Effect of Species Richness on Disease Risk

Dilution Effect and Underlying Mechanisms

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Table of Contents

Chapter 1: General Introduction

Infectious zoonotic diseases transmitted between human, wildlife and domestic animals have important impacts on livestock economies, wildlife conservation and public health [1]. In Southeast Asia alone, for examples, it has been estimated that highly pathogenic avian influenza (H5N1) virus outbreaks caused the death of 140 million domestic birds with economic losses at $10 billion [2]. After 1889, the introduced rinderpest virus rapidly reduced the ungulates in African savanna to 20% of their original abundance [3,4]. Ebola hemorrhagic fever has been considered as a big threat to African ape populations [5]. It has been estimated that infectious diseases are the most important threat to human health, responsible for 25% of worldwide mortality [6]. Over the last decades, about 20,000–30,000 cases of Lyme disease have been reported annually in USA and the average annual numbers of cases in Europe and Asia have been estimated at 65,467 and 3,450, respectively [7]. Although the epidemiology of human and livestock diseases are relatively well-studied [8], the epidemiology and ecology of wildlife diseases or human/livestock diseases with wildlife-human/livestock interface are largely unknown. In order to evaluate the risk of infectious diseases and take successful prophylactic measures, a clear understanding of the driving forces of the dynamics of these diseases is required.

Pathogen transmission can be affected by many biotic (e.g., host density, vector density etc.) and abiotic factors (e.g., climate etc.) [9,10]. Among these factors, host species richness has attracted much recent attention, because of interest in identifying and evaluating utilitarian functions of biodiversity [11,12]. Current studies argue that high species richness reduces risks of infectious diseases via a hypothesized 'dilution effect' [13,14,15], which presents an exciting convergence of public health concern and biodiversity conservation [11,14,16]. Although the dilution effect has been reported in many different diseases [17,18], its generality and mechanisms are still under active debate [11,12,19,20]. In this thesis, I contribute to a better understanding of the effect of species richness on disease risk by testing the dilution effect hypothesis and exploring the underlying mechanisms.

Mathematical models and host population size (or host density)

Mathematical host-pathogen models are able to generate conceptual understanding of disease dynamics [21]. Simplified host-pathogen models of disease dynamics occur in many forms [21]. The basic and well-known host-pathogen model is SIR (susceptible-infected-recovered) model and the transmission dynamics can be described as differential equations (Fig 1.1).

From the compartmental models, we can see that host abundance (host density for a consistent area) plays an important role in determining disease dynamics. For density-dependent transmitted diseases, host population size directly determines infection rate (dI/dt, Fig 1.1), and thus the infection prevalence. Take an example, the infection prevalence of *Phytophthora ramorum* in forest communities in western coasts

of USA was positively correlated with the density of its competent hosts, bay laurel and tanoak [22]. In addition, for density-dependent transmissions, there exists a threshold host population size under which the pathogen cannot invade the population [23]. Increasing host population size is able to increase the chances of pathogen invasion and disease outbreak [24]. Herd size or cattle density, for an example, is consistently identified to correlate with the probability of occurrence of bovine tuberculosis at herd level [25,26]. In frequency-dependent transmissions, infection rate is independent of host population size (Fig 1.1) and thus a high host population size cannot lead to a higher infection prevalence [24]. However, increasing host population size is capable of increasing the total number of infected hosts, since the number of infection is the product of infection prevalence and host population size.

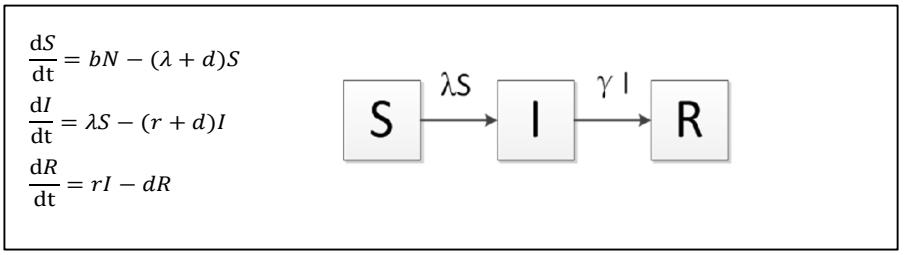

Figure 1.1: Structure of a classical SIR compartmental model. *N* represents the host population size. Hosts can be susceptible (*S*), infected (*I*) or recovered/immune (*R*). *b* and *d* respectively represent birth rate and mortality. Infected hosts can recover from an infection at a recovery rate *r*. The per capita transmission rate is determined by the force of infection, λ. The parameter *β* is the intraspecific transmission rate. For density-dependent transmission (Box 1), λ equals *βI*, whereas for frequency-dependent transmission, λ equals *βI*/*N*.

Box 1: Some definitions

Density-dependent transmission: transmission in which the number of new infections per unit time is proportional to the product of the density of infected hosts and the density of susceptible hosts.

Frequency-dependent transmission: transmission in which the number of new infections per unit time is proportional to the product of the density of infected hosts and the proportion (or frequency) of hosts that are susceptible.

Basic reproductive rate: R_0, the expected number of secondary cases caused by the first infectious individual in a wholly susceptible population

Generalist parasite: parasites that have been recorded as infecting more than one Family of related species.

Specialist parasite: parasites that had been recorded only from species within a single Family of related species.

Reservoir competence: a species' potential to support and transmit pathogens.

Zoonosis: an infectious disease which can be transmitted from animals to humans.

Infection prevalence: the proportion of a population found to have the infection.

Threshold population for invasion: the minimum host population size required for a disease to be able to invade a host population successfully.

Dilution effect and its mechanisms

Many pathogens are able to infect more than one host species, and these host species can differ strongly with respect to their abilities to support and transmit pathogens due to differences in their immune systems [27,28] or co-evolutionary histories with pathogens [27,29]. Also, host-pathogen interactions can be affected by the presence of interacting species, including predators, competitors, etc. within the

community [30,31]. Therefore, community structure and composition can largely influence the dynamics of infectious diseases [18,31].

With great pressures on nature resources and increasing global biodiversity loss, ecologists are reinforcing incentives for biodiversity conservation by outlining the ecosystem services it provides [32]. One of the ecosystem services could be protection against diseases [33]. This basic idea arose from 'zooprophylaxis' within the discipline of malaria epidemiology [34]. Zooprophylaxis describes the active use of livestock animals to divert vector (mosquito) bites away from humans to protect against malaria infection [35]. Then, Ostfeld and colleagues [36,37,38] reformulated this idea in their studies on Lyme disease and introduced 'dilution effect' with a restrictive definition referring to a community where higher species richness supports a greater fraction of low competency hosts, which leads to an increase in "waste" encounter events (between infected vectors and incompetent hosts) [17]. Now, the concept of 'dilution effect' has been extended and is used to describe the net effect of species diversity or species richness reducing disease risk by a variety of different mechanisms, because the restrictive definition leaves many mechanisms by which species diversity reduces disease risk undefined [17,31]. The dilution effect has been reported in a wide range of infectious diseases such as Lyme disease [39,40], West Nile encephalitis [41], Hantavirus pulmonary syndrome [42,43,44], schistosomiasis [45], trematode parasites [15,46] and so on [18]. However, it is still highly disputed whether the dilution effect generally occurs [11,12,19,20]. These critical studies suggested that the dilution effect is more likely idiosyncratic and applies only under certain circumstances [11,19].

Table 1.1: Mechanisms that can give rise to a dilution effect from changes in species richness.

Mechanism	Definition
Encounter reduction	Reduction in the rate of encounters between susceptible and infected hosts or between susceptible hosts and infected vectors.
Transmission reduction	Reduction in the probability of transmission of pathogen from infected hosts to susceptible hosts or vectors
Susceptible host regulation	Reduction in the abundance of susceptible hosts
Vector regulation	Reduction in density of infected vectors
Recovery augmentation	Faster disease recovery rate among infected hosts

By using standard infectious disease models in directly transmitted parasite and vector-borne infection, Keesing and colleagues [31] outlined five hypothetical mechanisms (Table 1.1) through which changes in species richness could influence infection risk. Among them, two mechanisms, encounter reduction and host regulation, have been well proved [17,41]. Encounter reduction describes the process that high species richness can reduce the encounter rates among competent hosts or between vectors and competent hosts by changing host behaviours, host home range and so on [31]. For examples, to test the dilution effect in Sin Nombre virus (SNV), Clay et al. monitored intra and interspecific encounters of deer mice (competent host for SNV) in the Great Basin Desert and found that higher species diversity reduced intraspecific contact rate between deer mice. This might because that the presence of other species may change the foraging behaviour of deer mice and force them to forage in less desirable areas [47]. Hass et al. found that incompetent host species in higher-diversity plant communities could dilute the transmission of *Phytophthora ramorum* since those incompetent hosts can act as physical barriers to pathogen spread and interfering with transmission pathways among competent hosts, bay laurel and tanoak [22]. Host regulation refers to that incompetent hosts in communities with high species richness can reduce the density or abundance of competent hosts by competition or predation, and thus reduce

disease risk [31]. For Lyme disease in USA, the density of competent host, white-footed mouse, is usually high in low-diversity communities due to the lack of competition, which leads to an increased disease transmission [28,39,48]. However, whether other mechanisms can cause a dilution effect is still conjectural or lack evidence from experimental and field studies [17].

Predictions of the dilution effect in mathematical models

The classical compartmental models for multi-host have been used to investigate the generality of the dilution effect [49,50,51].

$$\lambda_1 = \beta_{11} I_1 + \beta_{21} I_2 \qquad \text{(E 1.1)}$$

$$\lambda_1 = (\beta_{11} I_1 + \beta_{21} I_2)/(N_1 + N_2) \qquad \text{(E 1.2)}$$

Equation (E 1.1) describes the force of infection in a two-host system with density-dependent transmission, whereas equation (E 1.2) refers to frequency-dependent transmissions. Subscripts present different host species, a competent host species 1 and an incompetent host species 2. β_{11} is the intraspecific transmission rate, whereas β_{21} is the interspecific transmission rate (from host 2 to host 1). Using classical SIR models, Dobson investigated the effect of species richness on disease risk in both density-dependent and frequency-dependent transmission [49]. He found that the dilution effect is more likely to occur in frequency-dependent transmitted diseases while increasing host species richness always leads to increased R_0 (as a measure of disease risk, Box 1) in diseases with density-dependent transmission. These results are easy to understand. In frequency-dependent transmissions, adding an incompetent host species can increase the total host population (denominator in E 1.2), which may reduce the force of infection for the competent host and thus reduce disease risk. Whereas adding incompetent host species always increases the force of infection for the competent host (E 1.1) in density-dependent transmissions if incompetent hosts cannot change the density or behaviour of the competent host. However, if incompetent species is also able to reduce the density of competent hosts (e.g. through competition or predation) or change the behaviour of the competent host (e.g. reduce home range or foraging area of the competent host) in density-dependent transmissions, the dilution effect may also operates via mechanism 'host regulation' and 'encounter reduction', respectively [51].

Host heterogeneity in pathogen transmission

Species usually differ in their competence to support and transmit pathogens due to differences in their immune systems [27,28] or co-evolutionary histories with pathogens [27,29]. For many pathogens only a small fraction of host species is responsible for the majority of transmission [27]. Thus, knowing driving forces determining host competence for pathogens is beneficial to screen diverse host species to identify their roles in pathogen transmission [28,52].

Ecologists have begun to search for explanations for host heterogeneity, and linked species' reservoir competence to life-history traits [52,53]. Life-history theory generally suggests trade-offs between investment in self-maintenance (e.g., physiological resistance) and future reproduction [54]. The predictions derived from this theory suggest that short-lived hosts (i.e. species that usually have higher reproductive rates and smaller body sizes) tend to invest minimally in adaptive immunity [55,56], which may make them more competent for pathogens. Indeed, several studies have shown that lower specific

immune defence level or higher host reservoir competence could be related to higher fecundity [57], shorter developmental period [55], higher metabolic rate [52] and lower body mass [58].

On the other hand, studies linked species' local extinction risk to life-history traits. For example, fast-lived species generally have a higher reproduction rate and a higher population density, and therefore can recover more quickly from disturbances and thus experience lower local extinction risk [59,60]. Therefore, a species' reservoir competence and its local extinction risk might be negatively correlated and explained by similar underlying life-history traits. In this way, when species richness declines, species with higher competence for pathogens are more likely to remain in the community, and thereby increase the risk of transmitting these pathogens. This could be a causal mechanism underlying the dilution effect. However, before this thesis, few studies combined a species' local extinction risk, reservoir competence and life-history traits in their analyses.

Environmental and climatic factors related to pathogen transmission

Pathogen transmission can be conceived as the interactions between hosts, vectors (for vector-borne diseases) and pathogens [9]. Any environmental or climatic factor that affects any aspect of them (i.e. abundance, behaviour, longevity etc.) may have a potential to influence the dynamics of pathogen transmission. From a host perspective, changes in climate and environment can modify host distribution, host abundance and host community composition, and thus change transmission dynamics [9]. Extreme climate conditions can reduce host population and thus decrease disease risk, whereas increased habitat fragmentation can sometimes lead to a high concentration of host populations in small remaining fragments [37,39]. Environmental stresses may reduce immunity performance and make hosts more vulnerable to pathogen infection [61]. In addition, host habitat structure and topography can also influence the transmission dynamics through affecting host movements and dispersals [9,62,63]. From a pathogen perspective, climatic and environmental factors may even influence the persistence of the pathogens that can persist in the environment outside of either hosts or vectors. For example, studies have shown that *Mycobacterium bovis* (pathogenic agent for bovine tuberculosis) can survive longer under relatively colder and moister conditions [64,65]. For vector-borne diseases, climate and environmental factors that influence vector density, distribution and activity are able to affect the dynamics of pathogen transmission [9,66]. Previous studies showed that tick abundance, as an important predictor for the transmission risk of many tick-borne diseases, can be largely determined by climate and environment conditions [67,68,69]. Since these environmental and climatic factors might confuse the effect of species richness on disease risk, they must be taken into account to better understand the driving forces of pathogen transmission and disease risk.

Thesis outline

The main aim of this thesis was to investigate the effect of species richness on disease risk. Especially, I tested the current disputed hypothesis, the dilution effect hypothesis, and studied the underlying mechanisms. To achieve this, different approaches (spatial epidemiological, phylogenetic analyses and theoretical modelling) were used in a disease ecological framework.

In chapter 2, I test the dilution effect in bovine tuberculosis (BTB), an influential disease caused by *Mycobacterium bovis* and mainly spreads via aerosol transmission which is usually described as density-dependent transmission [70]. In Africa, BTB can infect a wide range of domestic and wildlife mammals,

and these mammals vary in their abilities to support and transmit *M. bovis*. Therefore, changes in mammal species richness may affect BTB risk. Using a spatial epidemiological approach and the data of BTB outbreaks provided by the World Organisation for Animal Health (OIE), I examine the effect of mammal species richness on BTB presence, as well as whether other factors are associated with BTB presence.

In chapter 3, I extend the detection of the dilution effect to the persistence and recurrence of BTB. Spatial pathogen dynamics has several distinct epidemiological phases including new establishment in a region, persistence, fade-out and recurrence (Fig. 1.2). In BTB, a few studies have indicated that the patterns of different phases might be driven by different determinants [70,71]. Improving understanding of the epidemiological processes underlying different BTB phases can lead to more effective control strategies and better targeted surveillance measures. Therefore, in this chapter, we test the dilution effect of mammal species richness and examine which other factors were associated with the persistence and recurrence of BTB in cattle.

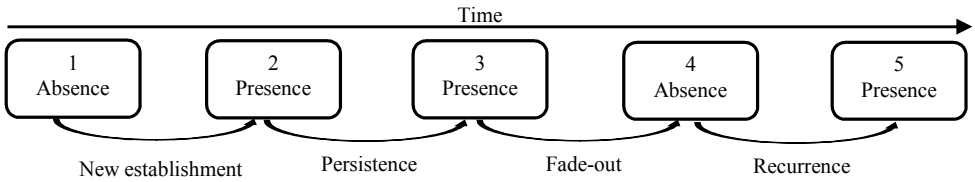

Figure 1.2: Illustration of different epidemiological phases. The period and BTB presence/absence status for an area are given within the rectangles. We assume no infection before the first period.

Next, I test the relationship between species' life-history traits and reservoir competence (as a measure of species' potential to support and transmit pathogens) in chapter 4. Considering species' local extinction risk can also be explained by life-history traits, understanding the relationships between these life-history traits and reservoir competence in a community is essential to better understand and predict how changes in species richness affect pathogen transmission in communities. The hypothesis, originated from the life-history theory, is that species' life-span is negatively correlated with reservoir competence. According to this hypothesis, species with low reservoir competence go extinct first with increasing species loss, whereas more competent hosts are more likely to remain in the communities and increase disease risk. Therefore, this hypothesis would be a central mechanism for the dilution effect.

In chapter 5, I conduct a modelling study to investigate the effect of connectivity on disease risk in metapopulations. Since species loss is often driven by habitat fragmentation [72], it is necessary to take habitat structure (connectivity) into account to get a better understanding of roles that fragmentation plays on pathogen transmission. In fragmented habitats, higher connectivity may dilute disease risk (dilution effect) via increasing species richness, or increase disease risk (facilitation effect) through increasing contact rates among patches. Therefore, we assume that the net impact of connectivity (fragmentation) is dependent on the relative importance of these two opposite effects.

Finally, chapter 6 reviews the key finding of previous chapters and ties them together in a discussion of the evidence and critiques for the dilution effect. Ultimately, suggestions are made for the future studies.

Chapter 2

Dilution Effect in Bovine Tuberculosis: Risk Factors for Regional Disease Occurrence in Africa

Zheng Y.X. Huang, Willem F. de Boer, Frank van Langevelde, Chi Xu, Karim Ben Jebara, Francesco Berlingieri and Herbert H.T. Prins

Changes in host diversity have been postulated to influence the risk of infectious diseases, including both the dilution and amplification effects. The dilution effect refers to a negative relationship between biodiversity and disease risk, whereas the amplification effect occurs when biodiversity increases disease risk. We tested these effects with an influential disease, bovine tuberculosis (BTB), which is widespread in many countries and causing severe economic losses. Based on the BTB outbreak data in cattle from 2005-2010, we also tested, using generalized linear mixed models, which other factors were associated with the regional BTB presence in cattle in Africa. The interdependencies of predictors and their correlations with BTB presence were examined using path analysis. Our results suggested a dilution effect, where increased mammal species richness was associated with reduced probability of BTB presence after adjustment for cattle density. In addition, our results also suggested that areas with BTB infection in the preceding year, higher cattle density, and larger percentage of area occupied by African buffalo were more likely to report BTB outbreaks. Climatic variables only indirectly influenced the risk of BTB presence through their effects on cattle density and wildlife distribution. Since most studies investigating the role of wildlife species on BTB transmission often only involve single species analysis, more efforts are needed to better understand the effect of the structure of wildlife communities on BTB dynamics.

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Introduction

Bovine tuberculosis (BTB), which is a chronic disease caused by *Mycobacterium bovis* and mainly spreads via aerosol transmission [70], not only infects a wide range of domestic and wildlife mammals, but also humans [73]. Although control programs have eliminated or nearly eliminated this disease from domestic animals in some developed countries, BTB is still widespread in Great Britain, Ireland, New Zealand and many developing countries, especially in Africa [74,75]. In fact, this zoonotic disease is still an important public health concern and can cause severe economic losses due to livestock death and trade restrictions [75].

Africa has the highest species richness of mammals [76] and many of these mammal species can be infected by *M. bovis*. Previous studies have been carried out to investigate the roles that wildlife species play on the dynamics of BTB transmission [75,77]. Wildlife hosts are usually classified as either maintenance hosts (such as African buffalo, *Syncerus caffer* and maybe also greater kudu, *Tragelaphus strepsiceros*), spillover hosts or dead-end hosts [74,75]. However, studies investigating the role of wildlife species often only involved single species analysis and neglected the effects of multiple hosts and community structure on the transmission of BTB [75].

Host diversity has been postulated to influence the risk of infectious diseases [31,78]. The effect of biodiversity on disease dynamics has attracted much current attention in the context of global biodiversity loss and increased emergence of infectious diseases [13,15,18,79]. In theory, changes in species richness or diversity in communities can lead to a dilution effect or amplification effect by changing the abundance of competent hosts or altering the encounter rates among competent hosts in a community [31]. The dilution effect, which suggests a negative relationship between biodiversity and disease risk, occurs when the incompetent host species are more likely to be present in high-diversity communities rather than in low-diversity communities. Species which are first lost from a community tend to be those that are less competent hosts [79], ultimately leaving a higher abundance of more competent species in low diversity systems due to release from competition or predation, and thereby increase the risk for disease transmission. On the contrary, the amplification effect occurs when there is a positive correlation between disease risk and species diversity. Even though mounting evidence of the dilution effect has been reported in many different diseases [18], whether the dilution effect generally occurs is still highly disputed [11,15].

Since the ability to transmit *M. bovis* varies among different mammal species, we expect that differences in mammal species diversity are probably able to affect the dynamics of BTB transmission. We assume that higher mammal species richness may provide more transmission pathways for *M. bovis*, thus facilitate the spread of the disease. On the other hand, higher mammal species richness may exert a dilution effect and lower BTB disease risk by increasing the abundance of incompetent hosts, which are able to interfere with the pathways of transmission or act as sinks for *M. bovis* and deflect BTB transmission away from the cattle. Here, we examine these two alternative hypotheses referring to the effect of mammal species richness on BTB risk in Africa.

Besides wildlife species, many other factors have identified to be responsible for facilitating BTB transmission [70,75]. At individual level, the prevalence of tuberculosis-like lesions has been found to increase with age in cattle and buffalo [80,81]. Different breeds of cattle also experience different risk of BTB [82]. At herd level, factors such as herd size and previous infection status have been identified to correlate with the probability of positive reaction to BTB test [25,26]. Cattle movements and purchase of

cattle have also been identified to facilitate BTB transmission [83,84,85,86]. However, the influence of risk factors on the dynamics of BTB transmission at larger scales, such as regional scale, is not well understood. A regional analysis of the dynamics of disease transmission can promote the understanding of the epidemiological process underlying the infection pattern, and might lead to important suggestions for regional control [87]. Moreover, studies on the influence of risk factors tend to concentrate mainly on industrialized countries, whereas the epidemiology of BTB in the developing world, especially in Africa, remains largely unknown [75]. Therefore, the present study aims to test whether the dilution or amplification effect of mammal species richness operates on BTB transmission, and examine the key factors associated with the regional BTB presence in cattle in Africa.

Materials and methods

Data collection and pre-processing

The data set of the BTB presence/absence in cattle in Africa from 2005 to 2010 was provided by the World Animal Health Information Database (WAHID) from the World Organisation for Animal Health (OIE). During this period, some countries reported the presence of BTB only at country-level, whereas other countries specified the presence/absence of BTB at a lower administrative level. The lowest administrative level of reporting was used as the level of analysis in this study. Only administrative areas that reported presence or absence in more than 2 consecutive years were used in the analyses. The compiled data set has 1355 rows of BTB presence/absence data covering 15 countries and 304 administration areas over the years 2005-2010 (Table S 2.1). Per year, 27.7% (± 11.40 % SD) of the administrative areas reported the presence of BTB.

Mammal species richness, which was defined as the total number of mammal species present in an administrative area, was calculated based on the geographical distribution of African mammals obtained from the African Mammal Databank (AMD), an atlas of medium to large mammals [88]. For each mammal species, the AMD includes two polygon coverage files respectively describing the distribution of suitable habitat and the distribution of species occurrence at a 1 x 1 km resolution [88]. The "actual distribution" for each species was calculated as the intersection of these two distribution maps [89]. Since the distribution data of small mammals are usually unreliable [90] and also small mammals are less often involved in BTB transmission in Africa, only species with an average body mass $\geq$ 2 kg from the AMD were used in the analysis. The presence (1) or absence (0) of each species was recorded in each administrative area using the "actual distribution" calculated from the AMD. The presence-absence data were compiled to calculate the total number of species, or species richness of each administrative area.

