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**DYNAMIQUE DES MALADIES DANS LES SYSTEMES
SOCIAUX COMPLEXES : EMERGENCE DES MALADIES
INFECTIEUSES CHEZ LES PRIMATES**

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A Gaston...

Résumé

Comprendre l'émergence et la propagation des maladies infectieuses chez les animaux sauvages est devenue une priorité en santé publique et en conservation. En combinant la collecte de données et le développement de modèles épidémiologiques, cette thèse s'est focalisée sur la compréhension de deux phénomènes clés: (i) étudier comment l'hétérogénéité au niveau des individus, des groupes, de la population et de l'environnement influence la propagation de parasites et (ii) quantifier la transmission de bactéries résistantes aux antibiotiques depuis l'homme vers les animaux sauvages dans trois aires protégées d'Afrique (Tsaobis NP- Namibie, Lopé NP-Gabon et Dzanga-Ndoki NP- République Centrafricaine). Les principaux résultats de ce travail montrent que : (1) De multiples facteurs incluant la température, la pluie, l'utilisation du territoire, le genre, l'âge et la condition physique influencent la richesse spécifique de parasites gastro-intestinaux chez le babouin chacma, (2) Les contacts entre animaux autour des points d'attractions de l'habitat peuvent influencer de manière importante la propagation spatio-temporelle d'une maladie, (3) La transmission d'entérobactéries résistantes semble avoir lieu depuis les humains ou le bétail vers les animaux sauvages dans des zones où le contact entre ces populations est élevé, (4) Le gradient de densité de gorilles produit par la chasse peut générer une augmentation de prévalence d'un parasite avec la distance au point d'introduction. Les conclusions de ce travail ouvrent de nouvelles possibilités pour l'étude des maladies émergentes chez les animaux sauvages.

Mots clés: Ecologie de la transmission, résistance aux antibiotiques, modèles épidémiologiques, gorilles de plaines de l'ouest, babouins chacma, *Escherichia coli*

Title : Disease dynamics in complex social systems: the emergence of infectious diseases in primates

Abstract

Understanding the emergence and spread of infectious disease in wild animal populations has become an important priority for both public health and animal conservation. Combining the collection of empirical data with the development of epidemiological models, this thesis focuses on understanding two key issues of wildlife epidemiology: (i) how heterogeneity at the individual, group, population and landscape level affects parasite spread (ii) investigating whether transmission of antibiotic resistant bacteria from humans to wildlife is occurring within three protected areas of Africa (Tsaobis NP-Namibia, Lope NP-Gabon and Dzanga-Ndoki NP-Central African Republic). The main findings of this work indicated that: (1) multiple-scale factors including temperature, rainfall, home range use, sex, age and body condition influence gastro-intestinal parasite richness among wild baboons; (2) animal contacts around 'habitat hotspots' can substantially influence the spatio-temporal dynamics of a disease; (3) antibiotic resistant enterobacteria seem to be spreading from humans/livestock to wildlife when the territory overlap between these two populations is expected to be high; (4) gradients in gorilla density created by bushmeat hunting can reverse the expected pattern of decreasing parasite prevalence with distance to human-spillover. The conclusions of this work open new possibilities for studying the mechanisms explaining the spread of emerging infectious diseases among wild animals.

Key words: wildlife epidemiology; disease ecology; antibiotic resistance; epidemiological-models; western lowland gorillas; chacma baboons; *Escherichia coli*

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Introduction

The incidence and emergence of infectious diseases (IDs) has increase in the past three decades, becoming a major threat for both human public health and biodiversity conservation (Daszak, 2000; Jones et al., 2008; Morens, Folkers & Fauci, 2004; Smith, Acevedo-Whitehouse & Pedersen, 2009). IDs remain a huge burden for human society as well as an increasing threat for the survival of several wild species. In humans for example, IDs account for more than 25 % of all deaths in the world a year and represent a huge burden for developing countries (Morens et al., 2004; WHO, 2002). Long time neglected, IDs are also being recognized as one of the major threats faced by endangered animal species (Daszak, 2000; Smith et al., 2009). In fact, diseases can cause direct species extinction or threaten species survival by extending the impact of other drivers of extinction such as habitat loss or global warming (de Castro & Bolker, 2005; Smith et al., 2009). One striking example is the spread of chytrid fungus, which is causing the worldwide decline of amphibian populations, with up to 90% population die-offs and local extinction (Berger et al., 1998; Daszak et al., 1999). Other examples include an up to 90% decline in the endangered Tasmanian devil (*Sarcophilus harrisi*) populations caused by facial tumour disease (Jones et al., 2007), and the death of a third of all remaining wild western lowland gorillas (*Gorilla gorilla gorilla*) due to outbreaks of Ebola virus (Bermejo et al., 2006; Walsh et al., 2003).

