*	95% confidence interval	Prevalence Ratio*	95% confidence interval
Mass	1.28	1.11, 1.49			1.27	1.096, 1.47	1.28	1.105, 1.48	1.29	1.01, 1.52
Biodiversity	.907	.826, .996	.929	.839, 1.029			.924	.831, 1.026	.920	.837, 1.011
PopDensMice	1.06	.944, 1.23	1.05	.928, 1.19	1.05	.916, 1.21			1.06	.924, 1.22
PopDensAllSmMammals	1.07	.935, 1.23	.986	.857, 1.14	1.06	.916, 1.22	1.05	.906, 1.22		

* The Prevalence ratio represents the effect of change by one standard deviation of the base variable on relative prevalence of parasites found.

Table 5. ALL MICE Models: three variable GEE models. Prevalence ratio of parasitism in GEE models with three variables tested on all dissected mice.

BASE VARIABLES	MODEL WITH MASS, BIODIVERSITY AND POPULATION DENSITY MICE		MODEL WITH MASS, BIODIVERSITY AND POPULATION DENSITY ALL SMALL MAMMALS		MODEL WITH MASS, POP DENSITY MICE AND POP DENSITY ALL SMALL MAMMALS		MODEL WITH BIODIVERSITY, POP DENSITY MICE AND POP DENSITY ALL SMALL MAMMALS	
	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals
Mass	1.27	1.1, 1.46	1.28	1.1, 1.50	1.3	1.10, 1.52		
Biodiversity	.939	.845, 1.04	.923	.819, 1.04			.93	.837, 1.033
Population density of mice	1.04	.910, 1.19			1.06	.924, 1.21	1.04	.894, 1.20
Population Density of all small mammals			.923	.836, 1.13	.97	.833, 1.13	1.05	.898, 1.22

* The Prevalence ratio reflects the effect of change by one standard deviation in the base variable on prevalence of parasites found.

Table 6. MATURE MICE ONLY Models; Crude estimates and two variable GEE models. Prevalence Ratio of parasitism for GEE models with one and two variables for the set of dissected mice including only mature mice.

BASE VARIABLES	CRUDE ESTIMATES		MODELS CONTAINING BASE VARIABLE **PLUS MASS**		MODELS CONTAINING BASE VARIABLE **PLUS BIODIV-ERSITY**		MODELS CONTAINING BASE VARIABLE **PLUS POP. DENSITY MICE**		MODELS CONTAINING BASE VARIABLE **PLUS POP. DENSITY ALL SMALL MAMMALS**	
	Prevalence Ratio*	95% Confidence Interval	Prevalence Ratio*	95% Confidence Interval	Prevalence Ratio*	95% Confidence Interval	Prevalence Ratio*	95% Confidence Interval	Prevalence Ratio*	95% Confidence Interval
Mass	1.128	.98, 1.3			1.11	.955, 1.28	1.124	.978, 1.29	1.114	.955, 1.015
Biodiversiy	.9	..833, .971	.918	.841, 1			.911	.831, .996	.917	.843, .998
PopDensMice	1.06	.928, 1.21	1.048	.922, 1.19	1.032	.913, 1.18			1.048	.919, 1.2
PopDens All Small Mammals	1.089	.955, 1.24	1.036	.895, 1.2	1	997, 1.01	1.076	.934, 1.24		

* Prevalence ratio reflects the effect of change by one standard deviation on the prevalence of parasites found.

Table 7. MATURE MICE ONLY Models: multiple variable GEE models. Prevalence Ratio of parasitism in GEE models with three and all variables on set of mature mice only of the dissected mice.