Other influencing variables were categorized into biotic and abiotic variables (Table 2.1). For biotic variables, cattle density and previous infection status have been linked to BTB risk at herd level in previous studies [25,85]. We tested whether these effects also influenced the probability of BTB presence at regional scale. Human population density was also used as an biotic predictor variable, as human population density could be considered as an indicator of trade activity [91], which has been proven to be positively related to BTB disease risk [83,84]. Besides, since maintenance hosts can maintain the pathogen in the community without any other species, and are able to play important roles in disease persistence, transmission and spread [74,77], we used the percentage of the area occupied by African buffalo and greater kudu as predictor variables to test the effects of these two maintenance host species. We calculated the mean cattle density (CattleD), the mean human population density (HumanD) and the percentage of the area occupied by African buffalo (Buffalo) or greater kudu (Kudu) for each

administrative area. We also collected information on the infection status in the preceding year (PreInf); if BTB presented in the preceding year, PreInf was specified as 1, otherwise, it was reported as 0. Since BTB disease can be transferred to a neighbouring area from an infected area through the borders they share, we calculated, for each administrative area, the percentage of the border that was shared with neighbouring infected areas in the preceding year (BorPre).

Table 2.1 Description and summary (mean ± SD) of the predictors used in the analysis, with unit, year and their predicted effects.

description of data sets	Abbreviation	predicted effect	unit	year	mean ± SD
biotic variables					
previous infection status	PreInf	positive	no unit	2005 - 2009	
mammal species richness	MSR	negative	no unit	1999	49.70 ± 29.23
cattle density	CattleD	positive	km^{-2}	2005	13.55 ± 18.70
human population density	HumanD	positive	km^{-2}	2006	334.5 ± 925.7
percentage of the border shared with previous infected areas	BorPre	positive	no unit	2006 - 2010	70.9 ± 35.0
percentage of area occupied by buffalo	Buffalo	positive	no unit	1999	36.3 ± 40.9
percentage of area occupied by kudu	Kudu	positive	no unit	1999	6.6 ± 20.1
abiotic variables					
annual mean temperature	TemMean	negative	ºC	1950 - 2000	23.91 ± 4.29
mean precipitation in driest month	PreDry	negative	mm	1950 - 2000	6.86 ± 8.41
mean annual aridity index	Aridity	negative	no unit	1950 - 2000	0.61 ± 0.34
interaction variables					
cattle density * mammal species richness	CattleMSR	negative	km^{-2}		
buffalo*mammal species richness	BufMSR	negative	no unit		
Kudu*mammal species richness	KuduMSR	negative	no unit		

Because *M. bovis* is associated with several wildlife species [74,77], environmental factors that can influence the distribution of wildlife populations may also play significant roles in disease transmission [64,91]. We assume that a lower mean precipitation in the driest month might be correlated with a higher probability for animals to assemble at water resources or under tree shaded areas [92,93], which can increase BTB transmission. Since previous studies have shown that *M. bovis* can survive longer under relatively colder conditions [92], we expect that lower annual mean temperature might increase the probability of the persistence of BTB. Therefore, the mean annual temperature (TemMean), the mean precipitation in the driest month (PreDry) and the mean annual aridity index (Aridity) were also calculated for each administrative area as abiotic predictor variables.

In addition, three interaction terms, mammal species richness associated with the three maintenance host species (cattle density, percentage of area occupied by African buffalo and greater kudu), were taken into account to test the prediction that higher mammal species richness might weaken the effects of the

maintenance hosts due to the dilution effect. The data for all predictor variables were acquired from existing databases (Table S 2.2). All data pre-processing analyses were conducted in ArcGIS 10.0.

Statistical analysis

Generalized linear mixed models with binary response (GLMM, logistic regression models) were used to examine the effects of predictors on the probability of BTB presence both for the whole period and for each year. Only the BTB presence/absence data from 2006 to 2010 were used as dependent variable, because the earliest year in the dataset was 2005, which was used as previous infection status (PreInf) for 2006. Before performing the GLMMs, we log-transformed MSR, Buffalo [log (Buffalo+0.5)], Kudu [log (Kudu+0.5)] and BorPre [log (BorPre+0.5)] to obtain distribution closer to normal distribution. Using GLMMs with country and year as random factors (in analyses for each year, only country was used as random factor), univariate analyses were first performed to identify the potential risk factors. The area of the unit (Area) was retained in the model as an obligate variable to correct for the effect of area size. Variables with a p-value of <0.25 were identified as potential risk factors which were used to construct multiple regression models. Before fitting the multiple regression models, we assessed the multi-collinearity by examining the variance inflation factor (VIF) of the candidate variables. The results of multi-collinearity test suggested little collinearity among variables. For analysis of the whole period, we constructed the final multiple model using both forward and backward selection procedures, where the Likelihood Ratio Test (LRT) was applied to test for difference in the fit of the nested models. The final model for the whole period was then fitted to each individual year to check for consistency of the effects of the predictors. After fitting the multiple regression models, we tested the spatial autocorrelation of the residuals using Moran's I index and found little evidence for spatial autocorrelation (Table S 2.3). In GLMMs with binary response, fully standardized regression coefficient (b^*_M) was used to compare the impacts of different variables [94].

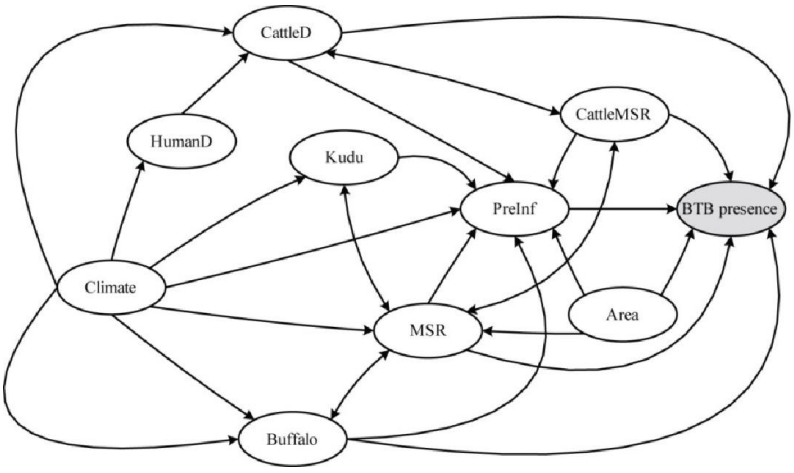

Figure 2.1: Conceptual path model of the potential relationships between risk factors and BTB presence.

Based on the results of the final multiple GLMM, a regression-based path analysis was conducted to examine the interdependencies of the predictors and their direct and indirect effects on BTB presence [95]. A recursive conceptual path model was constructed as Figure 2.1. Besides the variables TemMean, PreDry and Aridity, the mean annual precipitation (PreMean) and the mean temperature in the hottest

month (TemMax) were also included as climatic variables because they could be considered as measures for the suitability for human, cattle and wildlife. To reduce the climatic variables, we first conducted a factor analysis to extract the primary components. The final parsimonious path model was constructed based on W-statistic and Chi-squared test [96]. Again, the area of the unit (Area) was always retained in the model to correct for the effect of area size. Path coefficients were calculated also using fully standardized (logistic) regression coefficient to compare the strength of the effects on continuous and binary endogenous variables [94]. All statistical analyses were conducted in R 2.14.0 with appropriate packages.

Results

Regression analysis

The results of univariate models (Table 2.2) suggested that areas reporting BTB outbreak in the preceding year had a higher probability of reporting BTB occurrence than the areas without infection during the year before. As we predicted, a higher probability to report BTB presence was related to a higher cattle density (CattleD), a higher percentage of the border shared with neighbouring infected areas in the preceding (BorPre) and a higher occurrence of buffalo (Buffalo) and kudu (Kudu). Other factors did not show any significant relationships with BTB presence in the univariate analysis. The results of multi-collinearity test showed that the VIFs for all risk factors were smaller than 5, which indicated little collinearity among variables.

Table 2.2: Summary of the univariate analyses of risk factors associated with BTB presence in Africa for the whole period 2006-2010. (VIF is the variance inflation factor for scale variables)

variables	predicted effect	b	*p*	VIF
PreInf	positive	2.756	<0.001***	
CattleD	positive	0.022	<0.001***	1.32
Buffalo	positive	0.822	<0.001***	2.48
MSR	negative	-0.464	0.399	3.78
Kudu	positive	1.253	0.006**	1.89
HumanD	positive	0.000	0.750	1.20
BorPre	positive	0.462	<0.001***	1307
TemMean	negative	0.000	0.989	1.69
PreDry	negative	-0.023	0.113	3.12
Aridity	negative	-0.025	0.49	4.26

*** $p< 0.001$; ** $p< 0.01$; * $p<0.05$

Variables with a p-value of <0.25 were identified as potential risk factors which were used to construct multiple regression models. Using this criteria, 6 out of 10 variables, namely PreInf, CattleD, BorPre, Buffalo, Kudu and PreDry, were identified as potential predictors. In addition, in order to test the dilution effect of mammal species richness, we also included three interaction terms (Table 1) and mammal species richness (MSR) to construct the multiple regression models. Backward and forward selection procedures generated a similar final multiple regression model. This final model (Table 2.3) showed that the interaction of cattle density and mammal species richness (CattleMSR) was the only interaction term

which had a significant effect (b^*_M = -0.88; p = 0.001). The negative regression coefficient of CattleMSR indicated that the positive effect of cattle density was, as predicted, weaker under higher mammal species richness than under lower mammal species richness. The final model also identified previous infection status (PreInf), cattle density (CattleD), and the percentage of the area occupied by African buffalo (Buffalo) as significant risk factors in the prediction of regional BTB presence, all with positive regression coefficients as we predicted (Table 2). Cattle density (CattleD) had the strongest effect, with a b^*_M = 0.93 (p < 0.001). In addition, our analyses also identified that country was a significant random factor (χ^2= 70.5, p <0.001). The predictive accuracy of the final multiple model is 86.8% by using a cut-off of 0.5.

Table 2.3: Multiple regression analyses of risk factors associated with the probability of BTB presence in Africa for both the whole period 2006-2010 and each year. (b^*_M is the fully standardized regression coefficient)

year		**whole period**		**2006**	**2007**	**2008**	**2009**	**2010**
variables	**predict effect**	b^*_M	*p* - value	b^*_M	b^*_M	b^*_M	b^*_M	b^*_M
Area	positive	0.04	0.175	-0.08	-0.05	0.26*	-0.00	0.00*
PreInf	positive	0.37	0.000***	0.42***	0.36***	0.52***	0.37***	0.38***
CattleD	positive	0.93	0.000***	0.31	1.99***	1.64**	1.55*	1.56*
Buffalo	positive	0.21	0.000***	0.42***	0.15*	0.22	-0.04	0.05*
MSR	negative	-0.19	0.053	-0.26	-0.004	-0.14	0.02	0.02
CattleMSR	negative	-0.88	0.001***	-0.25	-1.97***	-1.63**	-1.62*	-1.61**

*** p <0.001; ** p <0.01; * p <0.05

The results of the regression analyses (Table 2.3) for each year identified that previous infection status (PreInf) was a strong predictor for the probability of BTB presence for all years. The interaction of cattle density and mammal species richness (CattleMSR) was negatively associated with BTB presence from 2007 to 2010. The results also suggested that cattle density (CattleD) showed a significant positive correlation with the probability of BTB presence for almost all years except for 2006. Only in 2006, 2007 and 2010, the percentage of area occupied by Buffalo (Buffalo) was identified to be positively associated with BTB presence (Table 2.3).

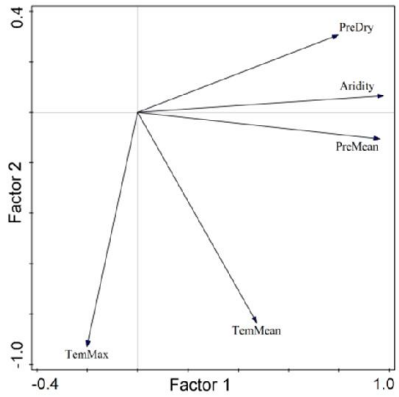

Figure 2.2: The results of the factor analysis for climatic variables. PreMean, PreMin and Aridity were heavily loaded on Factor 1, while TemMean and TemMax were heavily loaded on Factor 2

Path analysis

The results of factor analysis (Fig. 2.2) for the climatic variables showed that the first two component axes, Factor 1 and Factor 2, respectively explained 55.6% and 33.4% of the variation in climatic variables. PreMean, PreMin and Aridity were heavily loaded on Factor 1, whereas TemMean and TemMax were heavily loaded on Factor 2 (Fig. 2.2). A higher Factor 1 score was related to larger precipitation values while a higher Factor 2 score was associated with lower temperatures.

The path analysis confirmed the GLMM results that the interaction term CattleMSR had a negative total effect on BTB presence while previous infection status (PreInf), cattle density (CattleD) and the percentage of area occupied by buffalo (Buffalo) had positive total effects (Table 2.4, the final parsimonious path model is shown in Figure S 2.1). The human density (HumanD), the percentage of area occupied by kudu (Kudu), climatic Factor 1 and Factor 2 all showed positive indirect effects on BTB presence. Larger precipitation and lower temperatures were correlated to a higher risk of BTB presence through their effects on cattle density and the distribution pattern of wildlife (Figure S 2.1).

Table 2.4: Summary of the effects of predictors on the risk of BTB presence in path analysis

variables#	direct effect	indirect effect	total effect
Area	0	-0.04	-0.04
PreInf	0.37	0	0.37
CattleD	0.88	0.27	1.15
Buffalo	0.21	0.08	0.29
MSR	0	-0.11	-0.11
CattleMSR	-0.88	-0.24	-1.12
Kudu	0	0.09	0.09
HumanD	0	0.18	0.18
Factor 1	0	0.45	0.45
Factor 2	0	0.10	0.10

Variables are the area of the units (Area), previous infection status (PreInf), cattle density (CattleD), percentage of the area occupied by buffalo (Buffalo) and Kudu (Kudu), mammal species richness (MSR), human density (HumanD) and two climatic component factors (Factor 1 and Factor 2).

Discussion

Our study identified several significant risk factors that are correlated to the probability of BTB presence at regional scale in Africa. The results show that the positive effect of cattle density became weaker with increasing mammal species richness. Administration areas with previous BTB infection, higher cattle density and larger percentage of area occupied by buffalo were more likely to report BTB outbreaks. Climatic variables only indirectly influenced BTB presence through their effects on cattle density and wildlife distribution. In addition, the results show that the variation in BTB presence was partly explained by the country, a random factor.

For density-dependence disease systems, which are usually used to describe the transmission dynamics of direct-transmitted or aerosol-borne diseases, a threshold host density or a critical community size is required for successful pathogen establishment or pathogen persistence [23]. A higher cattle density

implies a higher contact rate of susceptible and infectious host individuals [97], which contributes to the persistence and spread of *M. bovis* [24]. Therefore, the probability of BTB presence is higher when cattle density increases.

A significant interaction between cattle density and mammal species richness was shown in our study. The positive effect of cattle density on BTB presence becomes weaker when mammal species richness increases. Since BTB presence rather than prevalence was used as the response variable, the negative interaction between cattle density and mammal species richness suggests that a higher cattle density is needed for the establishment and persistence of BTB when mammal species richness is higher [23]. Therefore, this result indicates that mammal species richness is able to dilute, rather than amplify, the impacts of cattle as maintenance host on the risk of BTB presence. This dilution effect of biodiversity on disease dynamics has attracted much attention in the context of global biodiversity loss and increased emergence of infectious diseases [13,15,18,78,79]. However, these studies investigating the biodiversity-disease relationships usually focus on vector-borne diseases or direct-transmitted plant diseases, while few studies were carried out on aerosol-borne or direct-transmitted animal diseases, except for hantavirus [18]. Our study detected, for the first time, the dilution effect of mammal species richness on the risk of BTB presence, an influential aerosol-borne disease.

Two main mechanisms have been proposed for the dilution effect. One is “encounter reduction” where the addition of alternative hosts may interfere with transmission pathways and reduce encounter rates between susceptible hosts and infected hosts, and the other is “susceptible host regulation” where interspecific competition or predation may limit the abundance of competent hosts [31]. The dilution effect we found in BTB, indicated by the negative interaction between mammal species richness and cattle density, can be possibly explained by the “encounter reduction”. Previous studies suggested that transmission becomes more frequency-dependent when local transmission is integrated together across spatial scales [98]. In BTB, although many wildlife species can become infected, most of them act as spillover or dead-end hosts, and transmit the pathogen inefficiently [74,77]. The presence of these incompetent hosts might reduce the contact rates among herds by acting as barriers to herd movement, and thus increase the threshold host density and critical community size. Therefore, a higher cattle density is needed for the establishment and persistence of BTB in areas with higher mammal species richness, which suggests an interactive effect between mammal species richness and cattle density as found in our study. Since we did not find the direct negative effect of mammal species richness on BTB presence, we cannot draw the conclusion whether “susceptible host regulation” operates in BTB in Africa. Certainly, we could not exclude the possibility that the dilution effect we detected is just caused by the correlations between mammal species richness and some unidentified factors that we did not include in our analyses. For example, areas with higher mammal species might be nature reserves or national parks, where fences are frequently used to prevent the encounter between wildlife species and livestock. These fences can also interfere with cattle movement and thus lead to a pseudo dilution effect. This certainly needs further research, and ideally experiments.

Our study also suggests that the previous infection status is a strong predictor in determining the probability of BTB presence. Being consistent with previous studies [85,99], our result indicates that BTB tends to occur repeatedly in the same area [85]. This result might be attributed to the endemicity of BTB in some areas. The difficulty in diagnosis in the early stage of the disease [92] and consistently failed control efforts [99] might also contribute to disease persistence. This result suggests that much more efforts should be made to control this infectious disease in those areas which experience BTB outbreaks.

We found that the percentage of area occupied by buffalo was a predictor for BTB presence for the whole period. As a maintenance host, African buffalo plays an important role in BTB transmission. Since African buffalo can remain infected and infectious for several years and transmit the pathogen through aerosol transmission [74], this widespread species could limit the efficiency of control measures. The movements of buffalo might also facilitate the spread of the disease, though few studies investigated this issue. Therefore, when a larger percentage of the area is covered by African buffalo, cattle will have more opportunities to come into contact with African buffalo, and thereby be more likely to experience a BTB outbreak. This result also coincides with previous studies in which the disease risk of BTB or the persistent of BTB in cattle were identified to be associated with other maintenance hosts, such as brushtail possum (*Trichosurus vulpecula*) in New Zealand [25], Eurasian badger (*Meles meles*) in UK and Ireland [26,100] and lechwe antelope (*Kobus leche*) in the Kafue basin of Zambia [73]. In addition, we did not find a significant direct effect of another maintenance host, the greater kudu. This might be attributed to the more limited distribution of greater kudu in Africa (13.2% administrative areas are occupied or partly occupied by greater kudu compared to 55.3% by African buffalo).

Although other variables, such as quality of veterinary service or used control measures, could not be taken into account because of lack or incompleteness of the data, the random factor country used in the analyses is capable of controlling, to some extent, for the variation caused by these country-level variables. We admit that conclusions are usually not easy to be drawn from large spatial scale studies because of the complexity of the natural environment and the difficulty of controlling confounding factors [101]. The quality of the BTB presence/absence data, especially the extent, the resolution and the accuracy, limit the precision and generality of our results. However, it is still an important step that our study was able, for the first time, to test for the importance of factors on BTB presence at regional level in Africa. Our study showed that the factors that play an important role in BTB transmission at herd level, like previous infection status and cattle density, might also be able to significantly influence BTB disease dynamics at regional level. In addition, despite the presence of many incompetent hosts in areas with higher mammal species richness, little evidence was found that these hosts facilitate pathogen transmission and spread the disease. On the contrary, increasing mammal species richness was correlated with lower chances of BTB presence in interaction with increasing cattle densities. Due to the limitations of our BTB data set, this dilution effect could not be tested at lower spatial levels in our study. Further research is needed to fully reveal the effect of mammal species richness on BTB transmission over different spatial scales. Since most studies investigating the role of wildlife species on BTB disease transmission often only involve single species analysis, it is worth increasing efforts to better understand the effect of structure of the wildlife community on BTB transmission [75].

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Supplementary Information 2

Table S 2.1: The names of the countries (with their country codes and the number of administrative areas) and years of the BTB presence data used in Chapter 2 and Chapter 3.

Country name	Country code	Years	Number of areas	Chapter 3
Angola	AGO	2005-2006	18	
Benin	BEN	2005-2010	77	Y
Cote D'Ivoire	CIV	2005-2007	49	
Cameroon	CMR	2005-2009	10	Y
Egypt	EGY	2005-2010	26	Y
Ghana	GHA	2005-2010	10	Y
Lesotho	LSO	2005-2010	10	
Morocco	MAR	2008-2010	7	Y
Mozambique	MOZ	2005-2010	10	Y
Malawi	MWI	2005-2010	3	Y
Nigeria	NGA	2005-2010	37	
Togo	TGO	2005-2010	5	Y
Tunisia	TUN	2005-2010	24	Y
South Africa	ZAF	2006-2010	9	Y
Zambia	ZMB	2006-2010	9	Y

Table S 2.2: Formats and sources of the original data for the variables we used in Chapter 2 and Chapter 3

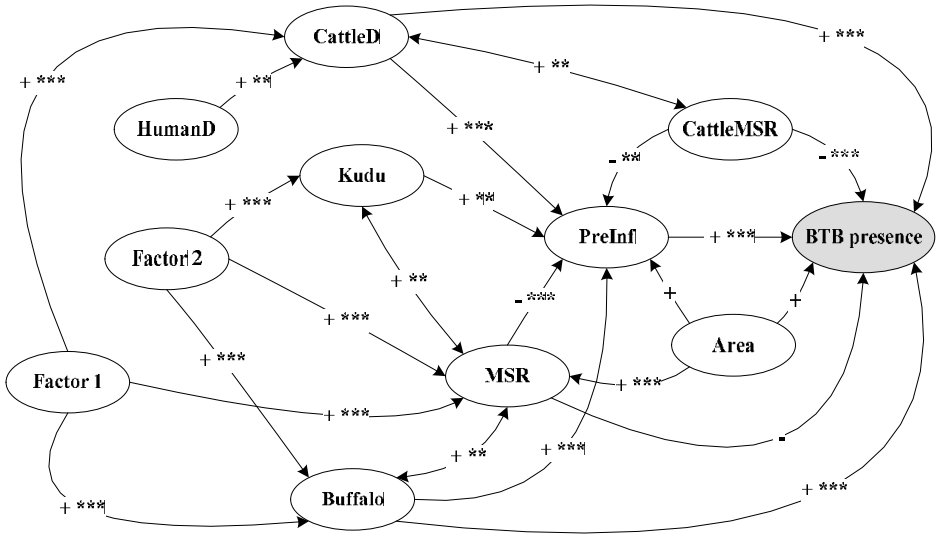
Datebase	Format	Variables	Data source	Web site links
Cattle density	raster	CattleD	FAO	http://www.fao.org/geonetwork/srv/en/metadata.show?id=12713
Human density	raster	HumanD	NASA	http://sedac.ciesin.columbia.edu/data/set/gpw-v3-population-density
BTB outbreaks	binary	PreInf, BorPre	OIE	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Mammal species distribution	polygon	MSR, Bufflao, Kudu	AMD	http://www.gisbau.uniroma1.it/amd/homepage.html
Mean temperature in each month	raster	TemMean TemMax	WorldClim	http://www.worldclim.org/current
Mean precipitation in each month	raster	PreDry PreMean	WorldClim	http://www.worldclim.org/current
Mean annual aridity index	raster	Aridity	CGIAR-CSI	http://csi.cgiar.org/aridity/
time-series datasets of variations in climate	raster	TemMean, TemMax, TemMin, ATemRng, BTemRng, RainMean, RainMin, RainCV, PreTemMean, PreRainMean, PreTemMax, PreRainMin	CRU	http://badc.nerc.ac.uk/view/badc.nerc.ac.uk__ATOM__dataent_1256223773328276

Table S 2.3: Moran's I values of residuals for the test of spatial autocorrelation in the final model both for the whole period and for each year. For the model of whole period, we calculated the Moran's I values also for different scales.