Several steps are needed to understand and ultimately control the spread of IDs in both human and wildlife populations. These steps involve the identification of a new pathogen (or one that is already circulating), understanding how diseases spread in a population, and the mechanisms favoring or limiting this spread. For example, Morse (1995) suggested that infectious disease emergence can be viewed as a two-step process: “1) Introduction of the agent into a new host population (whether the pathogen originated in the environment,

possibly in another species, or as a variant of an existing human infection), followed by 2) establishment and further dissemination within the new host population”. Even for a single pathogen, a full understanding of these two processes will require extensive multidisciplinary work. As host-parasite interactions tend to be complex systems, understanding the emergence and spread of infectious diseases will involve different approaches. Thus, this work concentrates mainly on improving our understanding of the factors influencing disease dynamics in wild animal populations, while utilizing the knowledge and tools generated in the study of infectious diseases among humans.

The origin of Emerging Infectious Diseases: the human-wildlife interface is crucial

A first and key step to understanding IDs is to identify their origin and cause, which can be achieved by active disease surveillance. ID surveillance first started for human diseases. Historically, IDs have caused disastrous death and genocides, including the black death plague and the importation of smallpox into Mexico with the Spanish colonization in 1520–1521, killing 10–15 million people and effectively ending Aztec civilization (Morens et al., 2004). However, disease surveillance has been increasingly developed after the identification of AIDS in the 80’s as an Emerging Infectious Disease (EID), defining an infectious disease whose incidence has increased in the past 20 years and threatens to increase in the near future (Morens et al., 2004). Overall, two major conclusions have been reached over the last decade regarding the origin of EIDs in humans: (i) most infectious EIDs are zoonotic (*i.e.* they originate and jump to humans from a domestic or wild animal) and (ii) landscape changes of human activities are driving their emergence (Daszak, Cunningham & Hyatt, 2001; Jones et al., 2008; Woolhouse & Gowtage-Sequeria, 2005). The origins of some EIDs have been relatively easy to establish (*e.g.* wildlife origin of SARS and swine flu epidemics: (Garten et

al., 2009; Li et al., 2005).. However, only the recent development of sophisticated molecular techniques along with extensive sampling effort has allowed us to elucidate the origin of other highly pandemic diseases. For example, great ape SIV has only been recently confirmed as the reservoir for human HIV. This was achieved via the isolation, amplification and sequencing of the virus' genetic material from fecal samples (Keele et al., 2006). Additionally, apes have been recognized as the reservoir for human malignant malaria using similar methods (Liu et al., 2010; Prugnolle et al., 2010; Rich et al., 2009), although this conclusion has been recently questioned by new data (Prugnolle et al., 2011). The role of wildlife in IDs has perhaps updated our priorities in the steps and gaps to be filled in order to understand how infectious diseases are emerging in humans. This in turn has benefited wildlife conservation because attention is now given to the understanding of diseases in wild animals, which has been incredibly neglected until recently in epidemiology.