BASE VARIABLES	MODEL WITH MASS, BIODIVERSITY AND POPULATION DENSITY MICE		MODEL WITH MASS, BIODIVERSITY AND POPULATION DENSITY ALL SMALL MAMMALS		MODEL WITH MASS, POP DENSITY MICE AND POP DENSITY ALL SMALL MAMMALS		MODEL WITH BIODIVERSITY, POP DENSITY MICE AND POP DENSITY ALL SMALL MAMMALS*		FULL MODEL: MASS, BIODIVERSITY, POP DENSITY OF MICE AND OF ALL SMALL MAMMALS	
	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals	Prevalence Ratio*	95% confidence Intervals
Mass	1.102	.957, 1.28	1.098	.938, 1.28	1.04	.984, 1.1			1.098	.939, 1.28
Biodiversity	.926	.839, 1.02	.922	.837, 1.015			.923*	.837, 1.018	.927	.835, 1.029
Population density of mice	1.023	.897, 1.17			1	.995, 1.01	1.02	.89, 1.16	1.020	.892, 1.17
Population Density of all small mammals			1.015	.864, 1.192	1	.996, 1.01	1.05	.904, 1.23	1.098	.854, 1.19

* The Prevalence ratio reflects the effect of change by one standard deviation in the base variable on prevalence of parasites found.

Table 8.

SNV Prevalence in Subset of Dissected Mice

	OVERALL	FOREST PARK	POWELL BUTTE PARK	TRYON CREEK PARK	TRNWR	OXBOW PARK
All Mice	.031	.042	.019	0	.047	.045
Mature Mice	.037	.058	.022	0	.065	.033

Table 9
Calculated Aggregations for Intensity (Parasite Load)

	OVERALL	FOREST PARK	POWELL BUTTE PARK	TRYON CREEK PARK	TRNWR	OXBOW PARK
All Mice	4.73	6.23	3.94	2.74	2.82	4.53
Mature Mice	4.67	6.28	3.83	2.93	2.9	3.59

[ii] Yates, T. L., J. N. Mills, et al. (2002). "The ecology and evolutionary history of an emergent disease: Hantavirus Pulmonary Syndrome." BioScience **52**(11): 10.

[iii] Personal communication

[iv] personal communication

[v] personal communication

[vi] Mills, J. N., T. G. Ksiazek, et al. (1999). "Long-Term Studies of Hantavirus Reservoir Populations in the Southwestern United States: A Synthesis." Emerging Infectious Diseases **5**(1): 8.

[vii] Netski, D., B. H. Thran, S. C. St Jeor. (1999) Sin Nombre Virus pathogenesis in Peromyscus maniculatus. J of Virology 73(1): 585

[viii] O'Connor, C. S., J. P. Hayes and S. C. St Jeor. (1997) Sin Nombre virus does not impair respiratory function of wild deer mice. J of Mammology 78(2): 661

[ix] Taylor, L. H., S. M. Latham, et al. (2001). "Risk factors for human disease emergence." Phil. Trans. R. Soc. Lond. B **356**: 7.

[x] Ibid

[xi] ibid

[xii] Daszak, P., A. Cunningham, et al. "Emerging Infectious Diseases of Wildlife--Threats to Biodiversity and Human Health." Science's Compass.

[xiii] Walters, M. J. (2006). "Do No Harm." Conservation in Practice **7**(4): 6.

[xiv] Daszak, P., A. A. Cunningham, A. D. Hyatt. (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78: 103

[xv] Anderson, R. M. and R. M. May (1982). "Coevolution of hosts and parasites." Parasitology **85**: 16.

[xvi] ibid

[xvii] May, R. M., S. Gupta, et al. (2001). "Infectious disease dynamics: what characterizes a successful invader?" Phil. Trans. R. Soc. Lond. B **356**: 10.

[xviii] Rudolf, V. H. W. and J. Antonovics (2005). "Species Coexistence and Pathogens with Frequency-Dependent Transmissions." The American Naturalist **166**(1): 6.

[xix] Menotti-Raymond, M. and S. J. O'Brien (1993). "Dating the Genetic Bottleneck of the African Cheetah." Proceedings of the National Academy of Sciences **90**: 5.

[xx] Daszak, P., A. A. Cunningham, et al. (2001). "Anthropogenic environmental change and the emergence of infectious diseases in wildlife." Acta Tropica **78**: 14.