Year	Final model for whole period				Final model for each year
Distance (k km)	global (0 - 8)	0 – 0.5	0-2	0-4	global (0 - 8)
2006	0.047***	0.035*	0.047**	0.042**	0.019*
2007	-0.009	0.011	-0.014	-0.003	-0.013
2008	-0.013	-0.012	-0.021	-0.009	-0.021
2009	-0.025	-0.071	-0.023	-0.015	-0.022
2010	-0.018	-0.061	-0.022	-0.010	-0.015

* $P < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Figure S 2.1: Final parsimonious path model and related risks. Arrows indicate the direction of the paths and the signs the direction of the associated coefficients.



Note:

Variables related to BTB presence, the area of the units (Area), previous infection status (PreInf), cattle density (CattleD), percentage of the area occupied by buffalo (Buffalo) and Kudu (Kudu), mammal species richness (MSR), human density (HumanD) and two climatic component factors (Factor 1 and Factor 2).

* $P < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Chapter 3

Dilution Effect and Identity Effect by Wildlife in the Persistence and Recurrence of Bovine Tuberculosis in Cattle

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Current theories on disease-diversity relationships predict a strong influence of host richness on disease transmission. In addition, identity effect, caused by the occurrence of particular species, can also modify disease risk. We tested the richness effect and the identity effects of mammal species on bovine tuberculosis (BTB), based on the regional BTB outbreak data in cattle from 2005-2010 in Africa. Besides, we also tested which other factors were associated with the regional BTB persistence and recurrence in cattle. Our results suggested a dilution effect, where higher mammal species richness was associated with reduced probabilities of BTB persistence and recurrence in interaction with cattle density. African buffalo had a positive effect on BTB recurrence and a positive interaction effect with cattle density on BTB persistence, indicating an additive positive identity effect of buffalo. The presence of greater kudu had no effect on BTB recurrence or BTB persistence. Climatic variables only act as risk factors for BTB persistence. In summary, our study identified both dilution effect and identity effect of wildlife and showed that BTB persistence and recurrence were correlated with different sets of risk factors. These results are relevant for more effective control strategies and better targeted surveillance measures in BTB.

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Introduction

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* is widespread in many countries, especially in Africa, and has caused significant economic losses [75]. Previous studies have identified various risk factors for BTB transmission at different levels, such as herd size, previous infection status and cattle movements at herd level [75,102]. Increasing evidence suggests that wildlife species play an important role in BTB dynamics as well [74,75,77,103,104]. This finding invokes further research efforts especially on multi-species transmission in livestock-wildlife interactions, as studies on the role of wildlife species often only involved single-species transmission [75,105].

It has been hypothesized that greater species diversity is able to reduce pathogen transmission, i.e. the dilution effect [13,15,31,50,106]. This dilution effect can operate through different mechanisms, such as decreasing encounter rates among competent hosts or regulating host abundance [31]. Although the dilution effect has been found in many different disease systems, it is still highly disputed whether the dilution effect generally occurs [79,106,107,108]. In Africa, many mammal species can be infected by *M. bovis*, and these species have been classified as maintenance hosts (such as African buffalo, Syncerus caffer), spillover hosts or dead-end hosts [74,77]. Since the competence to transmit *M. bovis* varies among different species, we expect that mammal species diversity can affect BTB transmission. We hypothesized that higher mammal species richness may trigger a dilution effect and reduce BTB risk by increasing the abundance of incompetent hosts which are able to interfere with the BTB transmission pathways. On the other hand, there might also be an identity effect, implying that the occurrence of particular species in the community changes the disease risk, either positively or negatively [109]. In this study, therefore, we tested the identity effect of buffalo, considering that the occurrence of maintenance host species might increase BTB risk because of their high competence in transmitting *M. bovis*. In addition, since studies have not reached an agreement on the role of greater kudu (Tragelaphus strepsiceros) in BTB transmission [74,75], we also tested for an identity effect of this species.

A previous study tested the dilution effect and found that mammal species richness was negatively associated with the probability of BTB presence [105]. However, spatial pathogen dynamics has several distinct epidemiological phases including new establishment in an area, persistence, fade-out and recurrence, and the patterns of different phases might be driven by different determinants [71,102]. Therefore, we tested the dilution effect as well as the identity effect in the persistence and recurrence of BTB and compared the differences in these two distinct phases. In addition to wildlife species, other factors are also responsible for facilitating BTB transmission, such as cattle density, the status of 'neighbourhoods', climate [75]. However, since studies usually investigated the effects of these factors at herd level and relatively fewer studies focus on regional scale [75], we also tested whether these variables act as risk factors for BTB risk at regional scale. The present study aimed at testing the dilution effect as well as the identity effects of two wildlife species (African buffalo and greater kudu) in the persistence and recurrence of BTB in cattle. We also examined which other factors were associated with BTB persistence and recurrence and tested whether they are associated with different sets of predictors.

Materials and methods

BTB data

Data of BTB in cattle in Africa from 2005 to 2010 were provided by the World Animal Health Information Database [110] from the World Organisation for Animal Health (OIE), which reported the status of BTB at

the administrative level every six months during this period. The lowest administrative level of reporting was used as the level of analysis in this study. Only countries with BTB outbreak histories were included in the analyses, since we focused on BTB recurrence and persistence. The compiled data set has 879 rows covering 11 countries and 190 administration areas (Table S 2.1).

We classified a disease fade-out if BTB was not reported in two consecutive reporting periods (therefore one year) following a presence. This criterion has been used in previous studies to identify BTB persistence and fade-out [111,112]. Therefore, a BTB presence in a specific period was considered as BTB recurrence if BTB was not reported in the administrative areas in the previous two reporting periods. BTB persistence was classified if a BTB presence was reported within a year (i.e. within maximally two 6-months reporting periods).

Table 3.1: Descriptions, abbreviations, units and summaries (mean ± SD) of the predictors used in the analysis.

Description of data sets	Abbreviation	Unit	Mean ± SD
Cattle density	CattleD	km^{-2}	13.6 ± 18.7
Mammal species richness	MSR	1	49.7 ± 29.2
Percentage of area occupied by buffalo	Buffalo	no unit	36.3 ± 40.9
Percentage of area occupied by kudu	Kudu	no unit	6.6 ± 20.1
Percentage of the border shared with previous infected areas	BorPre	no unit	70.9 ± 35.0
Climatic predictors			
Annual mean temperature	TemMean	ºC	24.9 ± 4.1
Mean temperature of the warmest month	TemMax	ºC	35.2 ± 3.4
Mean temperature of the coldest month	TemMin	ºC	14.9 ± 7.1
Temperature annual range	ATemRng	ºC	20.4 ± 6.8
Temperature bi-annual range	BTemRng	ºC	21.4 ± 7.0
Annual mean precipitation	RainMean	mm	84.0 ± 47.4
Mean precipitation in driest month in current year	RainMin	mm	3.1 ± 5.5
Annual coefficient of variation for precipitation	RainCV	no unit	0.94 ± 0.26
Mean temperature in preceding year	PreTemMean	ºC	24.8 ± 4.1
Mean precipitation in preceding year	PreRainMean	mm	82.6 ± 45.8
Mean temperature of the warmest month in preceding year	PreTemMax	ºC	35.2 ± 3.4
Mean precipitation in driest month in preceding year	PreRainMin	mm	2.8 ± 5.1
Interaction terms			
Cattle density * mammal species richness	CattleMSR	km^{-2}	
Cattle density * buffalo	CattleBuff	km^{-2}	
Cattle density * Kudu	CattleKudu	km^{-2}	

Wildlife data

Mammal species richness (MSR) were calculated from the African Mammal Databank (AMD), an atlas of medium to large mammals [88]. For each mammal species, the AMD includes two polygon coverage files respectively describing the distribution of suitable habitat and the distribution of species occurrence at a 1 x 1 km resolution [88]. The intersection of these two distribution maps was calculated as the "actual distribution" for each species [89]. Since small mammals are less often involved in BTB transmission in Africa, only species with an average body mass ≥ 2 kg from the AMD were used in the analysis. The presence or absence of each mammal species was recorded in each administrative area and were compiled to calculate the species

richness of each administrative area. Using species distribution maps from the AMD, the percentage of the area occupied by African buffalo (Buffalo) and greater kudu (Kudu) were calculated for each of the administrative areas to test the identity effects of these two species.

Other predictors

Many studies have identified that cattle density as an important predictor for BTB outbreaks [85,105,113,114,115]. The BTB status of 'neighbourhoods' might also play an important role in explaining and predicting BTB risk [116]. In addition, previous studies also indicated that climate can substantially influence BTB dynamics through the influence on the survival of *M. bovis* [64,65,75,113]. Therefore, we also included these variables to test their effect on BTB persistence and recurrence at regional scale.

From the Food and Agriculture Organization (FAO), we collected the data of cattle density in 2005, which had a resolution of 0.05 degree. We calculated the mean cattle density for each administrative area. To investigate the effect of 'neighbourhood', the percentage of the border that was shared with neighbouring infected areas in the preceding year (BorPre) was calculated based on the BTB presence/absence data from OIE. Twelve climatic variables (Table 3.1) representing temperature and precipitation conditions were calculated based on the Climate Research Unit (CRU) datasets [117]. CRU time-series datasets yield month-by-month variations in climate from 1900 to 2010. These are calculated per (0.5x0.5 degree) grid cell, compiled from an archive of monthly mean temperatures provided by more than 4000 weather stations distributed around the world. In addition, to investigate the effect of wildlife-livestock interactions, we included the interaction terms between cattle density and three wildlife variables, i.e., mammal species richness, areas occupied by buffalo and kudu, as potential predictors. The data for all predictor variables were acquired from existing databases (Table S 2.2). All data pre-processing analyses were conducted in ArcGIS 10.0.

Statistical analyses

Generalized linear mixed models (GLMM) with a binary response were used to examine the effects of predictors on BTB persistence and recurrence. In addition, country was included in the models as the random factor to control for possible differences between countries, because of the lack or incompleteness of the data of veterinary service and used control measures. Before performing the GLMMs, we log-transformed, log(x+0.5), Buffalo, Kudu and BorPre.

Using GLMMs, two approaches were applied to investigate the associations between predictors and BTB persistence or recurrence. First, we used a stepwise selection approach to construct a final multiple regression model. The area of the unit was retained in the model to correct for the effect of area size. We initially included cattle density in the model, since this predictor was shown to have significant impacts on both BTB persistence and recurrence. Then, other variables were added into the model by a standard-entry stepwise procedure, using a Likelihood Ratio Test (LRT) to test for the difference in the fit of the nested models. For highly correlated independent variables, only the one causing the largest change in the Log-Likelihood (LL) was added to the model to avoid multi-collinearity. We included interaction terms after including all main factors. Main terms were maintained in the model if they were included in a significant interaction term. Second, a 'removal approach' based on multiple regression frameworks [2,118] was used to test for the consistency of results obtained via the stepwise selection approach. In this approach, all variables, except for those causing multi-collinearity, were forced in the model. Then, the coefficient and the change in -2LL were estimated on the removal of each independent variable. We tested for the spatial autocorrelation of the

residuals (final models in stepwise selection approach and full models in removal approach) using Moran's I index and found little evidence of spatial autocorrelation for the models (Table S 3.1). The whole statistical process was conducted in R 2.15.1 with appropriate packages.

Results

Descriptive epidemiology

The regional prevalence of BTB was 36% during the study period (Table 3.2). The percentage of areas experiencing recurrence of BTB was 14%, and the percentage of areas with a BTB fade-out was 22% (persistence percentage was thus 88%).

Table 3.2: Infection prevalence, percentages of persistence and recurrence of bovine tuberculosis (BTB) in the African administrative areas during 2006-2010.

Year	No. of pathogen free area in preceding year	No. of areas with recurrence	Recurrence rate	No. of infected area in the preceding year	No. of areas with fade-out	Fade-out rate	Infection prevalence
2006	112	18	16 %	53	14	26 %	32 %
2007	114	18	16 %	60	11	18 %	34 %
2008	115	18	16 %	65	13	20 %	36 %
2009	110	14	13 %	70	21	30 %	39 %
2010	108	13	12 %	72	10	14 %	40 %
total	559	81	14 %	320	69	22 %	36 %

Table 3.3: Summary statistics (regression coefficient b ± SE; Adjusted Odds Ratio, AOR and 95% CI, calculated from the first and third quartiles, and p-value) for the predictors correlated with BTB persistence using a stepwise selection procedure and a removal approach (some non-significant variables in the removal approach were not listed).

Variables	Stepwise selection approach			Removal approach		
	b ± SE	AOR (95% CI)	p – value	b ± SE	Δ -2LL	p – value
CattleD	0.11± 0.03	7.54 (2.07 - 27.42)	0.002**	0.14±0.06	5.93	0.014*
PreTemMax	-0.25 ± 0.08	0.64 (0.45 - 0.87)	0.004**	-0.23±0.12	3.38	0.066
PreRainMin	-0.12 ± 0.05	0.64 (0.44 - 0.92)	0.019*	-0.16±0.06	7.11	0.008**
MSR	0.03 ± 0.02	3.29 (0.45 - 24.03)	0.241	0.02±0.03	0.26	0.613
Buffalo	-0.65 ± 0.41	0.24 (0.04 - 1.42)	0.116	-1.01±0.52	4.07	0.043*
CattleMSR	-0.004 ± 0.001	0.12 (0.03 - 0.44)	0.001***	-0.005±0.002	7.84	0.005**
CattleBuff	0.08 ± 0.03	3.15 (1.43 - 6.94)	0.005**	0.09±0.03	7.55	0.006**

* P< 0.05; ** p < 0.01; *** p < 0.001

Risk factors for BTB persistence

Both the stepwise selection approach and removal approach showed that cattle density was positively correlated to BTB persistence, while the interaction of cattle density and mammal species richness was negative (Table 3.3). Only one of the investigated maintenance hosts, buffalo, played a significant role in

BTB persistence, as indicated by the positive interaction with cattle density in both approaches. Removal approach also detected a positive main effect of buffalo. In addition, the mean precipitation of the driest month in the preceding year (PreRainMin) showed a negative relationship with BTB persistence in both approaches, and the mean temperature of the warmest month (PreTemMax) was negatively correlated with BTB persistence in the stepwise selection approach.

Risk factors for BTB recurrence

For BTB recurrence, the stepwise selection approach and removal approach generated similar results (Table 3.4). In contrast to BTB persistence, BTB recurrence was found to be correlated with a different set of predictors (Tables 3.3 and 3.4), highlighted by the positive main effect of the area occupied by Buffalo and the absence of climatic effects for BTB recurrence. Similar with BTB persistence, cattle density had a positive effect on BTB recurrence and there was a significant negative interaction between cattle density and mammal species richness.

Table 3.4: Summary statistics (regression coefficient b ± SE; Adjusted Odds Ratio, AOR and 95% CI, calculated from the first and third quartiles, and p-value) for the predictors correlated with BTB recurrence using a stepwise selection procedure and a removal approach (some non-significant variables in the removal approach were not listed).

Variables	Stepwise selection approach			Removal approach		
	b ± SE	AOR (95% CI)	*p* - value	b ± SE	Δ -2LL	p – value
CattleD	0.059 ± 0.016	2.47 (1.49 - 4.09)	< 0.001***	0.076 ±0.030	7.54	0.006**
MSR	0.006 ± 0.015	1.11 (0.65 - 1.88)	0.686	0.006 ±0.02	0.09	0.762
Buffalo	0.68 ± 0.20	4.44 (1.87 - 10.6)	< 0.001***	0.67 ±0.24	7.34	0.007**
CattleMSR	-0.0013 ± 0.0005	0.38 (0.18 - 0.77)	0.007**	-0.0018±0.0007	7.19	0.007**

* P< 0.05; ** p < 0.01; *** p < 0.001

Discussion

Despite large efforts to investigate the influences of wildlife on BTB transmission, the understanding of the impacts of livestock-wildlife interactions and wildlife community structure remains limited [74,75,77]. Here, we demonstrated that wildlife species play a substantial role in both the persistence and recurrence of BTB, and moreover, that these two distinct phases are correlated with different sets of risk factors.

In line with previous studies [85,113], our results showed that a higher cattle density facilitates both persistence and recurrence of BTB. This can be explained by the manner of BTB transmission, which is mainly through aerosol transmission [74,102], a density-dependent transmission [105]. Increasing host densities can lead to higher encounter rates of susceptible and infectious hosts, thereby promoting persistence and spread of the pathogen [24,111].

Recently, the influence of species diversity on disease dynamics has attracted great interest [31]. A well-known hypothesis is described as the “dilution effect”, which states that high species diversity can reduce disease risk [31]. The negative interaction between cattle density and mammal species richness in our study indicates the occurrence of a dilution effect in this BTB disease system. High mammal species richness can reduce the positive effect of cattle density on both BTB persistence and recurrence. This dilution effect is possibly explained by “encounter reduction”, which is described as that the addition of alternative hosts may

decrease the risk of pathogen transmission by reducing encounter rates between susceptible and infected hosts [31]. Many mammal species that can be infected by BTB are spillover or dead-end hosts and do not transmit the pathogen efficiently [74,77]. The presence of these non-competent wildlife species might act as barriers to herd movement of cattle and reduce encounter rates among herds, which leads to decreased probabilities of BTB persistence and recurrence in cattle. A few previous researches indicated that herbivores may forage in limited areas in the presence of other species, especially predators [119]. However, the understanding of effects of wildlife on livestock movements is still lacking and need more studies. In addition, we cannot exclude the possibility that the dilution effect we found might be just caused by some other predictors correlating with mammal species richness. For example, higher mammal species might occur in nature reserves where fences are frequently used to prevent the contact between wildlife and livestock. These fences are also able to interfere with cattle movements and thus lead to a 'pseudo' dilution effect.

Buffalo was found to have a positive identity effect on both BTB persistence and recurrence in cattle. The positive interaction between buffalo and cattle density in BTB persistence indicates that the positive effect of cattle density on BTB persistence was amplified under high buffalo occurrence. Theoretical studies suggested that the probability of pathogen persistence is indeed positively related to host density [24]. As maintenance host, buffalo can increase the effective encounter rates among cattle herds by frequently contacting different cattle herds, especially because they have similar habitat and dietary requirements, and thereby increase the effect of cattle density on pathogen persistence. For BTB recurrence, the positive association between buffalo and BTB recurrence might be caused by buffalo that spread the pathogen from infected regions to pathogen-free regions. Also, as a reservoir species, buffalo could preserve the pathogen and transmit it to cattle in the regions without cattle infection, triggering BTB recurrence in cattle. Previous studies suggested that maintenance hosts are more likely to remain in the community with decreased biodiversity [46,79], which indicated that the positive effect of maintenance host might also be underlay by the dilution effect. In this study, however, we did not find a significant negative correlation between mammal species richness and buffalo occurrence, suggesting the independence of the identity effect from the dilution effect. For greater kudu, neither BTB persistence nor recurrence was correlated with the presence of greater kudu, which might indicate that greater kudu is not a maintenance host for *M. bovis*.

Climate plays a role in the epidemiology of BTB through affecting the survival of *M. bovis* [64,65]. Extreme temperatures and precipitation (i.e., mean temperature of the warmest month and mean precipitation in driest month in the preceding year) were found to be correlated with BTB persistence. This is probably because hot and dry weather precludes long-time survival of *M. bovis* [64,65,75,113]. However, we did not find any significant relationship between climatic variables and BTB recurrence, which indicates that climate might exert little effect on pathogen re-establishment in cattle at this scale of analysis.

In summary, our study showed that wildlife substantially influences regional patterns of BTB persistence and recurrence. Mammal species richness was negatively correlated with BTB persistence and recurrence, suggesting the existence of a dilution effect, while buffalo as the maintenance host species had a positive identity effect. Our study also underlies the relevance of different ecological/climatic factors in determining BTB risk, and suggests that BTB persistence and recurrence are correlated with different sets of risk factors. These results are relevant for more effective control strategies and better targeted surveillance measures in BTB.

Acknowledgements

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Supplementary Information 3

Table S 3.1: Moran's I values of the test of spatial autocorrelation of the residuals in the final models of stepwise selection approach and full models of removal approach. We calculated the Moran's I values over different spatial scales.

	BTB persistence		BTB recurrence	
Distance (k km)	Stepwise selection	Removal approach	Stepwise selection	Removal approach
0 - 0.5	-0.014	-0.009	-0.006	-0.005
0 - 2	-0.021	-0.015*	-0.002	-0.003
0 - 4	-0.017	-0.012	-0.001	-0.0007
0 - 6	-0.016	-0.021	-0.0003	-0.0002
0 - 8 (global)	-0.016	-0.010	-0.0003	-0.001

* $P < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Chapter 4

Species' Life-history Traits Explain Interspecific Variation in Reservoir Competence: a Possible Mechanism Underlying the Dilution Effect

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Hosts species for multi-host pathogens show considerable variation in the species' reservoir competence, which is usually used to measure species' potential to maintain and transmit these pathogens. Although accumulating research has proposed a trade-off between life-history strategies and immune defences, only a few studies extended this to host species' reservoir competence. Using a phylogenetic comparative approach, we studied the relationships between some species' life-history traits and reservoir competence in three emerging infectious vector-borne disease systems, namely Lyme disease, West Nile Encephalitis (WNE) and Eastern Equine Encephalitis (EEE). The results showed that interspecific variation in reservoir competence could be partly explained by the species' life histories. Species with larger body mass (for hosts of Lyme disease and WNE) or smaller clutch size (for hosts of EEE) had a lower reservoir competence. Given that both larger body mass and smaller clutch size were linked to higher extinction risk of local populations, our study suggests that with decreasing biodiversity, species with a higher reservoir competence are more likely to remain in the community, and thereby increase the risk of transmitting these pathogens, which might be a possible mechanism underlying the dilution effect.

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Introduction

Diseases caused by multi-host pathogens are able to impact livestock productivity, agricultural economies, wildlife conservation and public health [120]. For many infectious multi-host pathogens, different host species, or even co-occurring host species in the same community, exhibit pronounced variation in their abilities to serve as reservoirs or transmit the pathogens [27,121]. Therefore, it is a major concern to better understand the dynamics of disease transmission, especially at community level, and the impact of differences in reservoir competence on infection risk [121].

Reservoir competence is usually used to measure a species' potential to serve as a reservoir for pathogens and transmit pathogens [122,123,124]. Recently, ecologists have begun to search for explanations for the interspecific variation in reservoir competence in the ecology and life histories of species [121,125]. Life history theory generally suggests trade-offs with investment in self-maintenance (e.g., physiological resistance) at the expense of other physiological activities, such as current reproduction and growth [54]. The predictions derived from this theory suggest that "fast-lived" species (i.e. species that follow a strategy aimed at growth and early reproduction) tend to invest minimally in adaptive immunity [55,56], which may make them more competent for pathogens [13], whereas "slow-lived" species with longer life spans and slower growth rates are hypothesized to invest more into costly immune defences. Several studies have shown that specific immune defence level could be related to life-history traits, such as fecundity [57] and developmental period [55]. However, only a few studies extended this trait-based approach to examine the relationships between the hosts' life-history traits and the potential to transmit pathogens (but see Cronin et al. [121]). Better understanding these relationships could help us to predict the species' reservoir competence and model disease dynamics at community level, which is relevant for human health, economic growth and wildlife conservation [120,121,125].

In this paper, we present a quantitative study relating life-history traits to the variation in species' reservoir competence for three vector-borne diseases: one tick-borne disease, Lyme disease and two mosquito transmitted diseases, West Nile Encephalitis (WNE) and Eastern Equine Encephalitis (EEE). We used the reservoir competence index (RCI) as a measure of the species' reservoir competence, which is considered to be a function of several epidemiological parameters, namely host susceptibility (probability of a host becoming infected by infected vectors), host infectivity (probability of a vector becoming infected, when feeding on an infected host), and duration of infectiousness (number of days that a host remains infectious) [28,121,122,124]. For species life-history traits, we used body mass, incubation time (gestation time for mammals), and clutch size (litter size for mammals). Incubation time and clutch size have been linked to the species' immune response [55], while body mass can serve as a surrogate for size-scaled life-history traits such as fecundity, metabolic requirements [126] and age at first breeding [127].