From the wildlife perspective, humans are the major driver of EIDs among wild animal populations. Modification of the landscape by humans is probably the number one driver of EIDs in wild animals (Daszak, 2000). Humans are also introducing pathogens into wild animal populations, referred to as “pathogen pollution” or human disease spillover (Dobson & Foufopoulos, 2001b). This last phenomenon includes the introduction of invasive species carrying new pathogens, the translocation of infected animals into new areas (*e.g.* during reintroduction programs), or the concentration of domestic animals such as dogs or cattle at the periphery of wildlife territory that subsequently spread pathogens into wildlife. For example, epidemics of rabies and distemper originated from dogs, resulting in the decline of several African carnivore populations such as wild dogs (Alexander & Appel, 1994; Lembo et al., 2007). Even human activities like tourism and scientific research are responsible for major epidemics in wild animals (*e.g.* human respiratory viruses' outbreaks in gorillas and chimpanzees: (Kondgen et al., 2008; Palacios et al., 2011). In some cases, parasite's spillover

from domestic animals can become endemic in wild populations, with the subsequent “spillback” from wildlife back to domestic animals. For example, brucellosis was first introduced into the bison of Yellowstone from cattle in 1917, however nowadays it is transmitted back to cattle by bison and elk (Kilpatrick, Gillin & Daszak, 2009). The origins of other more cryptic diseases are still very poorly understood. This is the surprising case of filoviruses such as Yellow fever, one of the most studied diseases in humans. For example, even if Yellow fever had been introduced into South America from Africa by humans, the wild animal hosts maintaining the circulation of the virus in the forest are poorly known, and the origin of recent devastating epidemics in howler monkeys is not understood (Holzmann et al., 2010). Similarly, although several Ebola strains have been identified and deadly epidemics reported among great apes, the natural reservoirs of the virus are still unknown, although bats are suspected as the main reservoir (Groseth et al., 2007; Leroy et al., 2005).

Overall, understanding the appearance or re-appearance of EIDs in both human and wildlife will require a broad understanding of disease transmission at the human-wildlife interface (Lloyd-Smith et al., 2009). In particular, disease surveillance should relocate its efforts into poorly studied areas with great potential for disease emergence, such as tropical forests (Jones et al., 2008), in order to fully understand and predict the conditions favouring their emergence.

Understanding disease spread in wild populations

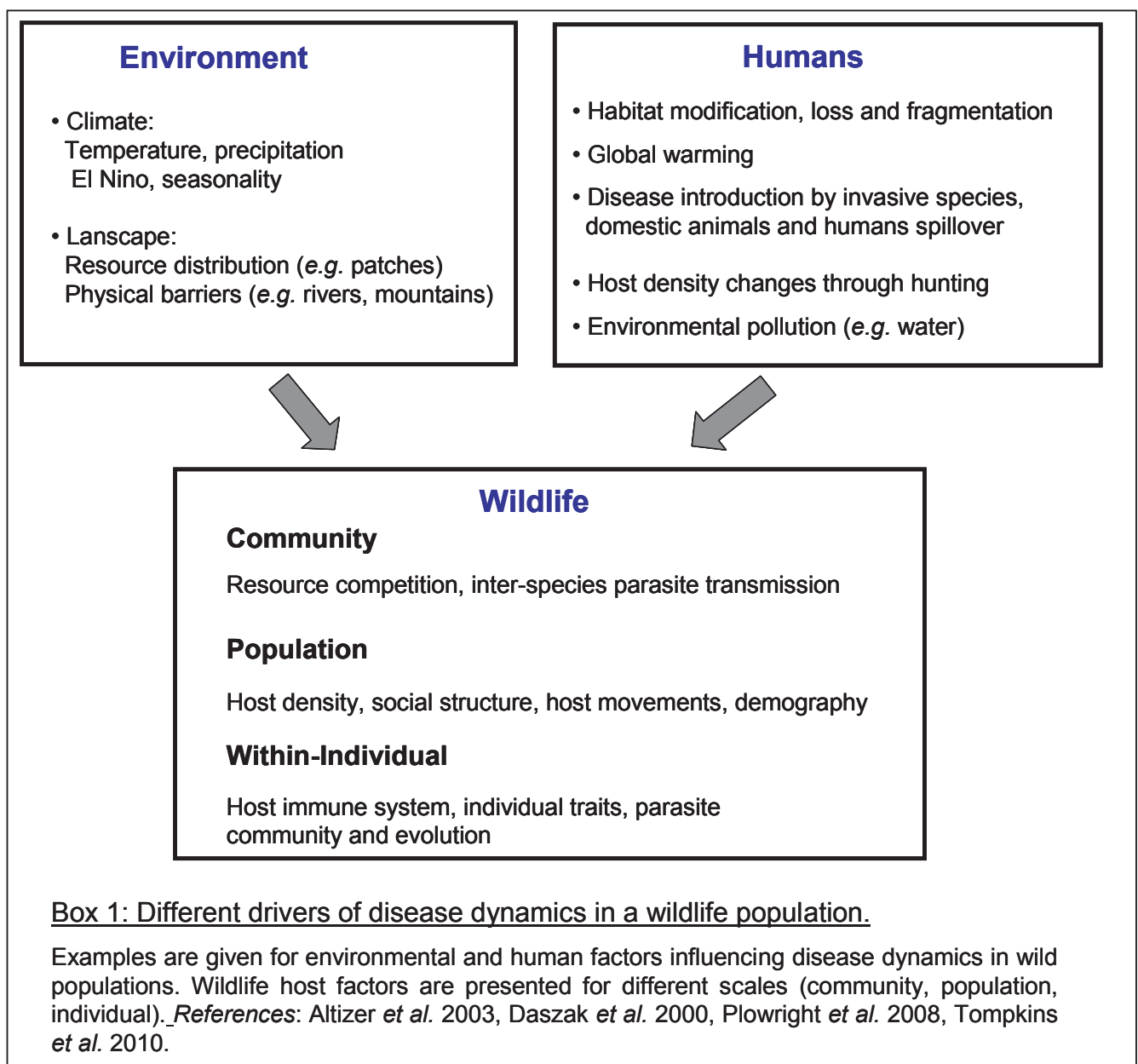
The second step towards understanding IDs is to describe how a given disease is going to spread after its introduction into a new population. Epidemiology has tackled this question as one of the oldest disciplines in biology (Anderson & May, 1991), going back to epidemiologists such as Hippocrates (460-377BC). As a holy grail, epidemiology’s ultimate goal is to predict and anticipate the spread of a disease. Thus, together with thousands of data