[xxi] Ibid.

[xxii] Ibid.

[xxiii] Dobson and Foufopoulos, 2001

[xxiv] Daszak, P., A. A. Cunningham, et al. (2001). "Anthropogenic environmental change and the emergence of infectious diseases in wildlife." Acta Tropica **78**: 14.

[xxv] Hahn, B. H., G. M. Shaw, et al. (2000). "AIDS as a Zoonosis: Scientific and Public Health Implications." Science **287**: 9.

[xxvi] Riley, S. P. D., J. Hadidian, et al. (1998). "Population density, survival, and rabies in raccoons in an urban national park." Canadian Journal of Zoology **76**: 11.

[xxvii] Rupprecht, C. E., J. S. Smith, et al. (1995). "The Ascension of Wildlife Rabies: A cause for Public Health Concern or Intervention?" Emerging Infectious Diseases **1**(4).

[xxviii]

[xxix] Ostfeld, R. S. and F. Keesing (2000). "Biodiversity and disease risk: the case of Lyme disease." Conservation Biology **14**: 6.

[xxx] Yates, T. L., J. N. Mills, et al. (2002). "The ecology and evolutionary history of an emergent disease: Hantavirus Pulmonary Syndrome." BioScience **52**(11): 10.

[xxxi] Altiser, S., C.L. Nunn, et al. (2003) "Social Organization and Parasite Risk in Mammals: Integrating Theory and Empirical Studies." Annu. Rev. Ecol. Evol. Syst. 34:517

[xxxii] Engelthaler, D. M., D. G. Mosley, et al. (1999). "Climatic and Environmental Patterns Associated with Hantavirus Pulmonary Syndrome, Four Corners Region, United States." Emerging Infectious Diseases **5**(1): 8.

[xxxiii] Ostfeld, R. S. and F. Keesing (2000). "Biodiversity and disease risk: the case of Lyme disease." Conservation Biology **14**: 6.

[xxxiv] Buskirk, J. V. and R. S. Ostfeld (1998). "Habitat heterogeneity, dispersal, and local risk of exposure to Lyme disease." Ecological Applications **8**(2): 14.

, Dobson, A. and J. Foulopoulos (2001). "Emerging Infectious pathogens of wildlife." Phil. Trans. R. Soc. Lond. B **356**: 1001-1012.

[xxxv] Ezenwa, V. O. (2004). "Parasite infection rates of impala (Aepyceros melampus) in fence game reserves in relation to reserve characteristics." Biological Conservation **118**: 5.

[xxxvi] Ezenwa, V. O. (2003). "Habitat Overlap and gastrointestinal parasitism in sympatric Afrcan bovids." Parasitology **126**: 10.

[xxxvii] Anderson, D. R., K. P. Burnham, et al. (1983). "Density Estimation of Small-mammal Populations using a trapping web and distance sampling methods." Ecology and Genetics of Host-Parasite Interactions **64**(4): 7.

[xxxviii] Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers and L. Thomas. (2001) Introduction to Distance Sampling. New York: Oxford University Press xv+432 pp

[xxxix] King, J. A. (1968). "Biology of Peromyscus (Rodentia)." Society of Mammologists Special Publication **special publication #2**.

[xl] Personal communication

[xli] Ostfeld, R. S. and F. Keesing (2000). "Biodiversity and disease risk: the case of Lyme disease." Conservation Biology **14**: 6.

Ezenwa, V. O. (2003). "Habitat Overlap and gastrointestinal parasitism in sympatric Afrcan bovids." Parasitology **126**: 10.

[xlii] J. H. Theis and R. G. Schwab. (1992) Seasonal prevalence of Taenia Taeniaeformis: relationship to age, sex, reproduction and abundance of an intermediate host (Peromyscus maniculatus). Journal of Wildlife Disease. 28(1): 42

[xliii] Ezenwa, V. O. (2003). "Habitat Overlap and gastrointestinal parasitism in sympatric Afrcan bovids." Parasitology **126**: 10.