In addition, a species' potential to serve as a reservoir or transmit pathogens may have a phylogenetic signal. Since the morphological and physiological traits of species which regulate interactions with pathogens are usually phylogenetically conserved [29], phylogenetic differences in reservoir competence may exist across different taxa [27]. Therefore, we use both a conventional and a phylogenetic comparative analysis to test the relationships between the life-history traits and reservoir competence. We expect reservoir competence to be negatively correlated with body mass and incubation time (gestation time for mammals) while positively correlated with clutch size (litter size for mammals).

Materials and methods

Data collection

We searched for reservoir competence data from published studies and found reservoir competence data for three vector-borne diseases (Table 4.1). For Lyme disease, we collected the data from studies about *Borrelia* and different tick vector species. Since different strains of pathogens and different tick vector species may influence host reservoir competence [128], we only used the data from those studies where the disease is caused by the etiologic agent *Borrelia burgdorferi* and transmitted by the vector *Ixodes scapularis* [122], while the numbers of host species in the data sets with respect to other strains of *Borrelia* or other tick vector species were too small. For Lyme disease, we used the species' realized reservoir competence (RRC), i.e. the product of the species' host susceptibility and host infectivity, as a measure for the species' reservoir competence [122] because of the lack of data on the duration of infectiousness. For WNE, we used two different data sets (Table 1, two data sets are referred as WNE-1 and WNE-2 respectively): the first data set determined the reservoir competence index and host infectivity for 25 native bird species of North America in experimental conditions [124], whereas the second described original raw experimental viremia data from different studies and recalculated the reservoir competence index for 44 bird species using a method to avoid inflation of average viremia and infectiousness by a single animal with a high-titred viremia [129]. For EEE, we used the published dataset of 10 bird species [123].

We collected life-history traits data (body mass, gestation/incubation time and litter/clutch size) from previous published studies or existing databases. Data sources are listed in Table S 3.1 and Table S 3.2.

Table 4.1: Disease parameters, studied taxon, number of host species used in the analysis of Lyme disease, West Nile Encephalitis (WNE) and Eastern Equine Encephalitis (EEE)

Disease	Host taxon	Disease parameter	Host number
Lyme disease	mammal	realized reservoir competence (RRC)	9
WNE-1	bird	reservoir competence index (RCI)	15
WNE-2	bird	reservoir competence index (RCI)	24
EEE	bird	reservoir competence index (RCI)	10

Phylogenetic tree

For WNE and EEE, we used a published phylogenetic tree of birds [130], which includes 169 avian and 2 out-group genera. If only one bird species in the disease data set did belong to a genus in the tree, the genus tip was considered as the tip of this species. If more than one bird species did belong to a genus in the tree, we added a new branch with length 0.0001 for each species to the genus tip, and then the genus tip became a node. For the bird species which did not belong to any genus in this phylogenetic tree, we checked if the tree included any genera sharing the same family with these bird species. Species which did not belong to any family derived from the genera in the tree were not used in the analysis. If there was only one genus in the tree sharing the same family with the bird species in the disease data, the genus tip was considered as the tip for this species. If a bird species shared the same family with more than one genus in the tree, we created a new 'family' tip [131]. Then this 'family tip' was used as the tip of the bird species in the disease dataset. For Lyme disease, we used a published phylogenetic tree including almost all extant mammal species [132]. Trees were transformed to ultrametric trees (Fig. 4.1) to perform the phylogenetic comparative analysis.

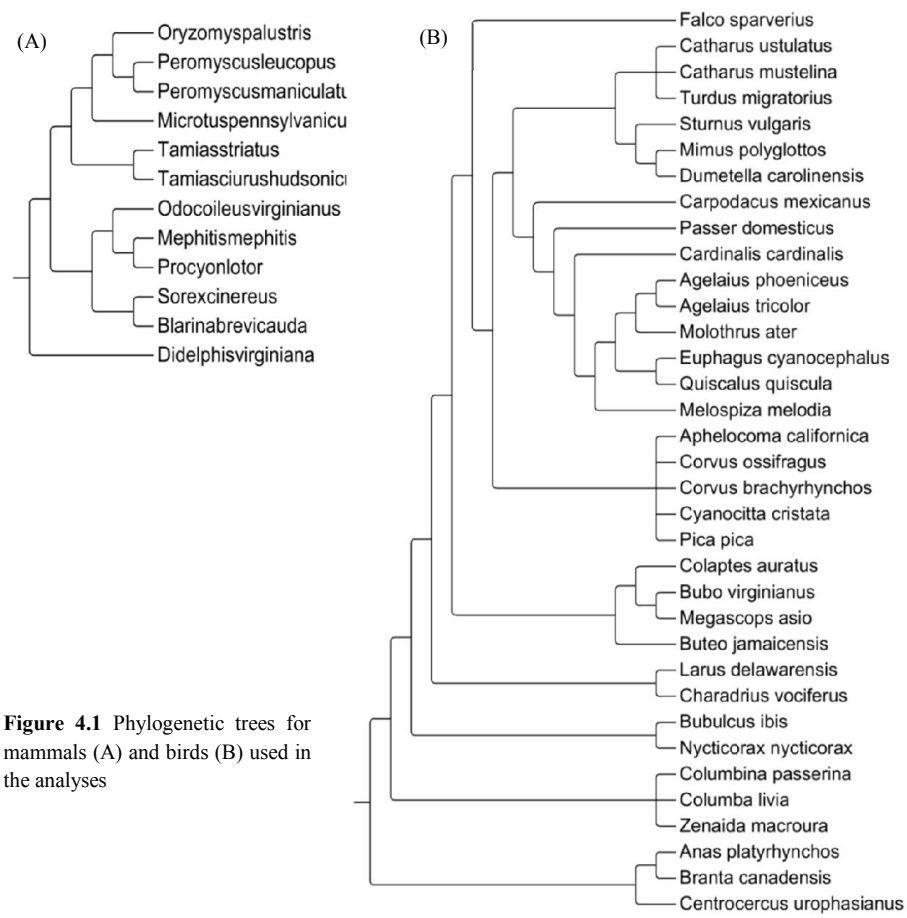

Figure 4.1 Phylogenetic trees for mammals (A) and birds (B) used in the analyses

4

Statistical analysis

In the datasets of WNE used in the study, there were several non-host bird species whose reservoir competences were zero. Non-hosts data were removed before analysis because within a community there are many non-host species which are often not included in reservoir competence studies, especially for the studies with respect to testing life history theory, since trade-offs between life-history traits versus immune defence against a specific pathogen might not occur in non-host species.

We log-transformed incubation time (gestation time for mammals) and body mass. We fitted models using reservoir competence as dependent variable and life-history traits as independent variables. We reported the results of a non-phylogenetic statistical analysis (assuming a star phylogeny [131]), and a phylogenetic comparative analysis under Brownian motion evolution. Since life-history traits were usually significantly correlated with each other and the relationship of a trait might be changed by adding other collinear variables in multiple regression models, we first conducted a factor analysis to extract the primary life-history axes, and reported the results of the univariate regressions using these extracted factor scores as independent variables. For Lyme disease, we first conducted our analyses using phylogenetic independent contrasts for all variables, then extracted the primary life-history axes from these

independent contrasts, and finally carried out regression analyses on these phylogenetically corrected responses and predictors [125]. For WNE and EEE, since the phylogenetic tree of birds was not fully dichotomous because of the lack of some branches' lengths (Fig. 4.1), we first conducted the factor analyses and then carried out the regression analyses using a phylogenetic GLS approach instead of the independent contrast approach [133]. After that, we also carried out univariate regression analyses to test for the impact of each life-history trait on the species' reservoir competence. All analyses were carried out in Canoco 5 and R 2·14·0 using the ape package [134].

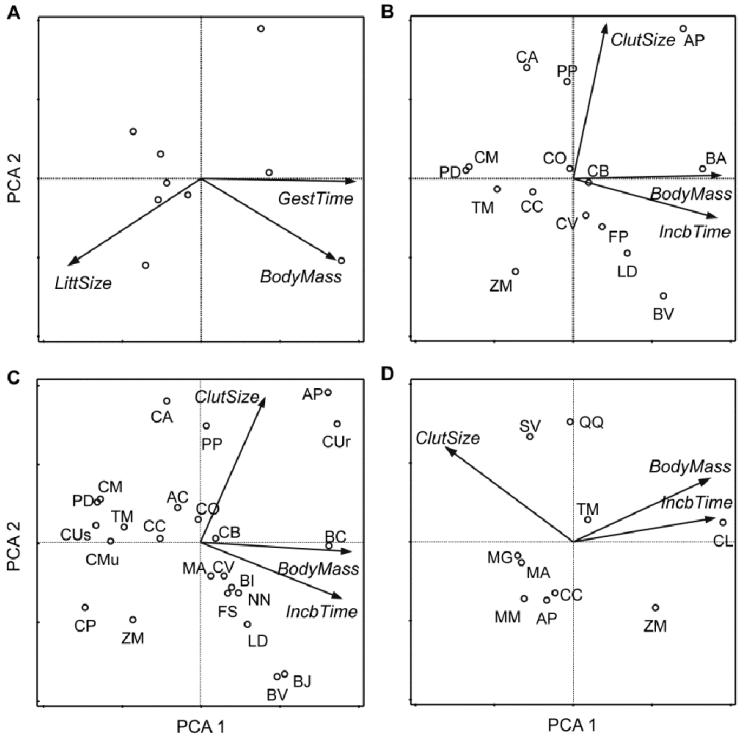

Figure 4.2: The results of factor analysis for (A) mammal hosts of Lyme disease; (B) bird hosts used in WNE-1; (C) bird hosts used in WNE-2 and (D) bird hosts of EEE. Species codes plotted in ordination space reflect the first two letters of the genus and species names (for Lyme disease, the species codes cannot be given because the species' names of the internal nodes were not available).

Results

Factor analysis

Factor analyses (Fig. 4.2) showed that the first component axis, Factor 1, explained a large percentage of the variance of the species' life-history traits: 78.5% for the hosts of Lyme disease, 57.2% for the hosts of WNE-1, 61.8% for the hosts of WNE-2 and 72.1% for the hosts of EEE. For Lyme disease and EEE, all three life-history traits were heavily loaded on Factor 1. Whereas for WNE-1 and WNE-2, only body mass and incubation time were heavily loaded on Factor 1, and clutch size was generally more extracted

on the second Factor. Host species with higher Factor 1 scores were generally those that have "slow-lived" characteristics, e.g. larger body mass, longer incubation/gestation time and smaller litter/clutch size (only in Lyme disease and EEE).

Regression analysis

The phylogenetic regression analyses of Factor 1 (Table 4.2) showed that the realized reservoir competence of Lyme disease, reservoir competence index in WNE-1 and EEE were all significantly negatively correlated to the Factor 1 scores. According to these results, higher Factor 1 scores referred to slower life histories, those species with higher reservoir competence tended to have fast life histories. The reservoir competence index in WNE-2 was not significantly associated to the Factor 1 scores (Table 4.2).

Table 4.2: Regression coefficient b, t-statistic and adjusted R^2 (only for conventional analysis) for the univariate linear regressions of the first primary component (Factor 1) for both non-phylogenetic and phylogenetic analysis of Lyme disease, West Nile Encephalitis (WNE) and Eastern Equine Encephalitis (EEE)

Disease, data resource	Disease parameters	Conventional analysis			Phylogenetic analysis	
		b	*t*	Adjusted R^2	*b*	*t*
Lyme disease (*n*=9)	RRC#	-0.10	-1.48	0.13	-0.14	-2.72*
WNE-1 (*n*=15)	RCI#	-0.21	-1.63	0.11	-1.22	-2.97*
WNE-2 (*n*=24)	RCI	-0.02	-0.21	-0.04	-0.48	-1.16
EEE (*n*=10)	RCI	-0.23	-1.98	0.24	-0.17	-2.71*

RRC: realized reservoir competence. RCI: reservoir competence index
*p ≤ 0.05, **p≤0.01

Table 4.3: Regression coefficient b, t-statistic and adjusted R^2 (only for conventional analysis) for the univariate linear regressions of each life-history traits for both non-phylogenetic and phylogenetic analysis of Lyme disease, West Nile Encephalitis (WNE) and Eastern Equine Encephalitis (EEE)

Disease, data resource	Disease parameters	Independent variables	Conventional analysis			Phylogenetic analysis	
			b	*t*	Adjusted R^2	*b*	*t*
Lyme disease (*n*=9)	RRC#	body mass	-0.18	-3.68**	0.61	-0.19	-3.55**
		gestation	-0.17	-1.32	0.08	-0.16	-1.23
		litter size	0.01	0.13	-0.14	0.01	0.24
WNE-1 (*n*=15)	RCI#	body mass	-0.49	-1.75	0.11	-1.54	-2.68*
		incubation	-0.36	-0.74	-0.03	2.64	0.40
		clutch size	-0.09	-0.81	-0.02	-0.36	-2.15
WNE-2 (*n*=24)	RCI	body mass	-0.01	-0.04	-0.03	-0.45	-0.87
		incubation	0.17	0.53	-0.02	13.57	1.44
		clutch size	0.06	0.80	-0.01	-0.29	-1.63
EEE (*n*=10)	RCI	body mass	-0.52	-1.02	0.01	-0.31	-0.78
		incubation	-1.87	-1.17	0.04	-0.25	-0.59
		clutch size	0.42	3.32*	0.53	0.37	2.92*

RRC: realized reservoir competence. RCI: reservoir competence index
*p ≤ 0.05, **p≤0.01

In regression analyses for each life-history trait, both non-phylogenetic and phylogenetic analysis showed that body mass was the strongest predictor for the species' realized reservoir competence of Lyme disease (Table 4.3). Species with a larger body mass tended to have a lower realized reservoir competence for Lyme disease. Neither gestation period nor litter size showed any significant relationship with realized

reservoir competence, though the coefficients were, as expected, negative for gestation and positive for litter size (Table 4.3).

For species' reservoir competence index of WNE-1, the phylogenetically corrected univariate regression showed significantly negative relationships with body mass (Table 4.3). Species with a larger body mass tended to have lower reservoir competence index for WNE. Whereas for the second WNE data set (WNE-2), no significant relationships between reservoir competence and life-history traits were found in the non-phylogenetic regression or in the phylogenetic regression (Table 4.3).

For EEE, both the results of the non-phylogenetic univariate regression and phylogenetic analysis showed that clutch size was a significant predictor for species' reservoir competence index (Table 4.3). Species with larger clutch size tend to have a higher reservoir competence for EEE. Neither body mass nor incubation time showed any significant relationships with reservoir competence, though the coefficients were, as expected, negative (Table 4.3).

Discussion

Our study focused on the relationships between life-history traits and species' reservoir competence for three vector-borne diseases. The results generally showed that life-history traits can partly explain interspecific variation in reservoir competence. Body mass is a strong predictor to the reservoir competence in Lyme disease and WNE-1. Larger-bodied species tend to have lower reservoir competence. The variation in birds' reservoir competence in EEE could be partly explained by clutch size. As we predicted, bird species with larger clutches tend to have a higher reservoir competence of EEE. For reservoir competence index in WNE-2, the lack of a significant relationship might be due to the different sources used in compiling this data set. The reservoir competence index can differ when measured under different conditions, since one component of reservoir competence index, the species' susceptibility, usually vary in space and over time [122].

Our findings build on an emerging body of studies on the relationships between life history theory and disease ecology. Instead of focusing on immunology, however, our study associated the species' potential to maintain and transmit pathogens with life-history traits. Life history theory suggests the existence of a trade-off between the immune system and life-history traits relating to growth and reproduction [135,136]. "Slow-lived" species tend to invest more in adaptive immunity because they probably encounter a greater number of infections overall, and are more likely to encounter the same pathogen, whereas "fast-lived" species which are in favour of growth and frequent reproduction tend to invest comparatively little in costly adaptive immunity [56,135]. Together with a previous study suggesting that species with a higher reservoir competence tend to favour cheaper, nonspecific immune defences that pathogens may be able to circumvent easily [58], the negative relationships between reservoir competence and life histories in our study support the predictions derived from life history theory. In addition, previous studies reported a strong positive relationship between natural antibody levels and incubation period in bird and mammal species [55,58], indicating that longer developmental times contribute to better adaptive immune systems. However, we did not find any significant relationship between incubation/gestation time and reservoir competence. This indicates that other factors, besides the effect of incubation period on adaptive immune system, might also influence species' reservoir competence, which needs to be studied in the future.

Recently several studies on life history theory proposed to discuss these physiological trade-offs between defence versus life histories in the context of a broader background, namely, the impact of biodiversity on

disease transmission [58,121,125]. Based on our results, one might expect that those species with a high reservoir competence are more likely to be those that are wide-distributed, since evidence is accumulating that species with faster life histories are more resistant to population decline and local extinction than "slow-lived" species [59,126]. Species with faster life histories (such as those with smaller body masses and larger clutch sizes) usually have lower energetic requirements and higher reproductive capacities, which make them more likely to be able to survive in remnant habitat patches with low biodiversity [59]. Also, some studies suggested that larger body mass usually associated with smaller population size [137], which also make them more vulnerable to biodiversity decline [138]. According to our findings that the species' reservoir competence can be partly explained by their life histories, species with slower life histories tend to have lower reservoir competence. Thus, the species which are first lost from a community when disturbed tend to be those that are less competent hosts, ultimately leaving a higher abundance of more competent species in low diversity systems due to release from competition or predation, and thereby increase the risk for disease transmission. This might be a possible mechanism underlying the dilution effect, the inverse relationship between biodiversity and disease risk, which has attracted much interest in the context of ongoing biodiversity losses and increased emergence of human and wildlife diseases [13,78,139].

In theory, high biodiversity might dilute or amplify disease risk by changing the relative abundance of competent hosts [31]. The amplification effect suggests a positive relationship between biodiversity and disease risk. Compared with rare studies that support the amplification effect [13], the dilution effect has been reported for quite a few different diseases [28,43,44,45,140,141,142,143,144]. Some studies have shown that the dilution effect generally occurs when competent host species survive and increase their local densities in disturbed low-diversity communities, while other ecologists criticised the dilution effect and claimed that the dilution effect only occurs under certain circumstances and depends on a specific community composition where incompetent host species are more likely to be present in high-diversity communities [107]. Together with a previous study which suggested that fast-lived amphibian species were particularly prone to infection and pathology of a virulent trematode parasite, Ribeiroia ondatrae [125], the results of our study might explain how the community composition changes under increasing species loss and how this affects the species' competence for the pathogen, triggering a dilution effect.

Our study highlights the importance of the association between life-history traits and species' potential to support and transmit pathogens and thus contributes to empirical evidence for life history theory. The results, in conjunction with findings of relationships between species' life histories and local extinction risk, suggest a possible mechanism why the dilution effect operates with decreases in biodiversity.

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Supplementary Information 4

Table S 4.1: Data sources for reservoir competence, mammals' life-history traits and birds' body mass

variables	sources or references
RRC for Lyme disease	LoGiudice K et al. 2003 (Table 1)
RCI for WNE-1	Komar N et al. 2003 (Table 10)
RCI for WNE-2	Kilpatrick AM. 2007 (Figure 2)
RCI for EEE	Komar N et al. 1999 (Table 3)
life-history traits for mammals	Jones KE. *Ecological Archives* E090-184-D1
body mass for birds	Olson et al. 2009

Table S 4.2: Body mass, clutch size and incubation period of birds used in the analysis

Species	Body mass (g)	Clutch size	Incubation period (day)	Sources and references
Agelaius phoeniceus	53.22	3.28	11.68	Bennett PM 2002
Agelaius tricolor	66.53	3.75	11.875	Bennett PM 2002
Anas platyrhynchos	1096.33	8.25	27.5	Bennett PM 2002; del Hoyo J et al. 1994
Aphelocoma californica	85.38	4.8	18.2	Martin TE 1995
Bubo virginianus	1205.96	2.25	30.15	Bennett PM 2002
Bubulcus ibis	345.43	3.67	23.85	Bennett PM 2002; Brown LH et al. 1982
Buteo jamaicensis	1121.90	2.43	32	Bennett PM 2002; del Hoyo J et al. 1994
Cardinalis cardinalis	44.70	3.37	12.5	Bennett PM 2002
Carpodacus mexicanus	21.39	4.33	13.53	Bennett PM 2002
Catharus mustelinus	49.65	3.43	13.23	Bennett PM 2002
Catharus ustulatus	31.19	3.66	12.5	Bennett PM 2002
Centrocercus urophasianus	2722.81	7.5	26	Bennett PM 2002
Charadrius vociferus	92.42	4	26.57	Bennett PM 2002
Colaptes auratus	136.21	6.47	12.625	Bennett PM 2002
Columba livia	332.47	2	17.25	Bennett PM 2002
Columbina passerina	30.29	2	13.5	Bennett PM 2002
Corvus brachyrhynchos	445.27	4.25	18.15	Bennett PM 2002
Corvus ossifragus	284.26	4.5	17	Bennett PM 2002
Cyanocitta cristata	74.56	4	17	Bennett PM 2002
Dumetella carolinensis	37.08	3.88	13.3	Bennett PM 2002
Euphagus cyanocephalus	63.19	5.09	12.75	Bennett PM 2002; Martin SG 2002
Falco sparverius	116.52	3.85	29.25	Bennett PM 2002
Larus delawarensis	517.06	3.07	26.1	Bennett PM 2002
Melospiza melodia	20.68	3.87	12.7	Bennett PM 2002; Arcese P et al. 2002
Mimus polyglottos	48.71	3.90	12.32	Bennett PM 2002
Molothrus ater	43.21	4	11.57	Bennett PM 2002; Lowther PE 1993
Nycticorax nycticorax	550.42	3.50	23	Bennett PM 2002
Megascops asio	184.50	4	26	Bennett PM 2002
Passer domesticus	26.98	4.15	12.51	Bennett PM 2002
Pica pica sericea	225.50	6.5	17	Madge S & Burn H 1999
Quiscalus quiscula	116.02	4.83	13.67	Bennett PM 2002
Sturnus vulgaris	81.70	5.12	12.375	Bennett PM 2002
Turdus migratorius	77.36	3.73	12.92	Bennett PM 2002
Zenaida macroura	119.76	2	14.8	Bennett PM 2002

Chapter 5

Dilution versus Facilitation: the Impact of Habitat Connectivity on Disease Risks in Metapopulations

Zheng Y.X. Huang, Frank van Langevelde, Herbert H.T. Prins, Willem F. de Boer

Several studies have been conducted to investigate the generality of the dilution effect, a highly disputed hypothesis that refers to the negative effect of species diversity on disease risk. However, these studies were conducted only in spatially homogeneous environments without considering habitat structure, which is surprising as species loss is often driven by habitat fragmentation. Using epidemiological metapopulation models, we linked fragmentation and habitat connectivity to the dilution effect and explored the effect of connectivity on disease risk. We showed that higher connectivity is not only able to increase disease risk (facilitation effect) through increasing contact rates among patches, but also able to dilute disease risk (dilution effect) via increasing species richness. When both effects operate, the net impact of connectivity depends on the dilution potential of the incompetent host. We also demonstrated that different risk indices react differently to increasing connectivity, and it is easier to detect a negative relationship between disease risk and species richness when using infection prevalence as the risk index than using the abundance of infection. Our study may reconcile the current debate on the dilution effect, and contributes to a better understanding of the generality of the dilution effect and the impacts of fragmentation on disease risks.