analyses, several statistical and predictive models have been developed in order to create a strong and large theoretical framework capable of capturing and predicting the dynamics of pathogens among different populations. Perhaps the most important contribution to epidemiology was the development of deterministic mass-action-based differential equations incorporating the major epidemiological features of a disease, and separating individuals into compartments according to their disease status (*e.g.*, Susceptible-Infectious-Recover model among many others) (Anderson & May, 1991; Bailey, 1975). Within these models applied mainly to human epidemics, the effectiveness of transmission by a pathogen is often given by calculating its basic reproductive rate (R_0), defined as the number of secondary cases one case generates on average over the course of their infectious period. If this number is higher than one, the disease is predicted to spread across the population (Heesterbeek, 2002). Likewise, other critical processes for disease spread have been widely proposed, such as density-dependent transmission (*i.e.* transmission increases with population density), or frequency-dependent transmission (*i.e.* transmission decreases with population size) (McCallum, Barlow & Hone, 2001). However, in wildlife populations, host features such as spatial or social structure are now questioning the effectiveness of these widely used concepts imported from human studies such as R_0 (Cross et al., 2007; Lloyd-Smith et al., 2005a). Addressing the question of disease thresholds in wild populations is challenged by the difficulty of collecting large sample sizes from wild populations, and could be achieved by developing a model that accounts for the specific features of wild populations that will influence parasite spread. Thus, to the initial general deterministic models developed for humans, a set of more “realistic” models are being developed considering different features related to the spread of disease in wild populations such as stochasticity, space (*e.g.* metapopulations), host demography and population contact structure (Dangerfield, Ross & Keeling, 2009; Fromont, Pontier & Langlais, 2003; Keeling & Eames, 2005; Swinton et al., 1998).