Submitted

Introduction

Habitat fragmentation caused by human disturbances has produced substantial negative impacts on biodiversity [72], and in turn on ecosystem functioning and services [145]. One of the ecosystem services could be protection against diseases [33]. Current studies argue that high species richness reduces the risk of infectious diseases via a hypothesized 'dilution effect' [13,14,40]. While this dilution effect presents an exciting convergence of public health concern and biodiversity conservation, its generality is still under active debate [11,19,20,146,147]. It has been reported that the dilution effect occurs in a wide range of infectious diseases [14], such as Lyme disease [39,40], West Nile encephalitis [41], bovine tuberculosis [148], Hantavirus pulmonary syndrome [43,44], and so on. In contrast, some researchers argued that the dilution effect is more likely idiosyncratic and applies only in certain circumstances [11,19].

Theoretical studies have been conducted to investigate the generality of the dilution effect. For example, it has been shown that the occurrence of the dilution effect may depend on the type of disease transmission [49,51]. Specifically, the dilution effect is more likely to operate in diseases with frequency-dependent transmission [50,51]. While for a density-dependent transmitted disease, high species richness generally increases the risk unless it can reduce the densities of competent hosts (e.g., through interspecific competition or predation) [51]. Different indices of disease risk may also react differently to the changes in species richness. By incorporating empirical laws of community assemblage, Roche et al. [149] found that high species richness can reduce infection prevalence while the number of total infected hosts in the community increases. Considering that species loss is often driven by habitat fragmentation, it is surprising that these studies on the disease-diversity debate were conducted in spatially homogeneous environments without considering habitat heterogeneity.

The landscape configuration can not only affect pathogen transmission through altering species richness and community assemblages [14,31], but also influence pathogen transmission dynamics by modifying contact rates among subpopulations [150,151]. Previous studies suggested that increasing connectivity among subpopulations almost universally facilitates disease transmission, allowing a pathogen to successfully invade a metapopulation [152] and increasing the prevalence and incidence of diseases in metapopulations [150,151]. We, therefore, hypothesize that increasing habitat connectivity can have both positive and negative effects on disease risk, depending on the interplay between enhanced pathogen transmission via promoting contact rates among subpopulations and the dilution effect through changes in host species richness. However, no study, to our knowledge, linked landscape structure and host movements to the disease-diversity debate. Incorporating these two mechanisms would be necessary in order to get a better understanding of the influence by fragmentation on disease risks and the generality of the dilution effect.

Here, we used simple stochastic models to linked fragmentation and habitat connectivity to the dilution effect, and investigate the effect of habitat connectivity on pathogen transmission in metapopulations. Our main objective is to examine the relative importance of the dilution and facilitation effects along a connectivity gradient and explore the scenarios when the positive and the negative relationships between species richness and disease risk can be detected. Overall, we demonstrate that disease risk can either decrease with increasing connectivity due to the dilution effect, or increase due to a facilition effect. The dilution effect of incompetent host can be overshaded by the facilitation effect of connectivity, and the net impact of connectivity depends on the dilution potential of the incompetent host.

Models and Methods

Pathogen transmission in the single-host system

We combined a classical SIR (susceptible-infectious-recovered) model with a Levins' metapopulation model to simulate the dynamics of directly transmitted pathogens. We assumed that the hosts were fully mixed in a certain number of patches with identical properties and homogeneous environment within, and that all patches were equally accessible to the hosts from other patches. The model reads as:

$$\frac{\mathrm{d}S_i}{\mathrm{dt}} = bN_i - (\lambda + d)S_i$$

$$\frac{\mathrm{d}I_i}{\mathrm{dt}} = \lambda S_i - (r + d)I_i$$

$$\frac{\mathrm{d}R_i}{\mathrm{dt}} = rI_i - dR_i$$

Where N_i represented the host population size in patch i, and hosts could be either susceptible (S_i), infected (I_i) or recoverd/immune (R_i). The host population increased with logistic growth, which included a constant birth rate b and a density-dependent mortality d within each patch. Host mortality was assumed to be independent of infection status. Infected hosts could be recovered from the infection at a recovery rate r. We assumed a frequency-dependent transmission, since the dilution effect is more likely to occur in this type of diseases [49,50,51]. Then, the force of infection λ was determined by intraspecific transmission rate β:

$$\lambda = \beta \frac{I_i}{N_i}$$

The pathogen transmission dynamics were modelled with a stochastic process. At each time step, the processes of birth, death, infection, recovery, movement and extinction were modelled sequentially [62,151]. The number of newly-born individuals followed a Poisson distribution, whereas the number of deaths, infections, and recoveries followed a binomial distribution. Newborns would neither die nor be infected [62].

Patches were connected to each other via host movements. The migration proportion, m, as a measure of connectivity, was used to describe how many individuals leave a patch at each time step. Therefore, the migration proportion represented the movement rates among subpopulations, with a higher value of migration proportion representing a higher frequency of movements [151], characteristic of a less fragmented landscape. This migration proportion was assumed to be the same regardless of the state of the individuals or the local population size in the patch. The number of emigrants for each patch at each time step also followed a binomial distribution, and all emigrants were distributed randomly over all patches.

In addition, we set a local extinction rate for the local population in each patch at each time step. This extinction rate was the inverse of persistence time, $T_p(i)$, which was a function of population size N_i and carrying capacity K [153]:

$$T_p(i) = \frac{N_i \times K}{N_i + K/a}$$

Where *a* was the adjusting parameter for persistence time. Higher values of *a* lead to a higher T_p and thus a lower local extinction risk.

Pathogen transmission in the two-host system

We further extended the abovementioned single-host model to a two-host system comprising one competent and one incompetent host species. Being consistent with previous studies [149,154], we assumed that competent host species had smaller body masses and shorter life-spans, because life-history theory generally suggests a trade-off between investment in self-maintenance (e.g., physiological resistance) and other physiological activities (e.g., reproduction and growth) [54]. In this way, compared to slow-lived species, fast-lived species (with smaller body massed and short life-spans) usually invest less in immunological defences, which makes them act as more competent hosts for pathogens [55,155]. Such trade-off has been confirmed for the hosts of Lyme disease [40,156,157], West Nile virus [157], Barley yellow dwarf virus [52] and trematode parasites [15,46].

We also assumed that birth rates and patches' carrying capacities were determined allometrically by the body masses of the species [158]. Therefore, the competent host species with smaller body mass had a higher birth rate b_1 than the incompetent host species b_2. The carrying capacity for the competent host species K_1 was higher than that of the incompetent host species K_2. In this way, the incompetent host would go extinct first with declining connectivity, which is consistent with empirical evidence [138,159,160].

Table 6.1: Description of model parameters and variables. Subscript 1 and 2 represent the competent and incompetent host species, respectively.

parameter	definition	value
b	Birth rate	$b_1 = 0.1$; $b_2 = 0.08$
d	Death rate	$d = bN/K$
β_1	Intraspecific transmission rate for competent host	0.9
β_2	Intraspecific transmission rate for incompetent host	[0.1, 0.9]
$\beta_{12} = \beta_{21}$	Interspecific transmission rate	$c(\frac{\beta_1+\beta_2}{2})$
c	Interspecific transmission scaling parameter	[0.01, 1]
r	Recovery rate	[0.1, 0.3]
K	Patch carrying capacity	$K_1 = 100$; $K_2 = 80$
$m_1 = m_2$	Migration proportion	[0, 0.05]
a	Local population extinction adjusting parameter	$a_1 = 5$; $a_2 = 3$

Interspecific transmission between the competent and incompetent host was assumed to be symmetrical and quantified as a scaled average of the intraspecific transmission rates [49,50]:

$$\beta_{12} = \beta_{21} = c\left(\frac{\beta_1 + \beta_2}{2}\right)$$

Where β_1 and β_2 were the intraspecific transmission rates, while β_{12} and β_{21} were the interspecific transmission rates, and c was a scaling parameter allowing us to adjust the magnitude of the interspecific transmission rates. The force of infection for the competent host, λ_1, could be calculated as [51]:

$$\lambda_1 = \beta_1 \frac{I_1}{N_1 + N_2} + \beta_{21} \frac{I_2}{N_1 + N_2}$$

The dilution effect here was caused by a mechanism called 'encounter reduction', which means that the addition of the incompetent host can reduce the chances of infected competent hosts to encounter susceptible competent hosts [31]. To investigate the relative importance of the dilution effect, different 'dilution potentials', defined as the competence of the additional host to reduce the disease risk [40], of the incompetent host were simulated by varying the intraspecific transmission rate of the incompetent host β_2 and the scaling parameter c. A higher dilution potential corresponds with a lower value of β_2 and/or c. Details for all parameters are provided in Table 6.1.

For reasons of simplicity, the migration proportions for the incompetent and competent hosts were assumed to be similar. Competition or predation were not considered in our model, which implied that the density of the competent and incompetent host populations were independent of each other.

Model analyses

The carrying capacity of a patch was set as 100, which was large enough for the infection to become endemic after the pathogen successfully invaded a metapopulation. Twenty-five patches were used in the simulations (we found that the number of patchs did not change the results qualitatively). We first ran the model without pathogens for 500 time steps to make sure the system reached a quasi-equilibrium. Then an infectious competent individual was added into a randomly selected occupied patch; and the transmission process was simulated with another 500 time steps so that the populations reached an approximate equilibrium. Since the infection and demographic processes are stochastic and can create large variation among runs of the model, we ran 1,000 repetitions for each parameter set [150]. We calculated the probability of pathogen invasion, i.e., the fraction of simulations where the pathogen successfully invaded the metapopulation, resulting in a persisting infection. We also quantified the disease risk for the simulations with successful pathogen invasion and persisting infection using three indicators, i.e., the mean infection prevalence of the competent host (i.e., the proportion of infected hosts in the whole metapopulation), the mean number of infectious competent hosts (NumInfC) and the total number of infectious individuals (TolInf, including both competent and incompetent host).

We first analysed the model behaviour in a single-host (competent host) system with different recovery rates and explored how the facilitation effect operated. Then, we extended our model to a two-host system and explored the relative importance of the dilution and facilitation effect on disease risk, respectively using a high ($\beta_2 = 0.1$, $c = 0.1$) and a low ($\beta_2 = 0.7$, $c = 0.7$) dilution potential for the incompetent host.

Results

Facilitation effect in single-host system

Our results show that the infection could not invade the metapopulation when habitat connectivity was very low (Fig. 5.1). At different recovery rates, the mean infection prevalence showed similar sigmoidal patterns in response to increasing migration proportion (Fig. 5.1), indicating a facilitative effect of connectivity on infection prevalence within a certain range. We defined the facilitation region as the range of the migration proportion where increasing migration proportion increased the infection prevalence. The upper threshold for this range was determined by the tangent of the curve where the infection prevalence increased less than 2% with an increase of 0.01 in migration proportion (we found

that slightly different criteria did not change the results qualitatively). We used infection prevalence to define the facilitation region because infection prevalence for frequency-dependent transmitted diseases is independent of host density, and thus its increase was only caused by increasing contact rates among subpopulations. The width of the facilitation region was enlarged with increasing recovery rate (shown by the dashed vertical lines in Fig. 5.1). In addition, our results also showed that the probability of pathogen invasion responded similarly to the increasing connectivity as the infection prevalence, showing sigmoidal patterns (Fig. 5.1).

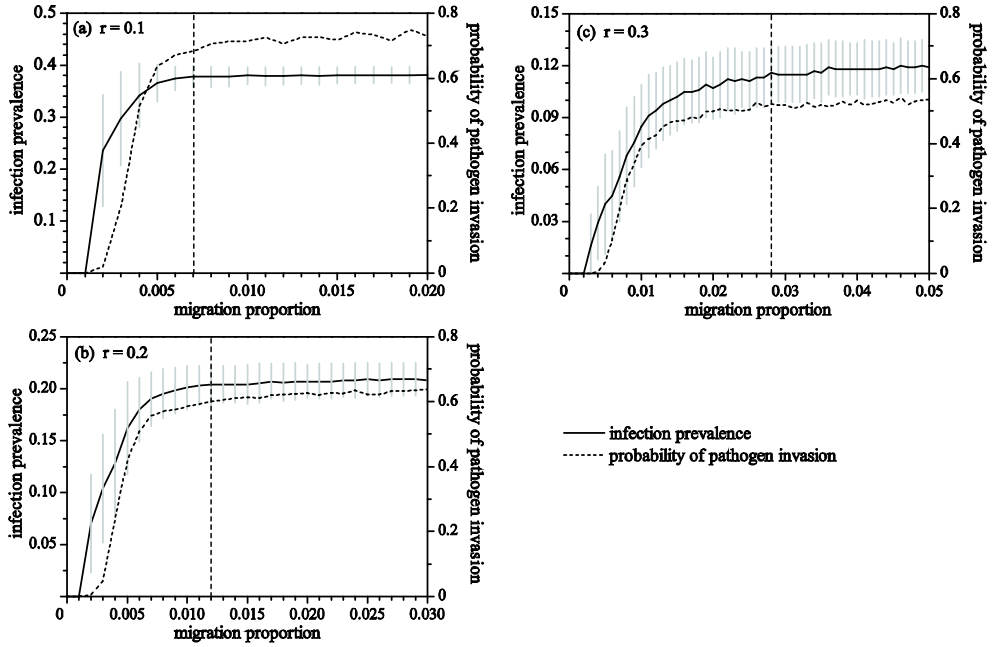

Figure 5.1: Changes in the probability of pathogen invasion and in the infection prevalence with increasing migration proportion under different recovery rates: (a) $r = 0.1$; (b) $r = 0.2$; (c) $r = 0.3$. Grey vertical lines indicate the standard deviation for the infection prevalence, and dashed vertical lines indicate the upper threshold of the facilitation region.

Population size changes and the dilution effect

The overall population sizes for competent and incompetent hosts reacted similarly to increasing connectivity (Fig. 5.2). Hosts could not invade the metapopulation under low connectivity until the connectivity reached a certain threshold. After that, host population size rapidly increased with increasing migration proportion, m, after which the growth rate slowed down. A higher connectivity threshold was needed for the incompetent host than for the competent host to invade the metapopulation because of its lower carrying capacity and higher local extinction risk. When the incompetent hosts invaded the system, the dilution effect started to operate. Given that the facilitation region for the competent host depended on the recovery rate, two distinct scenarios became apparent. One scenario is that the facilitation effect and dilution effect overlapped over a gradient of migration proportions (i.e., $r = 0.3$, shown as the shaded area in Fig. 5.2), the other scenario is that the facilitation effect operated at lower values of the migration proportion than the dilution effect (i.e., $r = 0.1$, Fig. 5.2). We explored how disease risks react to increasing connectivity in the two-host system under these two scenarios for both a low and a high dilution potential of the incompetent host.

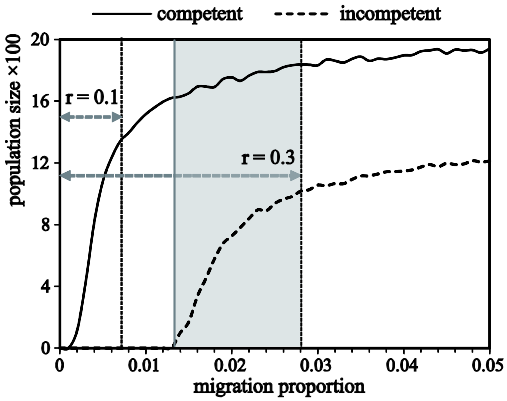

Figure 5.2: Changes in the equilibrium population sizes (after 500 time steps) of the two hosts with increasing migration proportion. The grey dashed arrows indicate the facilitation region under different recovery rates and the shaded area refers to the overlap region where both a dilution and a facilitation effect operate under the condition that the recovery rate $r = 0.3$. Dilution effect operates when the incompetent hosts invades the system.

Dilution versus facilitation in a two-host system

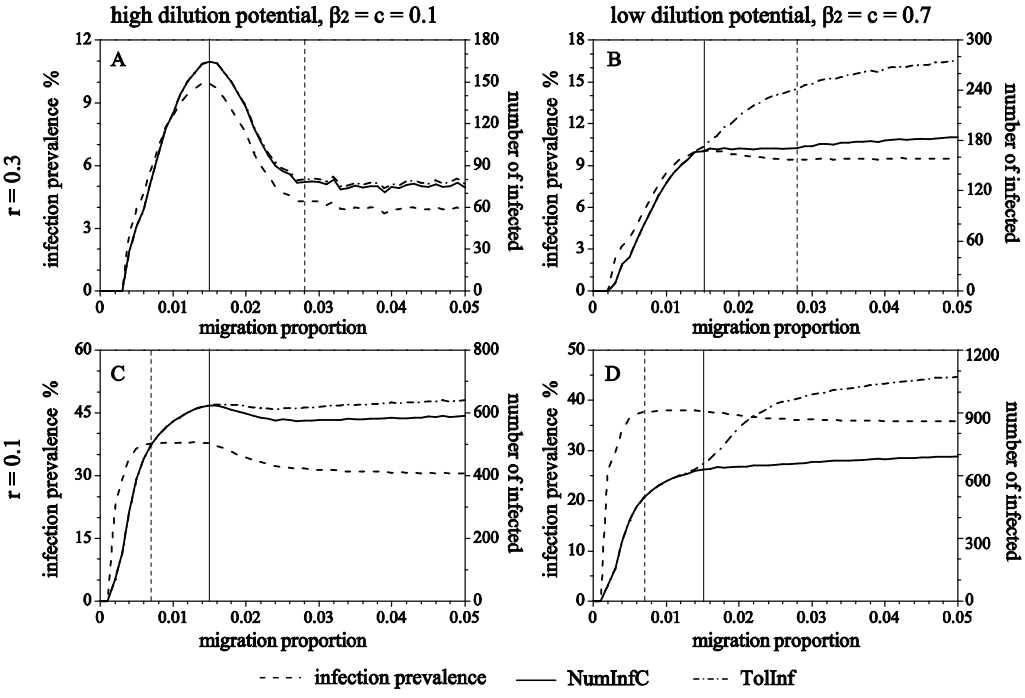

Figure 5.3: Changes in the infection prevalence of the competent host, number of infected competent hosts (NumInfC) and total number of infected hosts in the system (TolInf) with increasing migration proportions in a two-host system for different recovery rates and dilution potentials. Dashed vertical lines indicate the upper threshold of the facilitation region (see Fig 5.1); while the solid vertical lines indicate the start of the dilution effect (see Fig 5.2).

In the first scenario (r = 0.3) when the dilution effect and the facilitaiton effect overlapped and the incompetent host had a high dilution potential (β_2 = 0.1, c = 0.1, Fig 5.3A), the number of infected competent hosts (NumInC), the total number of infected individuals (TolInf, including both competent and incompetent host), and the infection prevalence of the competent host showed similar patterns. They first increased with migration proportion because of the facilitation effect, and then decreased due to the dilution effect to relatively stable levels. Hence, in the overlap region (with both the facilitation effect and the dilution effect), the faciliation effect was outweighed by the dilution effect. However, when the incompetent host had a low dilution potential (β_2 = 0.7, c = 0.7), different disease risk indicators showed different trends in the overlap region (Fig 5.3B), as the total number of infected individuals (TolInf) increased, the number of infected competent hosts (NumInfC) remained relatively stable, and the infection prevalence of the competent host decreased.

We then varied the dilution potential of the incompetent host (different combinations of c and β_2) to investigate the net effect of connectivity on disease risk, i.e., whether disease risk indicators increased or not in the overlap region when the facilitation and the dilution effect overlapped. Our results showed that the net effect of connectivity on disease risk in this region was determined by the dilution potential of the incomptent host (Fig 5.4). The lowest dilution potential (high values for β_2 and/or c) was required for the infection prevalence of the competent host to decrease.The number of infected competent host (NumInfC) and the total number of infected individuals (TolInf) needed higher dilution potentials to counteract the increasing host density (Fig 5.2) in the overlap region.

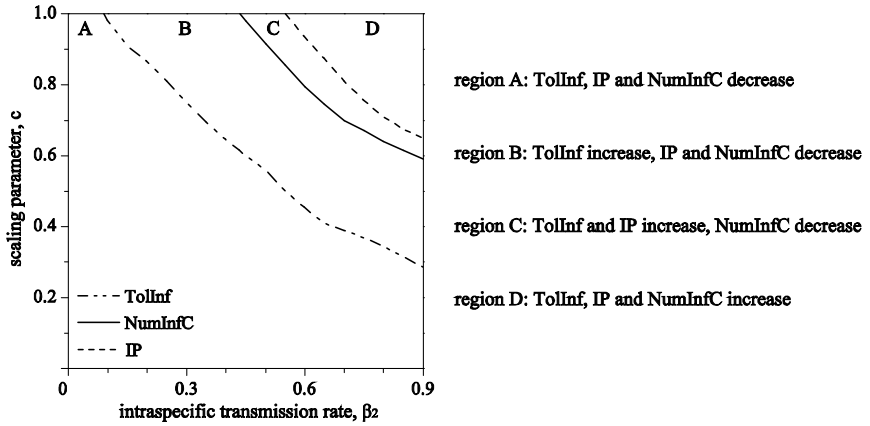

Figure 5.4: Thresholds for the combination of the intraspecific transmission rate for the competent host (β_2) and the interspecific transmission scaling parameter (c) under which the disease risks (the total number of infected individuals: TolInf, the number of infected competent hosts: NumInfC, and the infection prevalence of the competent hosts: IP) decrease in the overlap region, i.e. the range of migration proportions where both a facilitative and a dilution effect operate. Disease risks increase in the upper-right regions (lower dilution potential).

In the second scenario (r = 0.1) where the facilition effect and the dilution effect operate seperately, the infection prevalence of the competent host first increased rapidly with increasing connectivity because of the facilitation effect, and then remained stable until the dilution effect starts to operate (Fig 5.3C, D). After that, the infection prevalence decreased to a stable level, and a larger decrease rate of the infection prevalence occurs with higher dilution potential. The number of infected competent hosts (NumInfC) and the total number of infected individuals (TolInf) showed different patterns. They first increased due to the facilitation effect, and after the dilution effect starts to operate, they slightly decreased under a high

dilution potential of the incompetent host, whereas they both increased when the dilution potential is low (Fig 5.3C, D).

Discussion

Human-induced habitat fragmentation plays an important role in species loss [72], and in turn modifies disease dynamics [14,31]. Some studies have shown increased disease risk in fragmented habitats with reduced species richness [15,39,161], providing supportive evidence to the dilution effect. While others did not find such relationship and concluded that habitat framentation has a complex role in pathogen transmission [9,12,20,162]. Our modelling study shows that these apparent contradictions can be understood, and suggests that both the dilution and facilitation effect can operate with increasing habitat connectivity, and whether disease risks decrease or not depends on the dilution potential of the incompetent host.

Our results from the single-host system showed that the infection prevalence first increased within a certain range of migration proportion and then remained stable (Fig 5.1). This is because infectious individuals do not move frequently at low migration proportions, i.e. in a highly fragmented landscape, and thus could not encounter enough susceptible individuals and spread the pathogen, especially when there are many recovered and immune individuals and limited susceptibles. The infection prevalence was high when the metapopulation acted more like a homogeneous population at high migration proportions, which resulted in immediate access of susceptible individuals for infected individuals. A higher recovery rate led to a larger facilitation region as a higher recovery rate means shorter infection periods, and high infection prevalence could only be achieved in these circumstances when infectious individuals moved more frequently to encounter susceptible individuals within their infection period. For the probability of pathogen invasion, increasing the migration proportion means that infected individuals move more frequently and thus have higher probabilities to arrive in a fully susceptible patch and spread the pathogen within their infection periods.

In the two-host system, we have shown that disease risk in fragmented habitats does not always decrease due to the dilution effect when the connectivity increases, but can also increase because of the facilition effect. The net effect of connectivity reflects the relative importance of the dilution versus facilitaiton effect. This result might partly explain the current contradictions with regard to the existence of the dilution effect. For example, a recent study showed that higher disease risk of chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis*, was found in less fragmented landscapes with higher amphibian species richness [163]**,** wheres an experimental study on this emerging amphibian disease suggested a dilution effect where increased species richness reduced the disease risk [143]. We showed in this study, that the facilitation and dilution effects might co-occur. Although the dilution effect is expected to operate under a higher species richness (with less fragmentation), this effect could be outweighted by the facilitation effect caused by an increasing landscape connectivity. *B. dendrobatidis*, a driver of the global amphibian decline, can heavily infect quite a lot of amphabian species [164], which leads to a low dilution potentials of these hosts species, so that the facilitation effect might overshadow the dilution effect in habitats with high connectivity and high species richness.