Despite all the efforts of epidemiology and some success stories (*e.g.* eradication of foot-and-mouth disease from wildlife and livestock in some countries: Thomson et al. 2003), the spread of the majority of IDs in wildlife populations remains a puzzle and a tremendous challenge. Why? One obvious answer is the limited amount of empirical data available to understand the majority of IDs that are appearing. Another explanation is that host-parasite interactions are complex systems, because interactions operate at very different spatial and temporal scales. Thus, researchers trying to identify drivers of disease emergence are challenged by the complexity, scale, and natural variability of the systems involved (Plowright et al., 2008). For example, more than twenty plausible factors can be associated with the emergence and spread of Hendra virus among horses and humans (Plowright et al., 2008). A broad understanding of the general mechanisms affecting host-parasite interactions would therefore be an enormous step into our understanding of the dynamics of IDs. At the spatial scale, parasites interact with hosts at a large number of levels going from molecular and cell interactions within-hosts, between-host interactions at the population level, to between-species interactions at the community level. All of these levels of interaction generate what we ultimately observe as a parasite spread within a population (Tompkins et al., 2010). Thus, we also need to explore how one level of interaction (*e.g.* within-host dynamics) can scale into another level (*e.g.* population-level dynamics) (Ezenwa & Jolles, 2011). Likewise, at the temporal scale, some parasites can have immediate consequences on hosts (*e.g.* immediate immuno-response or sickness behavior), whilst others can affect species in the long term by affecting host fitness, reproduction or even evolutionary history (Freeland, 1976; Nunn et al., 2004). Interactions between hosts and parasites are also more and more recognized to step away from the “one host-one parasite” approach, as many parasites affect more than one host species and virtually every host is infected by more than one parasite species. Therefore, the “multiple hosts-multiple parasites” approach is being progressively

adopted (Bordes & Morand, 2011; Craft & Caillaud, 2011; Dobson & Andrew, 2004). Box 1 summarizes the main drivers of disease dynamics in a wild animal population at different scales.

The comprehension of the multiple factors influencing disease dynamics ranging from host ecology to parasite evolution has opened new areas of research updating our current paradigm of epidemiology. In fact, epidemiology is now being “infected” by inputs from many other disciplines. Some examples are the integration of spatial ecology, phylogeography, physics and evolutionary biology. This has recently given birth to new areas in this field such as spatial epidemiology, disease ecology, evolutionary epidemiology and conservation medicine (Daszak et al., 2004; Hudson et al., 2001; Ostfeld, Glass & Keesing, 2005; Wilson, 1992). Each of these disciplines brings new tools from other fields (*e.g.* landscape analysis, parasite phylogeny or network theory) that are improving our ability to understand the mechanism of host-parasite interactions at different scales, while trying to develop modelling tools that capture and predict the essential components of parasite spread at different scales.



Tools and methods for understanding disease dynamics in wildlife

In an ideal situation of disease surveillance, researchers would track each transmission and identify every infected or exposed individual through the description of symptoms. Humans would even come to the hospital themselves to be diagnosed. Perhaps, in some human epidemics, we are not that far from this ideal situation, and an accurate description of the disease spread in time and space can be approximated. For example, flu appears every winter

and hospitals saturate with children exhibiting running noses. The spread of diseases in wild animal populations is a completely different story. In most cases, we are confronted with a handful of samples collected during an outbreak. For example, diseases such as Ebola are detected only because animals start disappearing, and we are often able to track no more than a couple of groups of individuals out of a population composed of hundreds of groups. All of these factors limit our ability to understand the spread and drivers of the majority of IDs. Thus, this PhD focuses on studying processes and factors relevant to the spread of many IDs among wildlife, while focusing mostly on less virulent parasites or commensal bacteria. Our model systems include both gastro-intestinal parasites and antibiotic-resistant bacteria as case studies that can be used to understand different factors and mechanisms influencing IDs in wild populations. To link patterns of disease spread to the underlying mechanism generating the pattern, we also developed individual-based and Lotka-Volterra models that capture the main characteristics of a socially-structured host population. Altogether, the use and combination of these approaches aimed to increase our resolution and predictive power on the dynamics of diseases in wild animal populations.

PhD aims and objectives

The main purpose of this study was identify different individual, social and environmental factors influencing the spread of parasites within wildlife populations. In particular, this thesis focused in two general questions of wildlife epidemiology presented bellow.. From there, specific questions were answered, with each article of this manuscript providing some insight into these specific questions:

I. How do social and ecological factors affect the spread of parasites in wild primate populations?

1.1. What are the multi-scale factors influencing intestinal parasite richness in a wild population of baboons? : Article 1

1.2. Can transmission around habitat hotspots substantially affect disease dynamics? : Article 2

II. What are the consequences of human activities on the dynamics of bacterial transmission in wildlife populations living within a protected area?

2.1. Is bacteria being exchanged at the interface between humans/domestic animals and wildlife at the periphery of protected areas? : Articles 3, 4 and 5

2.2. Can bushmeat hunting affect the dynamics of human-introduced antibiotic resistant bacteria in a wild population by a density-dependent effect? : Article 5

In order to answer these questions, this study combined and integrated empirical data collection, statistical analyses and contact-network models. As such, this thesis had the ambition to be inserted within the typical multidisciplinary work needed to answer the complex questions faced within wildlife epidemiology. Therefore, it benefited from a wide range of collaborators and actors including local communities, park managers, microbiologists, quantitative ecologists, physicists, evolutionary biologists and behavioral ecologists.

I. Socio-ecological factors affecting parasite spread: heterogeneity is everywhere!

A key concept that is still making its way into epidemiology is that hosts, parasites and their environment are not homogenous systems. This variability, or heterogeneity, can therefore have important consequences for the spread of parasites in a wild population. In this work, we focused particularly on heterogeneity at the landscape and host level because they have been largely neglected in wildlife epidemiology until recently. Heterogeneity is a very familiar concept in evolution and ecology, with variation across individuals or environments being a major component of natural selection. However, host heterogeneity has only recently been considered as a key aspect of epidemiology, contradicting the “well-mixed homogeneous population” assumption of most classical epidemiological models. Below, it is first described how individual and environmental heterogeneity can affect parasite transmission. Then we explore how heterogeneity in host contacts influencing disease spread can itself be influenced by landscape features.

1. Individual heterogeneity in susceptibility to parasites

Although individual resistance to parasites is a key factor in understanding parasite spread amongst hosts, epidemiologists and disease ecologists have often neglected variation in host susceptibility (Beldomenico & Begon, 2010). Yet, a multitude of individual traits are now recognized to affect parasite infection including host age, sex, body condition, hormonal levels or host genetics (*e.g.* Major Histocompatibility Complex polymorphism), among many others (Deviche, Greiner & Manteca, 2001; Ezenwa, 2004a; Klein, 2004; Krasnov et al.,

2005; Moore & Wilson, 2002; Wilson et al., 2002). A detailed description of all these traits and their effects goes beyond the scope of this study and is an ongoing task in epidemiology. A very complete review of these factors is given by Nunn et al. (2006) for primate populations, which can be easily extrapolated for other hosts. Due to the small amount of long-term empirical data available for wild populations and a large number of studies reporting conflicting results, many of the predicted effects of these traits are yet to be confirmed by empirical data. Furthermore, some individual traits are predicted to have contradictory effects on parasite vulnerability. Among them, host age is expected to both increase parasite risk, by an accumulation of parasite exposure throughout lifespan, and decrease parasite susceptibility by reinforcing adaptive immunity in adults ((Wilson et al., 2002) and Fig 2 Article 1). Following the initial description of their effects, the mechanisms generating individual traits' heterogeneity need to be understood. For example, immunity investment is likely to be a result of a trade-off between immune defence and other physiological processes such as reproduction (Tompkins et al., 2010). As such, the 'terminal investment hypothesis' suggests that animals should invest more in current reproductive output if the chance of surviving until the next reproduction is low (Clutton-Brock, 1984). For example, the optimal strategy for accurate infection is to invest in immune defence and maximize survival probability whilst, for chronic infections, fitness may be maximized by investment in current reproduction. Within a population, individuals could have different strategies and thus, investment in immunity and parasite susceptibility in the population will be heterogeneous (Bonneaud et al., 2004). Other than by a trade-off, other traits result from a series of mechanisms that are still partially understood. For example; although it is expected that MHC diversity, linked to parasite resistance (Penn, Damjanovich & Potts, 2002), is mostly shaped by a co-evolution with multiple parasites (Wegner, Reusch & Kalbe, 2003),

could also be determined by a species mating such as monogamy (Sommer, Schwab & Ganzhorn, 2002).