In addition, we found that different indicators of disease risk (i.e., the proportion of infected and the number of infected individuals) show different trends over the connectivity gradient in metapopulations, suggesting that connectivity affects these indicators differently. If so, then this result makes the detection of a dilution effect more difficult. Many studies reported the dilution effect using infection prevalence as

the indicator of disease risk [38,40,41,43,44]. However, several studies argued that sometimes the density of infected individuals might be a more direct measure of disease risk [19,20,146], especially for the risk for humans when the wildlife-human contacts are density-dependent [149]. According to our results, if the density of infected individuals is used as the risk indicator, the dilution effect might disappear, or a positive correlation between disease risk and species richness can be found because higher dilution potentials of incompetent hosts are needed to counteract the facilitation effect of connectivity. Hence, we recommend reporting both the prevalence and the density of infected individuals to better understand the determinants of disease risk.

In the simulation models, we made a number of simplifying assumptions to make the analysis tractable. First, we assumed that the incompetent host has a similar migration proportion as the competent host, which might not be realistic. However, we thought that relaxing this assumption will not qualitively affect our conclusions, since it can only change the threshold of connectivity for colonization of the incompetent host and make the overlap region (the range of connectivity where both a facilitation and a dilution effect operate) move over the gradient of connectivity. Also, we did not consider the effect of competition or predation. If the additional host can reduce the density of incompetent hosts, another mechanism for the dilution effect, 'host regulation' [31], would operate and lead to a stronger dilution effect [49,51]. In this way, it would be more likely to detect a negative correlation between species richness and disease risk.

In addition, we assumed a negative relationship between a species' reservoir competence and its local extinction risk, which is central in the dilution effect hypothesis [12,50,157]. Although this negative relationship, which is based on life-history theory, has been documented in several studies (see review in [50]), its universal application is still under debate [12,50]. Weak correlations between host reservior competence and local extinction risk can create inconsistent effects of host species richness on disease risk [50]. To better compare disease risk under different levels of connectivities, we also assumed that seasonal factors did not influence disease transmission and host demographic variables. These seasonal patterns directly modify susceptible recruitment and transmission patterns [165,166] and thus may potentially influence our results. Therefore, a natural next step would be to relax these assumptions to increase our understanding of the roles that connectivity plays in pathogen transmission.

In general, our study shows that even when the dilution effect operates in a system, the impact of fragmentation on disease risk cannot be easily predicted because connectivity is able to trigger simultaneously a facilitation and a dilution effect. Our study reconciles the current debate on the dilution effect, and contributes to a better understanding of the impacts by habitat fragmentation on disease risk. We show that a facilitation and a dilution effect can operate simultaneously, so that both an increase or a decrease in disease risk can be expected when habitat fragmentation increases.

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Chapter 6

Synthesis: Evidence and Critiques on the Dilution Effect

With ongoing species loss and increasing emergence of infectious diseases, the interest in identifying the effect of species richness on disease risk has been accumulating [12,15,50,167]. Species richness and community composition can influence disease dynamics because many pathogens infect multiple host species that vary in terms of their reservoir competence [18,27,157]. Recent studies suggested that high species richness can reduce disease risk in communities via a dilution effect [15,18,21,31,168]. The dilution effect presents an exciting convergence of conservation and public health interests [11,12,146], and has been reported in many different disease systems [17,18,22,41,42,43,44,45,144,169]. However, uncertainty persists over its generality and its underlying mechanisms [11,15,19]. Understanding the effect of species richness on disease risk is critical to predict changes in pathogen transmission under increasing species loss, and may provide new insights with regard to interventions and control measures.

Previous studies have formulated three prerequisites for the dilution effect (Fig 6.1) [11,18]. They are: 1) host species differ in their reservoir competence; 2) higher quality hosts dominate in species-poor communities, whereas lower quality hosts occur mainly in more diverse communities; 3) species with low reservoir competence reduce disease risk through several mechanisms (mainly through host regulation and encounter reduction, see Table 1.1). However, recent critical studies argued that the last two prerequisites are usually not fulfilled (Fig 6.1) [11,12,19,20,50,146].

In this thesis, I first tested the dilution effect in bovine tuberculosis (BTB) (Chapter 2 & 3). Then, species' reservoir competence was linked to life-history traits to provide support for the second prerequisite in Chapter 4. Finally, in Chapter 5 the effect of habitat connectivity on disease risk was studied using an epidemiological metapopulation model, as species loss is often driven by human disturbance and habitat fragmentation. The results from these chapters can be either interpreted as support (Chapter 2, 3 & 4) or critique (Chapter 5) for the dilution effect. In this Synthesis, these results are brought together in order to gain a better understanding of the mechanisms and generality of the dilution effect. Based on these findings, I here discuss the evidence for and critiques on the dilution effect. Lastly, I draw the main conclusion and suggest a focus for future studies.

Association between local extinction risk and reservoir competence

One of the central prerequisites for the dilution effect is that a species' local extinction risk negatively correlates with its competence to support and transmit pathogens [11,12,18,50]. In this way, species with a lower reservoir competence are expected to go extinct first with increasing species loss, whereas species with a higher reservoir competence are more likely to remain in the community and increase pathogen transmission [18,50]. However, before this thesis, few studies provided support for this prerequisite (critique 2 in Fig. 6.1). Understanding the relationship between a species local extinction risk and their reservoir competence is essential to predict disease dynamics with community changes and understand the generality of the dilution effect.

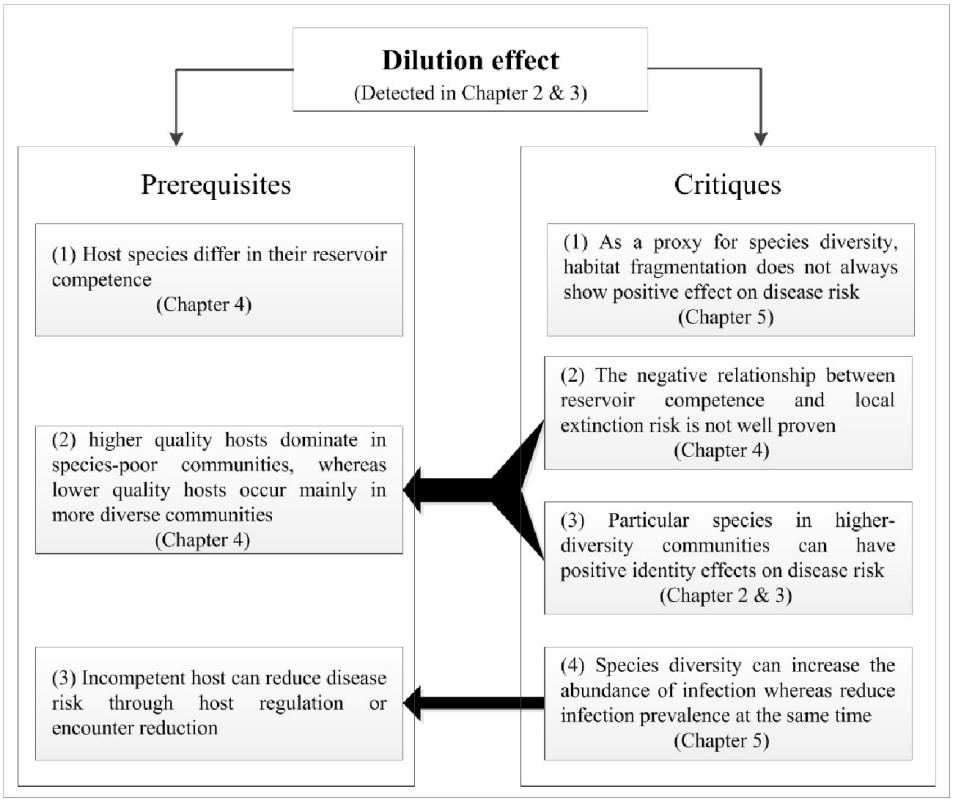

Figure 6.1: Illustration of the prerequisites for and critiques (following the arrows) on the dilution effect.

The variation in a species' reservoir competence can be partly explained by life-history traits [52]. Life-history theory suggests trade-offs with investment in self-maintenance (e.g., physiological resistance) at the expense of other physiological activities, such as current reproduction and growth [54]. Slow-lived species tend to invest more in adaptive immunity because they probably encounter a greater number of infections overall, and are more likely to encounter the same pathogen, whereas fast-lived species which are in favour of growth and frequent reproduction tend to invest comparatively little in costly adaptive immunity and thus usually have a higher competence for pathogens [56,135]. In Chapter 4, the relationships between reservoir competence and life-history traits were tested in three vector-borne

diseases, namely Lyme disease, West Nile encephalitis (WNE) and Eastern Equine encephalitis (EEE). Our results support the life-history theory and show that species with larger body mass (for hosts of Lyme disease and WNE) or smaller clutch size (for hosts of EEE) have lower reservoir competences. Besides my study, such a relationship between life-history traits and reservoir competence has recently also been documented in Barley yellow dwarf virus [52], *Trypanosoma cruzi* infections [53] and trematode parasites [15,46]. On the other hand, an increasing number of studies showed that the risk of extinction is not randomly distributed over the spectrum of species, and some life-history traits predispose species' population declines in human disturbed ecosystems [170,171]. For example, species with longer developmental time and lower fecundity are less able to compensate by increase reproductive output during periods with a relatively higher mortality, and are therefore more vulnerable to population decline within disturbed communities [172,173]. In addition, a larger body mass usually correlates to a larger home range or area requirement [59,174], a lower population density [59,175] and a lower fecundity [126], all of which are associated with a higher local extinction risk [59,174]. Some the life-history traits (such as body mass, clutch size in Chapter 4.) that are correlated to species' reservoir competence might be also associated with the species' local extinction risk. Therefore, a species' reservoir competence and its local extinction risk might be negatively correlated and explained by similar underlying life-history traits (Fig 6.2), and those species with a high reservoir competence are more likely to be those that are widely distributed.

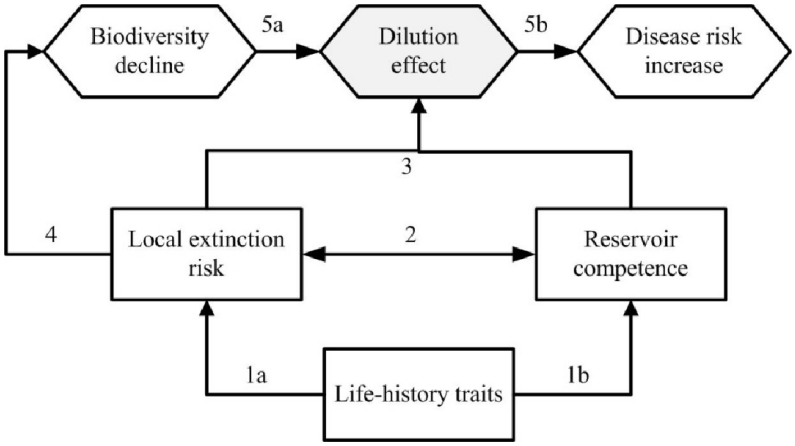

Figure 6.2: Competence-extinction relationship explains the dilution effect. Hexagons are ecological processes. Rectangles are factors that affect ecological processes or correlate to other factors. 1a, 1b: Species' reservoir competence and local extinction risk are linked through life-history traits; 2: Parasite local adaptation also leads to negative relationship between reservoir competence and local extinction risk; 3: Dilution effect occurs because species with lower local extinction risk have higher reservoir competence; 4: Local extinction risk determines which species are lost when biodiversity declines; 5a, 5b: Biodiversity declines increase disease risks through reducing the dilution effect.

The covariance between species' reservoir competence and local extinction risk might also arise due to parasite local adaptation [50]. Parasites can be driven by the selective pressure of losing hosts during community disassembly to evolve to infect the most abundant or widespread host [176,177], which also leads to a negative relationship between reservoir competence and local extinction risk (Fig 6.2). Currently, some studies have suggested that increased abundance of fast-lived species is a common result of human-induced global change [178,179,180,181,182,183]. Shift in host community composition

towards fast-lived species suggested by such a relationship might be a mechanism by which global change increases pathogen transmission rates [52] and hence support the dilution effect.

Although both life-history theory and the local adaptation mechanism provide theoretical grounds for a negative correlation between a species' reservoir competence and local extinction risk, few study directly tested the relationships between reservoir competence and local extinction risks. Studies also pointed out that many stochastic factors are able to influence community composition [184,185,186], which might weaken this negative relationship. The uncertainty in the negative correlation between reservior competence and local extinction risk can create inconsistent effects of host species richness on disease risk, making the dilution effect disappear or even a positive effect of species diversity emerges [50]. Therefore, I suggest to directly test this relationship in future studies, which would be beneficial to better predict disease dynamics in relation to biodiversity declines.

Dilution effect and identity effect

Previous studies have suggested two mechanisms by which species diversity influence ecosystem functioning, the complementarity effect and the identity effect [185,187,188,189]. The complementarity effect influences ecosystem properties through the interactions (or absence of interactions) among species, whereas the identity effect is due to the presence of a key species with a particularly higher or lower contribution than average in communities [187,188]. The identity effect can be caused by a selection effect or a sampling effect whereby increasing species richness results in an increasing probability of such a key species [187,190,191]. In Chapter 2 and 3, the dilution effect of mammal species richness and the identity effect of African buffalo on the presence, persistence and recurrence of bovine tuberculosis (BTB) were tested at regional scale. The results show that mammal species richness has a possible dilution effect whereas African buffalo as a maintenance host species has a positive identity effect on BTB presence, persistence and recurrence. The results indicate that the identity effect and the dilution effect of species richness can operate simultaneously, which is consistent with previous studies regarding the effect of species diversity on ecosystem productivity [187,192] and nutrient retention [193].

Currently, a critique of the dilution effect (critique 3 in Fig. 6.1) is that disease risk might be increased in high-diversity communities due to the presence of some particular species which are heavily disease-prone or main feeders for vectors [11,20]. For example, the presence of deer, as an important bloodmeal host for ticks, can largely increase tick abundance and thus may increase the risk of tick-borne diseases, such as Lyme disease [194,195]. These critical studies argued that increasing species richness can lead to an increased probability of the presence of such a particular species. The dilution effect may be overshaded by the positive identity effects of these species, even a positve relationship between species richness and disease risk can be detected [11,20]. However, others argued that the sampling effect might be a statistic artefact and is not widespread in natural communities, because the communities are arguably not random assemblages of species [196,197]. In my BTB studies (Chapter 2 & 3), I detected a positive correlation between species richness and the occurrence of buffalo ($r = 0.66$, $p < 0.01$), which indicate that the species identity effect could act as an additional mechanism by which species richness influences disease risk.

Regardless of the debate on the identity effect, it is desirable to seperate this effect from other effects in interpreting the effects of species diversity on disease risk, since it is beneficial to better understand the effect caused by increasing species richness or a changing community composition on disease risk [190]. Recently some other studies on the disease-diversity relationship also started to test for a species identity

effect on disease risk. For example, using manipulative experiments, Venesky and co-workers found that amphibian species richness was a significant negative predictor for the abundance of *Batrachochytrium dendrobatidis* (*Bd*), whereas the presence of *Bufo terrestris* was able to amplify *Bd* abundance because of its high susceptibility [167]. When examining the effects of tree species richness on the risk of foliar fungal pathogens in Germany, Hantsch and colleagues found that tree species richness was negatively correlated with the pathogen load of common mildew species, while the presence of *Quercus* as a particular disease-prone species was correlated with a high pathogen load at plot level [191]. These studies, together with the BTB studies in Chapter 2 and 3, are among the first to test the species' identity effect with regard to the effect of species diversity on disease risk.

The prevalence and the abundance of infection

To quantify the risk of an endemic disease, scientists usually use the prevalence and the abundance of infection (either infected hosts or infected vectors) to study the dynamics of pathogen transmission. When testing the effect of species richness, many studies with regard to the dilution effect used the infection prevalence as an index of disease risk [38,40,41,43,44]. For examples, in the Great Basin Desert there was a negative relationship between rodent species diversity and the infection prevalence of the Sin Nombre virus infection in deer mice [44]. The prevalence of the West Nile virus infection in mosquito vectors increased with decreasing bird diversity in North America [41]. Along areas at the western coast of the USA, the infection prevalence of *Phytophthora ramorum* for competent hosts, bay laurel and tanoak, was negatively correlated with tree species richness in forest communities [22]. However, many studies suggested that the number of infected individuals might be a more suitable indicator for disease risk to humans since wildlife-human contacts are usually density-dependent [19,20,146]. However, infection prevalence and the number of infection may show different relationships with species diversity. For example, a theoretical study incorporating empirical laws of community assemblage found that high species richness can reduce the infection prevalence while the number of total infected hosts in the community increases [78]. Hence, I suggest that both the prevalence and the abundance of infection should be reported to better understand the determinants of disease risk.

Another critique on the dilution effect (critique 4 in Fig. 6.1) exists when species diversity has a negative effect on infection prevalence whereas no or even a positive effect on the abundance of infection. The abundance of infection, as the product of infection prevalence and host/vector population size, may increase when host/vector population increases, even if the infection prevalence decreases. When testing the effect of connectivity on disease risk in metapopulations, I demonstrated that different indicators of disease risk (infection prevalence and number of infected hosts) react differently to increasing connectivity (Chapter 5). When the infection prevalence decreases with increasing connectivity (along with increasing species richness), the number of infected hosts can also increase since increasing connectivity is expected to increase host abundance. Therefore, the negative correlation of species richness with infection prevalence would be easier to be detected than with the number of infected hosts (Chapter 5). For vector-borne diseases, a higher species richness may provide more bloodmeals for vectors and increase vector abundance, especially tick species which are sit-and-wait species relying on host movement to facilitate encounters with hosts for feeding [11,20,198]. In this way, the abundance of infected vectors, as an index for human disease risk, can increase with increasing species richness even if the infection prevalence of vectors decreases [11,20,38]. Therefore, it seems that for both direct-transmitted and vector-borne diseases, it is easier to detect a dilution effect using the proportion of infection as risk indicator than using the abundance of infection.

Fragmentation and dilution effect

Habitat fragmentation can considerably change the dynamics of disease transmission through modifying host movements, host/vector density, community composition and micro-environments [9]. Controversal results have been found in previous empirical studies investigating the effect of habitat fragmentation on disease risk, even in the same disease systems [19]. For example, nymphal infection prevalence and the density of infected nymphs for Lyme disease were positively correlated with fragmentation in New York & Connecticut [39,161], whereas no relationship was found across New York, Connecticut and New Jersey [48]. These inconsistent results indicate that habitat framentation may have a complex role in pathogen transmission.

Habitat fragmentation can cause species loss [72], which may lead to an increased disease risk due to a reduced dilution effect [31,39]. On the other hand, reduced connectivity among fragmented patches can limit host movements and thus the transmission of pathogens carried by hosts [151,199]. Combining these two effects of fragmentation, I investigated how connectivity affects disease risk in metapopulations in chapter 5. I found that disease risks may show non-linear relationships with connectivity. The net impact of connectivity depends on the relative importance of the dilution effect (by increased species richness) versus the facilitation effect (by increasing contact rates among patches). These results indicate that the effect of species richness is not only a consequence of host species richness, but also of the landscape configuration. Besides contact rates among patches, fragmentation also influences host density and vector density which can affect disease risk [9]. For example, when mapping the risk of Lyme disease, Estrada-Peña found that tick density was higher in habitats with higher connectivity [67], which can counteract the dilution effect in these habitats [66]. Therefore, the dilution effect, if it occurs, may be confused by other effects of factors which change community composition. A future challenge for studies investigating the effect of species richness on disease risk will be to include the effects of those factors modifying species richness.

Outlook

The dilution effect hypothesis has been investigated in many studies with different diseases, but its generality and mechanisms are still highly disputed. The uncertainty in the effect of species richness may be caused by the confounding factors which can influence pathogen transmission. Many climatic and environmental factors can largely affect pathogen transmission through modifying host/vector density, species susceptibility/infectivity, pathogen survival and so on [9]. One direction for future studies with regard to the disease-diversity relationship is to use manipulative experiments, which currently only a few studies conducted [146,167], to control for these confounding factors. Moreover, manipulative experiments and well-designed studies are also needed to test the underlying mechanisms by which species diversity influence disease risk [17,167]. For example, by monitoring the intra- and interspecific encounters of deer mice in foraging arenas at five sites in Great Basin Desert to test the dilution effect of rodent species diversity in Sin Nombre virus and explore the underlying mechanisms, Clay and colleagues detected a dilution effect and suggested the encounter reduction mechanism that intraspecific interactions between deer mice was reduced with increased diversity [47].

When investigating the effect of species richness on disease risk at a higher level, such as landscape level, future studies should take into account habitat configuration and environmental context, since they are able to influence pathogen transmission and community composition simultaneously. This thesis, for example, has demonstrated that habitat fragmentation can affect disease risk in a complex way. I propose

that the advances in studies of patch connectivity (e.g., graph theory), together with classical epidemiological models and field studies, can provide powerful tools to increase our understanding of the epidemiological processes underlying the infection pattern at landscape level.

In addition, increasing studies have shown that reservoir competence not only varies in different species, but also shows genetic heterogeneity that different genotypes of a same species may show different competence to transmit pathogens [18,200,201,202]. For example, a mesocosm study showed that the prevalence of *Octosporea bayeri* in *Daphnia magna* water fleas was consistently lower in host populations with higher than lower genetic diversity, which might be caused by the encounter reduction mechanism that those less susceptible *Daphnia* genotypes act as dead-end hosts when they filter parasites that might otherwise infect susceptible genotypes [203]. A next important step in the field of disease ecology could be to investigate the effect of genetic diversity on the dynamics of pathogen transmission.

Conclusion

Understanding the effect of species richness on disease risk in communities is essential to predict disease dynamics with ongoing biodiversity declines. The aim of this thesis is to test a controversial hypothesis, the dilution effect, and investigate the underlying mechanisms. I showed that there is a possible dilution effect of mammal species richness on the presence, recurrence and persistence of BTB. African buffalo can exert a positive identity effect that increase BTB risk (Chapter 2 & 3). I suggest that the identity effect could be an additional mechanism by which species richness influences disease risk. I also demonstrated that the variation in host competence to support and transmit pathogens can be partly explained by species' life-history traits that are linked to species' local extinction risk, which provides support for the dilution effect hypothesis (Chapter 4). In chapter 5, I showed that habitat connectivity can both increase or decrease disease risk, and the net impact of connectivity on disease risk was dependent on the relative importance of the dilution effect (due to the increasing species richness) versus the facilitation effect (caused by increasing contact rates among patches). I propose that future studies investigating the effect of species richness on disease risk should also consider those factors (such as fragmentation) that can simultaneously affect pathogen transmission and community composition, in order to get a better understanding of the roles played by such factors. In addition, different indicators of disease risk (e.g., prevalence and abundance of infections) may show different relationship with species richness (Chapter 5). This might be one of the reasons that inconsistent results were suggested by previous studies regarding to the dilution effect. In general, the results of this thesis support the dilution effect hypothesis and indicate under what conditions it can be found. I propose that a future direction would be to conduct manipulative experiments to study the effect of species richness on disease risk and the underlying mechanisms.

References

1. Cleaveland S, Laurenson MK, Taylor LH (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 356: 991-999.
2. Gilbert M, Xiao XM, Pfeiffer DU, Epprecht M, Boles S, et al. (2008) Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. Proceedings of the National Academy of Sciences of the United States of America 105: 4769-4774.
3. Hudson PJ, Dobson AP, Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? Trends in Ecology & Evolution 21: 381-385.
4. Prins HHT, van der Jeugd HP (1993) Herbivore Population Crashes and Woodland Structure in East-Africa. Journal of Ecology 81: 305-314.
5. Nunn CL, Thrall PH, Stewart K, Harcourt AH (2008) Emerging infectious diseases and animal social systems. Evolutionary Ecology 22: 519-543.
6. Organization WH (1999) Removing obstacles to healthy development: report on infectious diseases. Geneva: World Health Organization.
7. Levy S (2013) The Lyme Disease Debate Host Biodiversity and Human Disease Risk. Environmental Health Perspectives 121: A120-A125.
8. Riley S (2007) Large-scale spatial-transmission models of infectious disease. Science 316: 1298-1301.
9. Estrada-Peña A, Ostfeld RS, Peterson AT, Poulin R, de la Fuente J (2014) Effects of environmental change on zoonotic disease risk: an ecological primer. Trends in Parasitology.
10. Tompkins DM, Dunn AM, Smith MJ, Telfer S (2011) Wildlife diseases: from individuals to ecosystems. Journal of Animal Ecology 80: 19-38.
11. Randolph SE, Dobson ADM (2012) Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139: 847-863.
12. Young H, Griffin RH, Wood CL, Nunn CL (2013) Does habitat disturbance increase infectious disease risk for primates? Ecology Letters 16: 656-663.
13. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, et al. (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468: 647-652.
14. Ostfeld RS, Keesing F (2012) Effects of Host Diversity on Infectious Disease. Annual Review of Ecology Evolution and Systematics 43: 157-182.
15. Johnson PTJ, Preston DL, Hoverman JT, Richgels KLD (2013) Biodiversity decreases disease through predictable changes in host community competence. Nature 494: 230-233.
16. Bonds MH, Dobson AP, Keenan DC (2012) Disease Ecology, Biodiversity, and the Latitudinal Gradient in Income. Plos Biology 10.
17. Johnson PTJ, Thieltges DW (2010) Diversity, decoys and the dilution effect: how ecological communities affect disease risk. Journal of Experimental Biology 213: 961-970.
18. Ostfeld RS, Keesing F (2012) Effects of Host Diversity on Infectious Disease. Annual Review of Ecology, Evolution, and Systematics, Vol 43 43: 157-182.
19. Salkeld DJ, Padgett KA, Jones JH (2013) A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16: 679-686.
20. Wood CL, Lafferty KD (2013) Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology & Evolution 28: 239-247.
21. Keeling MJ, Rohani P (2008) Modeling infectious diseases in humans and animals: Princeton University Press.

22. Haas SE, Hooten MB, Rizzo DM, Meentemeyer RK (2011) Forest species diversity reduces disease risk in a generalist plant pathogen invasion. Ecology Letters 14: 1108-1116.
23. Holt RD, Dobson AP, Begon M, Bowers RG, Schauber EM (2003) Parasite establishment in host communities. Ecology Letters 6: 837-842.
24. Lloyd-Smith JO, Cross PC, Briggs CJ, Daugherty M, Getz WM, et al. (2005) Should we expect population thresholds for wildlife disease? TRENDS in Ecology & Evolution 20: 511-519.
25. Porphyre T, Stevenson MA, McKenzie J (2008) Risk factors for bovine tuberculosis in New Zealand cattle farms and their relationship with possum control strategies. Preventive Veterinary Medicine 86: 93-106.
26. Mathews F, Macdonald DW, Taylor GM, Gelling M, Norman RA, et al. (2006) Bovine tuberculosis (Mycobacterium bovis) in British farmland wildlife: the importance to agriculture. Proceedings of the Royal Society B-Biological Sciences 273: 357-365.
27. Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD (2006) Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society B-Biological Sciences 273: 2327-2333.
28. LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F (2003) The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proc Natl Acad Sci U S A 100: 567-571.
29. Gilbert GS, Webb CO (2007) Phylogenetic signal in plant pathogen-host range. Proceedings of the National Academy of Sciences of the United States of America 104: 4979-4983.
30. Holt RD, Roy M (2007) Predation can increase the prevalence of infectious disease. American Naturalist 169: 690-699.
31. Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. Ecology Letters 9: 485-498.
32. Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441: 629-632.
33. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, et al. (2012) Biodiversity loss and its impact on humanity. Nature 486: 59-67.
34. Macdonald G (1956) Epidemiological basis of malaria control. Bulletin of the World Health Organization 15: 613.
35. Hess A, Hayes RO (1970) Relative potentials of domestic animals for zooprophylaxis against mosquito vectors of encephalitis. American Journal of Tropical Medicine and Hygiene 19: 327-334.
36. Ostfeld R, Keesing F (2000) The function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology-Revue Canadienne De Zoologie 78: 2061-2078.
37. Ostfeld RS, Keesing F (2000) Biodiversity and disease risk: The case of lyme disease. Conservation Biology 14: 722-728.
38. Schmidt KA, Ostfeld RS (2001) Biodiversity and the dilution effect in disease ecology. Ecology 82: 609-619.
39. Allan BF, Keesing F, Ostfeld RS (2003) Effect of forest fragmentation on Lyme disease risk. Conservation Biology 17: 267-272.
40. LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences of the United States of America 100: 567-571.
41. Allan BF, Langerhans RB, Ryberg WA, Landesman WJ, Griffin NW, et al. (2009) Ecological correlates of risk and incidence of West Nile virus in the United States. Oecologia 158: 699-708.
42. Dizney LJ, Ruedas LA (2009) Increased Host Species Diversity and Decreased Prevalence of Sin Nombre Virus. Emerging Infectious Diseases 15: 1012-1018.
43. Suzan G, Marce E, Giermakowski JT, Mills JN, Ceballos G, et al. (2009) Experimental Evidence for Reduced Rodent Diversity Causing Increased Hantavirus Prevalence. PLoS One 4.
44. Clay CA, Lehmer EM, Jeor SS, Dearing MD (2009) Sin Nombre Virus and Rodent Species Diversity: A Test of the Dilution and Amplification Hypotheses. PLoS One 4.
45. Johnson PTJ, Lund PJ, Hartson RB, Yoshino TP (2009) Community diversity reduces Schistosoma mansoni transmission, host pathology and human infection risk. Proceedings of the Royal Society B-Biological Sciences 276: 1657-1663.

46. Johnson PTJ, Rohr JR, Hoverman JT, Kellermanns E, Bowerman J, et al. (2012) Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. Ecology Letters 15: 235-242.
47. Clay CA, Lehmer EM, St Jeor S, Dearing MD (2009) Testing Mechanisms of the Dilution Effect: Deer Mice Encounter Rates, Sin Nombre Virus Prevalence and Species Diversity. Ecohealth 6: 250-259.
48. Logiudice K, Duerr STK, Newhouse MJ, Schmidt KA, Killilea ME, et al. (2008) Impact of Host Community Composition on Lyme Disease Risk. Ecology 89: 2841-2849.
49. Dobson A (2004) Population dynamics of pathogens with multiple host species. American Naturalist 164: S64-S78.
50. Joseph MB, Mihaljevic JR, Orlofske SA, Paull SH (2013) Does life history mediate changing disease risk when communities disassemble? Ecology Letters 16: 1405-1412.
51. Rudolf VHW, Antonovics J (2005) Species coexistence and pathogens with frequency-dependent transmission. American Naturalist 166: 112-118.
52. Cronin JP, Welsh ME, Dekkers MG, Abercrombie ST, Mitchell CE (2010) Host physiological phenotype explains pathogen reservoir potential. Ecology Letters 13: 1221-1232.
53. Gottdenker NL, Chaves LF, Calzada JE, Saldana A, Carroll CR (2012) Host Life History Strategy, Species Diversity, and Habitat Influence Trypanosoma cruzi Vector Infection in Changing Landscapes. PLoS Neglected Tropical Diseases 6.
54. Miller MR, White A, Boots M (2007) Host life span and the evolution of resistance characteristics. Evolution 61: 2-14.
55. Lee KA, Wikelski M, Robinson WD, Robinson TR, Klasing KC (2008) Constitutive immune defences correlate with life-history variables in tropical birds. Journal of Animal Ecology 77: 356-363.
56. Martin LB, Hasselquist D, Wikelski M (2006) Investment in immune defense is linked to pace of life in house sparrows. Oecologia 147: 565-575.
57. Webster JP, Woolhouse MEJ (1999) Cost of resistance: relationship between reduced fertility and increased resistance in a snail-schistosome host-parasite system. Proceedings of the Royal Society of London Series B-Biological Sciences 266: 391-396.
58. Previtali MA, Ostfeld RS, Keesing F, Jolles AE, Hanselmann R, et al. (2012) Relationship between pace of life and immune responses in wild rodents. Oikos: no-no.
59. Cardillo M (2003) Biological determinants of extinction risk: why are smaller species less vulnerable? Animal Conservation 6: 63-69.
60. Davidson AD, Hamilton MJ, Boyer AG, Brown JH, Ceballos G (2009) Multiple ecological pathways to extinction in mammals. Proceedings of the National Academy of Sciences of the United States of America 106: 10702-10705.
61. Hicks TA, McGlone JJ, Whisnant CS, Kattesh HG, Norman RL (1998) Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. Journal of Animal Science 76: 474-483.
62. Jesse M, Ezanno P, Davis S, Heesterbeek JAP (2008) A fully coupled, mechanistic model for infectious disease dynamics in a metapopulation: Movement and epidemic duration. J Theor Biol 254: 331-338.
63. Jesse M, Heesterbeek H (2011) Divide and conquer? Persistence of infectious agents in spatial metapopulations of hosts. J Theor Biol 275: 12-20.
64. Wint GRW, Robinson TP, Bourn DM, Durr PA, Hay SI, et al. (2002) Mapping bovine tuberculosis in Great Britain using environmental data. Trends in Microbiology 10: 441-444.
65. Rhodes SG, De Leij FAAM, Dale JW (2007) Protozoa as an environmental reservoir of bovine tuberculosis. Trends in Microbiology 15: 338-339.
66. Estrada-Pena A (2009) Diluting the dilution effect: a spatial Lyme model provides evidence for the importance of habitat fragmentation with regard to the risk of infection. Geospatial Health 3: 143-155.
67. Estrada-Pena A (2003) The relationships between habitat topology, critical scales of connectivity and tick abundance Ixodes ricinus in a heterogeneous landscape in northern Spain. Ecography 26: 661-671.
68. Oorebeek M, Kleindorfer S (2008) Climate or host availability: what determines the seasonal abundance of ticks? Parasitology Research 103: 871-875.

69. Lindgren E, Talleklint L, Polfeldt T (2000) Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick Ixodes ricinus. Environmental Health Perspectives 108: 119-123.
70. Skuce RA, Allen AR, McDowell SW (2012) Herd-level risk factors for bovine tuberculosis: a literature review. Vet Med Int 2012: 621210.
71. Allepuz A, Casal J, Napp S, Saez M, Alba A, et al. (2011) Analysis of the spatial variation of Bovine tuberculosis disease risk in Spain (2006-2009). Preventive Veterinary Medicine 100: 44-52.
72. Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annual Review of Ecology Evolution and Systematics 34: 487-515.
73. Munyeme M, Muma JB, Skjerve E, Nambota AM, Phiri IGK, et al. (2008) Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia. Preventive Veterinary Medicine 85: 317-328.
74. Renwick AR, White PCL, Bengis RG (2007) bovine tuberculosis in southern Africa wildlife: a multi-species host-pathogen system. Epidemiology and Infection 135: 529-540.
75. Humblet MF, Boschiroli ML, Saegerman C (2009) Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Veterinary Research 40.
76. Olff H, Ritchie ME, Prins HHT (2002) Global environmental controls of diversity in large herbivores. Nature 415: 901-904.
77. Corner LAL (2006) The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: How to assess the risk. Veterinary Microbiology 112: 303-312.
78. Roche B, Dobson AP, Guegan JF, Rohani P (2012) Linking community and disease ecology: the impact of biodiversity on pathogen transmission. Philosophical Transactions of the Royal Society B-Biological Sciences 367: 2807-2813.
79. Huang ZYX, de Boer WF, van Langevelde F, Olson V, Blackburn TM, et al. (2013) Species' life-history traits explain interspecific variation in reservoir competence: a possible mechanism underlying the dilution effect. Plos One 8: e54341.
80. Cleaveland S, Shaw DJ, Mfinanga SG, Shirima G, Kazwala RR, et al. (2007) Mycobacterium bovis in rural Tanzania: Risk factors for infection in human and cattle populations. Tuberculosis 87: 30-43.
81. Caron A, Cross PC, Du Toit JT (2003) Ecological implications of bovine tuberculosis in African buffalo herds. Ecological Applications 13: 1338-1345.
82. Omer MK, Skjerve E, Woldehiwet Z, Holstad G (2001) A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea. Tropical Animal Health and Production 33: 295-303.
83. Oloya J, Muma JB, Opuda-Asibo J, Djonne B, Kazwala R, et al. (2007) Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda. Preventive Veterinary Medicine 80: 318-329.
84. Tschopp R, Schelling E, Hattendorf J, Aseffa A, Zinsstag J (2009) Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. Preventive Veterinary Medicine 89: 205-211.
85. Gilbert M, Mitchell A, Bourn D, Mawdsley J, Cliton-Hadley R, et al. (2005) Cattle movements and bovine tuberculosis in Great Britain. Nature 435: 491-496.
86. Green DM, Kiss IZ, Mitchell AP, Kao RR (2008) Estimates for local and movement-based transmission of bovine tuberculosis in British cattle. Proceedings of the Royal Society B-Biological Sciences 275: 1001-1005.
87. Glavanakov S, White DJ, Caraco T, Lapenis A, Robinson GR, et al. (2001) Lyme disease in New York State: Spatial pattern at a regional scale. American Journal of Tropical Medicine and Hygiene 65: 538-545.
88. Biotani L (1998) A databank for the conservation and management of the African mammals. In: Biotani L, editor. Rome, Italy: Institute of Applied Ecology.
89. Boitani L, Sinibaldi I, Corsi F, De Biase A, Carranza ID, et al. (2008) Distribution of medium- to large-sized African mammals based on habitat suitability models. Biodiversity and Conservation 17: 605-621.
90. Prins HHT, Olff H (1998) Species richness of African grazer assemblages: towards a functional explanation. In: Brown ND, Prins HHT, editors. Dynamics of tropical communities. Oxford: Blackwell Science. pp. 449 - 490.

91. Si YL, Wang TJ, Skidmore AK, de Boer WF, Li L, et al. (2010) Environmental Factors Influencing the Spread of the Highly Pathogenic Avian Influenza H5N1 Virus in wild birds in Europe. Ecology and Society 15.
92. Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I (2004) Bovine tuberculosis: an old disease but a new threat to Africa. International Journal of Tuberculosis and Lung Disease 8: 924-937.
93. de Boer WF, Vis MJP, de Knegt HJ, Rowles C, Kohi EM, et al. (2010) Spatial distribution of lion kills determined by the water dependency of prey species. Journal of Mammalogy 91: 1280-1286.
94. Menard S (2004) Six approaches to calculating standardized logistic regression coefficients. American Statistician 58: 218-223.
95. Rigdon EE (1997) LISREL approaches to interaction effects in multiple regression - Jaccard,J, Wan,CK. Journal of Marketing Research 34: 537-540.
96. Epstein LH, Wisniewski L, Weng R (1994) Child and parent psychological problems influence child weight control. Obes Res 2: 509-515.
97. Barlow ND, Kean JM, Hickling G, Livingstone PG, Robson AB (1997) A simulation model for the spread of bovine tuberculosis within New Zealand cattle herds. Preventive Veterinary Medicine 32: 57-75.
98. Levin SA, Grenfell B, Hastings A, Perelson AS (1997) Mathematical and computational challenges in population biology and ecosystems science. Science 275: 334-343.
99. White PCL, Benhin JKA (2004) Factors influencing the incidence and scale of bovine tuberculosis in cattle in southwest England. Preventive Veterinary Medicine 63: 1-7.
100. Woodroffe R, Donnelly CA, Cox DR, Bourne FJ, Cheeseman CL, et al. (2006) Effects of culling on badger Meles meles spatial organization: implications for the control of bovine tuberculosis. Journal of Applied Ecology 43: 1-10.
101. Ostfeld RS, Glass GE, Keesing F (2005) Spatial epidemiology: an emerging (or re-emerging) discipline. TRENDS in Ecology & Evolution 20: 328-336.
102. Skuce RA, Allen AR, McDowell SW (2012) Herd-level risk factors for bovine tuberculosis: a literature review. Veterinary Medicine International 2012: 621210.
103. De Garine-Wichatitsky M, Caron A, Kock R, Tschopp R, Munyeme M, et al. (2013) A review of bovine tuberculosis at the wildlife-livestock-human interface in sub-Saharan Africa. Epidemiology and Infection 141: 1342-1356.
104. O'Brien DJ, Schmitt SM, Rudolph BA, Nugent G (2011) Recent advances in the management of bovine tuberculosis in free-ranging wildlife. Veterinary Microbiology 151: 23-33.
105. Huang ZYX, de Boer WF, van Langevelde F, Xu C, Ben Jebara K, et al. (2013) Dilution effect in bovine tuberculosis: risk factors for regional disease occurrence in Africa. Proceedings of the Royal Society B-Biological Sciences 280: 20130624.
106. Ostfeld RS, Keesing F (2012) Effects of host diversity on infectious disease. Annual Review of Ecology, Evolution, and Systematics 43: 157-182.
107. Randolph SE, Dobson AD (2012) Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology: 1-17.
108. Ostfeld RS (2013) A Candide response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. Parasitology 140: 1196-1198.
109. Hantsch L, Braun U, Scherer-Lorenzen M, Bruelheide H (2013) Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. Ecosphere 4: art81.
110. Ben Jebara K, Caceres P, Berlingieri F, Weber-Vintzel L (2012) Ten years' work on the World Organisation for Animal Health (OIE) Worldwide Animal Disease Notification System. Preventive Veterinary Medicine 107: 149-159.
111. Brooks-Pollock E, Keeling M (2009) Herd size and bovine tuberculosis persistence in cattle farms in Great Britain. Preventive Veterinary Medicine 92: 360-365.
112. Karolemeas K, McKinley TJ, Clifton-Hadley RS, Goodchild AV, Mitchell A, et al. (2011) Recurrence of bovine tuberculosis breakdowns in Great Britain: Risk factors and prediction. Preventive Veterinary Medicine 102: 22-29.

113. Humblet MF, Gilbert M, Govaerts M, Fauville-Dufaux M, Walravens K, et al. (2010) New Assessment of Bovine Tuberculosis Risk Factors in Belgium Based on Nationwide Molecular Epidemiology. Journal of Clinical Microbiology 48: 2802-2808.
114. Mathews F, Lovett L, Rushton S, Macdonald DW (2006) Bovine tuberculosis in cattle: Reduced risk on wildlife-friendly farms. Biology Letters 2: 271-274.
115. Vial F, Donnelly CA (2012) Localized reactive badger culling increases risk of bovine tuberculosis in nearby cattle herds. Biology Letters 8: 50-53.
116. White PW, Martin SW, De Jong MCM, O'Keeffe JJ, More SJ, et al. (2013) The importance of 'neighbourhood' in the persistence of bovine tuberculosis in Irish cattle herds. Preventive Veterinary Medicine 110: 346-355.
117. Harris I, Jones PD, Osborn TJ, Lister DH (2013) Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. International Journal of Climatology: in press.
118. Martin V, Pfeiffer DU, Zhou XY, Xiao XM, Prosser DJ, et al. (2011) Spatial Distribution and Risk Factors of Highly Pathogenic Avian Influenza (HPAI) H5N1 in China. PLOS Pathogens 7.
119. Howery LD, DeLiberto TJ (2004) Indirect effects of carnivores on livestock foraging behavior and production. Sheep & Goat Research Journal: 8.
120. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, et al. (2008) Global trends in emerging infectious diseases. Nature 451: 990-U994.
121. Cronin JP, Welsh ME, Dekkers MG, Abercrombie ST, Mitchell CE (2010) Host physiological phenotype explains pathogen reservoir potential. Ecol Lett 13: 1221-1232.
122. Brunner JL, LoGiudice K, Ostfeld RS (2008) Estimating reservoir competence of *Borrelia burgdorferi* hosts: Prevalence and infectivity, sensitivity, and specificity. Journal of Medical Entomology 45: 139-147.
123. Komar N, Dohm DJ, Turell MJ, Spielman A (1999) Eastern equine encephalitis virus in birds: Relative competence of European starlings (*Sturnus vulgaris*). American Journal of Tropical Medicine and Hygiene 60: 387-391.
124. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, et al. (2003) Experimental infection of north American birds with the New York 1999 strain of West Nile virus. Emerging Infectious Diseases 9: 311-322.
125. Johnson PT, Rohr JR, Hoverman JT, Kellermanns E, Bowerman J, et al. (2012) Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. Ecol Lett 15: 235-242.
126. McKenzie NL, Burbidge AA, Baynes A, Brereton RN, Dickman CR, et al. (2007) Analysis of factors implicated in the recent decline of Australia's mammal fauna. Journal of Biogeography 34: 597-611.
127. Cunningham AA, Dobson AP, Hudson PJ (2012) Disease invasion: impacts on biodiversity and human health Introduction. Philosophical Transactions of the Royal Society B-Biological Sciences 367: 2804-2806.
128. Eisen L, Dolan MC, Piesman J, Lane RS (2003) Vector competence of *Ixodes pacificus* and *I. spinipalpis* (Acari : ixodidae), and reservoir competence of the dusky-footed woodrat (*Neotoma fuscipes*) and the deer mouse (*Peromyscus maniculatus*), for *Borrelia bissettii*. Journal of Medical Entomology 40: 311-320.
129. Kilpatrick AM, LaDeau SL, Marra PP (2007) Ecology of west nile virus transmission and its impact on birds in the western hemisphere. Auk 124: 1121-1136.
130. Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, et al. (2008) A phylogenomic study of birds reveals their evolutionary history. Science 320: 1763-1768.
131. Felsenstein J (1985) Phylogenies and the comparative method. American Naturalist 125: 1-15.
132. Bininda-Emonds ORP, Cardillo M, Jones KE, MacPhee RDE, Beck RMD, et al. (2007) The delayed rise of present-day mammals. Nature 446: 507-512.
133. Martins EP, Hansen TF (1997) Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. American Naturalist 149: 646-667.
134. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289-290.
135. Lee KA (2006) Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology 46: 1000-1015.

136. Rantala MJ, Roff DA (2005) An analysis of trade-offs in immune function, body size and development time in the Mediterranean Field Cricket, Gryllus bimaculatus. Functional Ecology 19: 323-330.
137. Robinson JG, Redford KH (1986) Body Size, Diet, and Population-Density of Neotropical Forest Mammals. American Naturalist 128: 665-680.
138. Henle K, Davies KF, Kleyer M, Margules C, Settele J (2004) Predictors of species sensitivity to fragmentation. Biodiversity and Conservation 13: 207-251.
139. Wood CL, Lafferty KD (2012) Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology and Evulotion In press.
140. Allan BF, Keesing F, S. Ostfeld RS (2003) Effects of forest fragmentation on Lyme disease risk. Conserv Biol 17: 267-272.
141. Allan BF, Langerhans RB, Ryberg WA, Landesman WJ, Griffin NW, et al. (2009) Ecological correlates of risk and incidence of West Nile virus in the United States. Oecologia 158: 699-708.
142. Haas SE, Hooten MB, Rizzo DM, Meentemeyer RK (2011) Forest species diversity reduces disease risk in a generalist plant pathogen invasion. Ecol Lett 14: 1108-1116.
143. Searle CL, Biga LM, Spatafora JW, Blaustein AR (2011) A dilution effect in the emerging amphibian pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences of the United States of America 108: 16322-16326.
144. Swaddle JP, Calos SE (2008) Increased avian diversity is associated with lower incidence of human West Nile infection: observation of the dilution effect. PLoS One 3: e2488.
145. Mace GM, Norris K, Fitter AH (2012) Biodiversity and ecosystem services: a multilayered relationship. Trends in Ecology & Evolution 27: 19-26.
146. Wood CL, Lafferty KD, DeLeo G, Young HS, Hudson PJ, et al. (2014) Does biodiversity protect humans against infectious disease? Ecology 95: 817-832.
147. Begon M, Ostfeld R, Keesing F, Eviner V (2008) Effects of host diversity on disease dynamics. Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems: Princeton University Press.
148. Huang ZYX, de Boer WF, van Langevelde F, Xu C, Ben Jebara K, et al. (2013) Dilution effect in bovine tuberculosis: risk factors for regional disease occurrence in Africa. Proceedings of the Royal Society B-Biological Sciences 280.
149. Roche B, Dobson AP, Guegan JF, Rohani P (2012) Linking community and disease ecology: the impact of biodiversity on pathogen transmission. Philosophical Transactions Of The Royal Society Of London Series B-biologic 367: 2807-2813.
150. Cross PC, Lloyd-Smith JO, Johnson PLF, Getz WM (2005) Duelling timescales of host movement and disease recovery determine invasion of disease in structured populations. Ecology Letters 8: 587-595.
151. Hess G (1996) Disease in metapopulation models: Implications for conservation. Ecology 77: 1617-1632.
152. Colizza V, Vespignani A (2007) Invasion threshold in heterogeneous metapopulation networks. Physical Review Letters 99.
153. Hakoyama H, Iwasa Y (2000) Extinction risk of a density-dependent population estimated from a time series of population size. J Theor Biol 204: 337-359.
154. Roche B, Rohani P, Dobson AP, Guegan JF (2013) The Impact of Community Organization on Vector-Borne Pathogens. American Naturalist 181: 1-11.
155. Martin II LB, Hasselquist D, Wikelski M (2006) Investment in immune defense is linked to pace of life in house sparrows. Oecologia 147: 565-575.
156. Previtali MA, Ostfeld RS, Keesing F, Jolles AE, Hanselmann R, et al. (2012) Relationship between pace of life and immune responses in wild rodents. Oikos 121: 1483-1492.
157. Huang ZY, de Boer WF, van Langevelde F, Olson V, Blackburn TM, et al. (2013) Species' life-history traits explain interspecific variation in reservoir competence: a possible mechanism underlying the dilution effect. PLoS ONE 8: e54341.
158. DeLeo GA, Dobson AP (1996) Allometry and simple epidemic models for microparasites. Nature 379: 720-722.

159. Cardillo M (2003) Biological determinants of extinction risk: why are smaller species less vulnerable? Animal Conservation 6: 63-69.
160. Boyle SA, Smith AT (2010) Can landscape and species characteristics predict primate presence in forest fragments in the Brazilian Amazon? Biological Conservation 143: 1134-1143.
161. Brownstein JS, Skelly DK, Holford TR, Fish D (2005) Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. Oecologia 146: 469-475.
162. Lafferty KD, Wood CL (2013) It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. Trends in Ecology & Evolution 28: 503-504.
163. Becker CG, Zamudio KR (2011) Tropical amphibian populations experience higher disease risk in natural habitats. Proceedings of the National Academy of Sciences of the United States of America 108: 9893-9898.
164. Fisher MC, Garner TWJ, Walker SF (2009) Global Emergence of Batrachochytrium dendrobatidis and Amphibian Chytridiomycosis in Space, Time, and Host. Annual Review of Microbiology 63: 291-310.
165. Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, et al. (2006) Seasonality and the dynamics of infectious diseases. Ecology Letters 9: 467-484.
166. Keeling MJ, Rohani P, Grenfell BT (2001) Seasonally forced disease dynamics explored as switching between attractors. Physica D 148: 317-335.
167. Venesky M, Liu X, Sauer E, Rohr J (2013) Linking manipulative experiments to field data to test the dilution effect. Integrative and Comparative Biology 53: E385-E385.
168. Ostfeld RS (2009) Biodiversity loss and the rise of zoonotic pathogens. Clinical Microbiology and Infection 15: 40-43.
169. Johnson PTJ, Preston DL, Hoverman JT, Henderson JS, Paull SH, et al. (2012) Species diversity reduces parasite infection through cross-generational effects on host abundance. Ecology 93: 56-64.
170. Bennett PM, Owens IPF (2002) The evolutionary ecology of birds. Oxford: UK: Oxford University Press.
171. Schipper J, Chanson JS, Chiozza F, Cox NA, Hoffmann M, et al. (2008) The status of the world's land and marine mammals: Diversity, threat, and knowledge. Science 322: 225-230.
172. Lees AC, Peres CA (2008) Avian life-history determinants of local extinction risk in a hyper-fragmented neotropical forest landscape. Animal Conservation 11: 128-137.
173. Purvis A, Gittleman JL, Cowlishaw G, Mace GM (2000) Predicting extinction risk in declining species. Proceedings of the Royal Society B-Biological Sciences 267: 1947-1952.
174. Biedermann R (2003) Body size and area-incidence relationships: is there a general pattern? Global Ecology and Biogeography 12: 381-387.
175. Blackburn TM, Brown VK, Doube BM, Greenwood JJD, Lawton JH, et al. (1993) The relationship between abundance and body-size in natural animal assemblages. Journal of Animal Ecology 62: 519-528.
176. Lively CM, Dybdahl MF (2000) Parasite adaptation to locally common host genotypes. Nature 405: 679-681.
177. Lajeunesse MJ, Forbes MR (2002) Host range and local parasite adaptation. Proceedings of the Royal Society B-Biological Sciences 269: 703-710.
178. Clark CM, Cleland EE, Collins SL, Fargione JE, Gough L, et al. (2007) Environmental and plant community determinants of species loss following nitrogen enrichment. Ecology Letters 10: 596-607.
179. Fynn R, Morris C, Kirkman K (2005) Plant strategies and trait trade-offs influence trends in competitive ability along gradients of soil fertility and disturbance. Journal of Ecology 93: 384-394.
180. Leishman MR, Haslehurst T, Ares A, Baruch Z (2007) Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. New Phytologist 176: 635-643.
181. Ceballos G, Ehrlich PR (2002) Mammal population losses and the extinction crisis. Science 296: 904-907.
182. Cardillo M, Mace GM, Jones KE, Bielby J, Bininda-Emonds ORP, et al. (2005) Multiple causes of high extinction risk in large mammal species. Science 309: 1239-1241.
183. Collen B, Bykova E, Ling S, Milner-Gulland EJ, Purvis A (2006) Extinction risk: A comparative analysis of central Asian vertebrates. Biodiversity and Conservation 15: 1859-1871.
184. Loreau M (2000) Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91: 3-17.

185. Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, et al. (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75: 3-35.
186. Mouquet N, Moore JL, Loreau M (2002) Plant species richness and community productivity: why the mechanism that promotes coexistence matters. Ecology Letters 5: 56-65.
187. Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412: 72-76.
188. Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, et al. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294: 804-808.
189. Turnbull LA, Levine JM, Loreau M, Hector A (2013) Coexistence, niches and biodiversity effects on ecosystem functioning. Ecology Letters 16: 116-127.
190. Loreau M (1998) Separating sampling and other effects in biodiversity experiments. Oikos 82: 600-602.
191. Hantsch L, Braun U, Scherer-Lorenzen M, Bruelheide H (2013) Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. Ecosphere 4.
192. Tilman D (2001) Effects of diversity and composition on grassland stability and productivity. Ecology: Achievement and Challenge: 183-210.
193. Reich PB, Knops J, Tilman D, Craine J, Ellsworth D, et al. (2001) Plant diversity enhances ecosystem responses to elevated CO2 and nitrogen deposition. Nature 410: 809-810.
194. Burri C, Bastic V, Maeder G, Patalas E, Gern L (2011) Microclimate and the Zoonotic Cycle of Tick-Borne Encephalitis Virus in Switzerland. Journal of Medical Entomology 48: 615-627.
195. Jensen PM, Hansen H, Frandsen F (2000) Spatial risk assessment for Lyme borreliosis in Denmark. Scandinavian journal of infectious diseases 32: 545-550.
196. Aarssen LW (1997) High productivity in grassland ecosystems: effected by species diversity or productive species? Oiko 80: 183-184.
197. Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110: 449-460.
198. Ogden NH, Tsao JI (2009) Biodiversity and Lyme disease: dilution or amplification? Epidemics 1: 196-206.
199. McCallum H, Dobson A (2002) Disease, habitat fragmentation and conservation. Proceedings of the Royal Society B-Biological Sciences 269: 2041-2049.
200. Goedbloed DJ (2013) Population genetics and disease ecology of European wild boar: Wageningen University.
201. Savage AE, Zamudio KR (2011) MHC genotypes associate with resistance to a frog-killing fungus. Proceedings of the National Academy of Sciences of the United States of America 108: 16705-16710.
202. Bishop SC, Stear MJ (2003) Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. Veterinary Parasitology 115: 147-166.
203. Altermatt F, Ebert D (2008) Genetic diversity of Daphnia magna populations enhances resistance to parasites. Ecol Lett 11: 918-928.

Summary

Many pathogens infect multiple host species which can differ in their reservoir competence. Consequently the species richness and composition of the host community can considerably influence the dynamics of disease transmission. Recently, an increasing number of studies reported the existence of a dilution effect whereby high host species richness reduces the disease risk. However, the generality of the dilution effect and its mechanisms are still highly debated. In this thesis, I tested the existence of a dilution effect in bovine tuberculosis (BTB) and investigated the underlying mechanisms of the dilution effect.

I detected a possible dilution effect in BTB, where higher mammal species richness reduced the probability of occurrence of BTB at a regional level in Africa, after correcting for cattle density (Chapter 2). This dilution effect might be caused by encounter reduction, i.e. the presence of non-competent mammal species might act as barriers to herd movement of cattle and reduce encounter rates among herds, which leads to a decreased probability of BTB outbreaks. Then I extended the study of the BTB dilution effect to the analysis of BTB persistence and recurrence (Chapter 3). The results showed that mammal species richness was also negatively correlated with the BTB persistence and recurrence. Besides, I demonstrated that the presence of African buffalo, as a maintenance host for *Mycobacterium bovis* (the causative agent of BTB), had a positive identity effect and increased the risk of BTB persistence and recurrence, whereas greater kudu distribution was not correlated with BTB persistence or recurrence. In addition, BTB persistence and recurrence were correlated with different sets of risk factors.

In Chapter 4, I showed that interspecific variation in species' reservoir competence could be partly explained by life-history traits in three vector borne diseases, namely Lyme disease, West Nile Encephalitis (WNE) and Eastern Equine Encephalitis (EEE). Species with larger body mass (for hosts of Lyme disease and WNE) or smaller clutch size (for hosts of EEE) had a lower reservoir competence. Given that both larger body mass and smaller clutch size are linked to higher extinction risk of local populations, the results indicate that species with a higher reservoir competence are more likely to remain in the community when biodiversity declines, and thereby potentially increase the risk of transmitting these pathogens. This might be a possible mechanism underlying the dilution effect.

Combing the results about the relationships between species' reservoir competence and life-history traits, I constructed a compartmental model to investigate the effect of connectivity on the risk of directly transmitted diseases in metapopulations (Chapter 5). I showed that different indicators of disease risk (infection prevalence and number of infected individuals) reacted differently to increasing connectivity. Higher connectivity can not only decrease disease risk due to the dilution effect by increasing species richness, but can also increase disease risk through increasing contact rates among patches (facilitation effect). The net impact of connectivity depends on the relative importance of the dilution versus facilitation effect. These results may reconcile the current debate on the dilution effect, and contributes to a better understanding of the impacts of fragmentation on disease risks and the generality of the dilution effect.

Finally, I combined these findings and reviewed the evidence and critiques on the dilution effect (Chapter 6). Latest studies (also the BTB study in this thesis) tried to test species identity effects, caused by particular species in communities, and found that the identity effect and dilution effect can operate simultaneously in the host community. I suggest that the identity effect could act as an additional mechanism explaining the effect of species richness on disease risk. A weak correlation between host reservoir competence and local extinction risk can create inconsistent effects of host species richness on disease risk. Moreover, different indicators of disease risk may react differently to the changes in species richness. This could also be one of the reasons for the controversial results from previous studies that used different indicators (e.g., prevalence or number of infection) of disease risk. In conclusion, this thesis presents both evidence and critique for the existence of the dilution effect. Since factors may simultaneously influence community compostion and the characteristics of pathogen transmission (e.g., susceptibility, survival of pathogen etc.), future studies should also consider these factors, rather than only species richness, to better understand the effect of species richness on disease risk.

Samenvatting

Veel ziekteverwekkers infecteren meerdere gastheren en deze gastheren kunnen verschillen in hun capaciteit om de ziekteverwekker over te dragen. Als gevolg hiervan kan de soortenrijkdom en de samenstelling van de gastheergemeenschap een grote invloed hebben op de dynamiek van de overdracht van ziektes. Een toenemend aantal studies laat zien dat een hogere soortenrijkdom het ziekterisico verlaagd, wat in het Engels het "dilution effect" wordt genoemd (wat vertaald kan worden als het verdunningsseffect). Desondanks is er veel debat over de algemeenheid en de mechanismes van dit verdunningseffect. In dit proefschrift heb ik getest of er aanwijzingen zijn voor een verdunningseffect bij rundertuberculose (BTB) en onderzocht ik mechanismes die het verdunningseffect kunnen veroorzaken.

Ik vond een mogelijk verdunningseffect op regionale schaal voor BTB in Afrika, waarbij een hogere zoogdiersoortenrijkdom het voorkomen van BTB verlaagde als het effect gecorrigeerd wordt voor de dichtheid waarmee koeien worden gehouden (Hoofdstuk 2). Dit verdunningseffect zou kunnen worden veroorzaakt door een verlaging van de kans op het tegenkomen van andere dieren, dat wil zeggen, de aanwezigheid van niet-competente zoogdiersoorten zou als een barrière voor bewegingen van kuddes van koeien kunnen werken. Hierdoor is er minder contact tussen verschillende kuddes, wat zou kunnen leiden tot een afname van het aantal BTB uitbraken. Ik heb dit onderzoek uitgebreid met een analyse waarbij het aanhouden van en de terugkeer van BTB uitbraken werd onderzocht (Hoofdstuk 3). De resultaten van deze analyse lieten zien dat zoogdiersoortenrijkdom ook negatief gecorreleerd was met deze twee parameters. Daarnaast liet ik zien dat de aanwezigheid van de Afrikaanse buffel, die als gastheer onder andere verantwoordelijk is voor de permanente aanwezigheid van *Mycobacterium bovis* (de ziekteverwekker van BTB) in de dierengemeenschap, een positief identiteitseffect had op zowel het aanhouden van, als het terugkeren van BTB uitbraken. Verrassend was dat de aanwezigheid van de grote koedoe niet gecorreleerd was met het aanhouden van en het terugkeren van BTB. Bovendien liet ik zien dat de aanhouding van en de terugkeer van BTB uitbraken gecorreleerd was met verschillende risicofactoren.

In Hoofdstuk 4, heb ik aangetoond dat variatie tussen soorten in hun capaciteit om ziekteverwekkers over te dragen gedeeltelijk kan worden verklaard door levensloopkarakteristieken. Ik heb dit onderzocht voor drie verschillende vector-overdraagbare aandoeningen, namelijk de ziekte van Lyme, West-Nijlziekte (WNE) en Eastern Equine Encephalitis (EEE). Soorten met een hoger lichaamsgewicht (voor gastheren van de ziekte van Lyme en WNE) of met een kleinere legselgrootte (voor gastheren van EEE) hadden een lagere capaciteit om de ziekte over te dragen. Gegeven het feit dat dieren met een hoger lichaamsgewicht en een kleinere legselgrootte een grotere kans hebben om lokaal uit te sterven, tonen deze resultaten aan dat het waarschijnlijk de soorten zijn met een hoge capaciteit om ziekteverwekkers over te dragen die overblijven in een gemeenschap als de biodiversiteit afneemt. Dit zou als gevolg kunnen hebben dat het risico om de ziekteverwekker over te dragen groter wordt, wat één van de onderliggende mechanismes zou kunnen zijn van het verdunningseffect.

Hierna heb ik de resultaten van de relatie tussen de capaciteit om ziekteverwekkers over te dragen en de levensloopkarakteristieken van een soort gecombineerd in een compartimentenmodel, waarmee ik het effect van landschapsverbindingen op het risico op direct overdraagbare aandoeningen in metapopulaties heb getest (Hoofdstuk 5). Ik vond dat verschillende ziekterisico indicatoren (de infectieprevalentie en het aantal geïnfecteerde individuen) anders reageerden op een toename in landschapsverbindingen. Een hoger aantal verbindingen kon zowel leiden tot een afname in het ziekterisico dankzij een verdunningseffect door een verhoogde soortenrijkdom, als tot een toename van het ziekterisico dankzij een toename in het contact tussen dieren uit verschillende plekken (het facilitatie-effect). Het netto effect van landschapsverbindingen hangt af van het relatieve belang van zowel het verdunningseffect als het facilitatie-effect. Deze resultaten verbeteren ons inzicht met betrekking tot de invloed van landschapsversnippering op ziekterisico en de algemeenheid van het verdunningseffect, en dragen ze bij om beter te begrijpen waarom sommige studies wel, en andere studies geen verdunngineffect vinden.

In het laatste hoofdstuk (Hoofdstuk 6) combineerde ik mijn bevindingen en gaf ik een overzicht van de bewijzen voor, en de kritiek op het verdunningseffect. De meest recente studies (waaronder de studie van BTB in dit proefschrift) hebben geprobeerd om het identiteitseffect van een soort te testen. Dit identiteitseffect wordt veroorzaakt door specifieke soorten in gemeenschappen. Uit deze studies bleek dat het identiteitseffect en het verdunningseffect gelijktijdig kunnen voorkomen in een gemeenschap. Ik stel voor dat het identiteitseffect kan werken als een extra mechanisme wat het effect van soortenrijkdom op ziekterisico zou kunnen verklaren. Een zwakke correlatie tussen de capaciteit van een gastheer om ziekteverwekkers over te dragen en het risico voor die soort om lokaal uit te sterven zou een reden kunnen zijn voor inconsistente relaties tussen gastheersoortenrijkdom en ziekterisico. Bovendien kunnen verschillende ziekterisico-indicatoren anders reageren op veranderingen in soortenrijkdom. Dit zou kunnen verklaren waarom studies die naar verschillende indicatoren kijken (bijvoorbeeld de prevalentie of het aantal infecties) andere resultaten vinden. Samenvattend presenteert dit proefschrift zowel bewijs voor, als kritiek tegen een verdunningseffect. Omdat factoren tegelijkertijd invloed kunnen hebben op zowel de samenstelling van de dierengemeenschap als karakteristieken die invloed hebben op de overdacht van ziekteverwekkers (bijvoorbeeld vatbaarheid en de overleving van de ziekteverwekker), zouden toekomstige studies ook deze factoren mee moeten nemen bij het bestuderen van de relatie tussen soortenrijkdom en ziekterisico.

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Curriculum vitae

Zheng Huang was born on 15 Februry 1985 in Anhui province China, and grew up in the small forestry farm in Anhui. After completing high school in 2003, he enrolled in Nanjing University, China. He received his Becholar's degree in biotechnology in 2007, after which he stayed at Nanjing University and started with MSc. In his MSc thesis, supervised by Mao-song Liu, he studied plant adaptation and interspecies interactions in arid environments (Western China), especially the tree-grass interactions. During his degree he also participated in several research projects on wetlands restoration and helped local Forestry Administration (Jiangsu Province) with the second nationwide wetland inventory of China.

After completion of his MSc study at Nanjing Univeristy in 2007, he successfully applied for a scholarship from Chinese Scholarship Council, and moved to Wageningen University to start his PhD study at Rersource Ecology Group under supervision of Prof. Herbert Prins, Dr. Fred de Boer and Dr. Frank van Langevelde. His PhD research focused on the role of species richness in disease dynamics. He used both spatial epidemiological/ecological statistics and theoretical modelling to test the dilution effect and investigate the underlying mechanisms. The results of his research culminated in this thesis. During this PhD period, he also worked on species richness pattern in collaboration with Chi Xu from Nanjing University, China. In November 2013, he went to the Department of Ecology and Evoutionary Biology of Princeton University as a Visiting Research Collaborator for two months to improve his modelling skills. After completing his PhD project, he will go back to China, and try to find a position to continue his academic career on ecology.

List of Publications

Huang ZYX, van Langevelde F, Prins HHT, de Boer WF. 2014. Dilution versus facilitation: the impact of habitat connectivity on disease risks in metapopulations. Submitted.

Huang ZYX, Xu C, van Langevelde F, Prins HHT, Ben Jebara K, de Boer WF. 2014. Dilution effect and identity effect by wildlife in the persistence and recurrence of bovine tuberculosis. *Parasitology*. 141: 981-987

Xu C & **Huang ZYX**, Chi T, Chen BJW, Zhang M, Liu M. 2014. Can local landscape attributes explain species richness patterns at macroecological scales? *Global Ecology and Biogeography* 23: 436-445

Huang ZYX, de Boer WF, van Langevelde F, Xu C, Ben Jebara K, Berlingieri F, Prins HHT. 2013. Dilution effect in bovine tuberculosis: risk factors for regional disease occurrence in Africa. *Proceedings of the Royal Society B: Biological Series* 280: 20130624

Huang ZYX, de Boer WF, van Langevelde F, Olson V, Blackburn TM, Prins HHT. 2013. Species' life-history traits explain interspecific variation in reservoir competence: a possible mechanism underlying the dilution effect. *PloS One* 8(1): e54341

Huang Z, Liu M, Chen B, Uriankhai T, Xu C, Zhang M. 2010. Distribution and interspecific correlation of root biomass density in an arid *Elaeagnus angustifolia* - *Achnatherum splendens* community. *Acta Ecologica Sinica* 30: 45–49

Xu C, Liu M, Zhang M, Chen B, **Huang Z**, Uriankhai T, Sheng S. 2011. The spatial pattern of grasses in relation to tree effects in an arid savannah community: Inferring the relative importance of canopy and root effect. *Journal of arid environment* 75: 953-959

Xu C, Liu M, Yang XJ, Sheng S, Zhang M, **Huang Z**. 2010. Detecting the spatial differentiation in settlement change rates during rapid urbanization in the Nanjing metropolitan region, China. *Environmental Monitoring and Assessments* 171: 457-470.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- The relationship between biodiversity and disease risk

Writing of project proposal (4.5 ECTS)

- Infectious diseases and mammalian species richness in Africa: is the dilution effect true?

Post-graduate courses (4.8 ECTS)

- Geostatistics; PE&RC (2010)
- Generalized linear models; PE&RC (2012)
- Mixed linear models; PE&RC (2012)
- Bayesian statistics; PE&RC (2012)
- The art of crop modelling; PE&RC (2013)

Laboratory training and working visits (3 ECTS)

- Infectious disease modelling and simulation; Princeton University, USA (2013)

Invited review of (unpublished) journal manuscript (1 ECTS)

- African Journal of Microbiology Research: epidemiology of bovine tuberculosis in Butajira, Southern Ethiopia: a cross-sectional abattoir-based study (2013)

Deficiency, refresh, brush-up courses (3 ECTS)

- Ecological methods (2010)

Competence strengthening / skills courses (1.7 ECTS)

- PhD Competence assessment; WGS (2011)
- Voice matters – voice and presentation skills; WGS (2013)
- Interpersonal communication for PhD students; WGS (2013)
- Data management; WGS (2013)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Weekend (2010)
- PE&RC Day (2013)

Discussion groups / local seminars / other scientific meetings (5.4 ECTS)

- Ecological theory and application discussion group (2010-2014)
- The R users group (2012-2013)
- Wageningen Evolutionary and Ecology Seminar (WEES) (2013-2014)

International symposia, workshops and conferences (8.5 ECTS)

- The Annual Conference of the Society for Tropical Ecology; Germany (2012)
- The 11th International Mammaloogical Congress; UK (2013)
- The Netherlands Annual Ecological Meeting; the Netherlands (2013)
- The Netherlands Annual Ecological Meeting; the Netherlands (2014)

Lecturing / supervision of practical's / tutorials (3 ECTS)

- Animal ecology (2013)
- Ecological methods (2013)

Supervision of one MSc student

- Risk factors for African swine fever in Africa (2014)

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