2. Environmental heterogeneity

At a larger scale, ecological factors have also long been considered as an important driver of disease transmission (Ostfeld et al., 2005). Because human changes in both the landscape and the climate are among the most important drivers of disease emergence, the importance of ecological factors in disease dynamics has received increasing attention, developing the discipline of disease ecology (Hudson et al., 2001). For example, “spatial epidemiology” focuses on identifying landscape features such as vegetation, precipitation and rainfall that will influence the distribution of vectors, pathogen reservoirs, and disease cases, in order to predict future distribution of specific pathogens (Ostfeld et al., 2005). This has mainly been achieved due to recent developments of remote sensing for environmental monitoring such as satellite imagery. For example, at the continental scale, Lyme disease occurrence has been associated with temperature and satellite-derived measures of vegetation such as Normalized Derived Vegetation Index (NDVI) (Estrada-Pena, 2002), while hanta virus has been linked to vegetation distribution (Glass et al., 2000). At a more local scale, the distribution of resources in the landscape and their seasonal nature can also be important in host aggregation, movement and exposure to pathogens (Altizer et al., 2006). However, this is only beginning to be explored in epidemiology (Ostfeld et al., 2005).

3. Adding social structure to the mixture...

The social structure of a population will largely contribute to generate different levels of heterogeneity both at the individual and population level (Altizer et al., 2003). First, individual social status is expected to be linked to both stress-related immunity and resource

acquisition, generating another mechanism of individual heterogeneity to parasite susceptibility (Nunn *et al.* 2006). Second, the spatial aggregation of the population into social groups will have direct consequences for disease dynamics. For example, social group size has been positively correlated to helminth diversity and malaria prevalence in primates (Davies *et al.*, 1991; Vitone, Altizer & Nunn, 2004) and parasite intensity in mammals (Côté & Poulin, 1995). Similarly, for a given population density, an optimal group size is predicted to maximize survival from a disease (Watve & Jog, 1997). Third, group's ranging behavior will interact with environmental heterogeneity to influence parasite exposure through heterogeneity in group range size and in the intensity of habitat use (Bordes *et al.*, 2009; Nunn *et al.*, 2011). Finally, social structure will affect migration between groups, and groups may be acting as biological islands with local adaptation of each group member to the parasites of its group (Freeland, 1979). Overall, whether it is by creating new forms of heterogeneity or by interacting with other drivers like the environment; social structure needs to be systematically considered when studying the features influencing disease dynamics in wildlife populations.

4. What are the socio-ecological factors influencing intestinal parasite richness in a wild population of baboons? : Article 1

In this thesis, we took advantage of a unique data set describing gastrointestinal parasites in a population of chacma baboons (*Papio ursinus*), comprising fecal samples from 86 individuals across two groups collected over two dry seasons in a desert population. Analyzing the data using linear mixed-effect models (LMMs) with temporal autocorrelation, we explored eight possible determinants of parasite richness factors at the population, group and individual level. A summary of the results are given in table 1 and more details about the methods and results are given in Article 1. These findings indicate that a range of factors influence host

parasite richness through both encounter and infection probabilities, but individual-level processes may be more important than within-year variations at the group or population level. In particular, we found that individual age had the strongest influence on parasite richness compared to any other variable. Our study is particularly relevant for the dynamics of infectious diseases in terms of exposure to different parasites. For example, home range intensity of use was correlated to parasite richness, this was most likely because a more extensive use of the home range increased exposure to parasites residing in the environment. Also, environmental exposure to parasites was, as expected, associated with higher temperatures and rainfall. In fact, both high temperatures and rainfall seemed to trigger an increase in parasite richness one or two weeks later, possibly by increasing the reproduction and/or survival of parasite free-living stages. Finally, our study did not show evidence that parasite exposure increases with a higher resource intake among dominant individuals, or animals with better body condition. Overall, this study indicated that both environmental and individual heterogeneity at a fine local scale can drive individual parasite susceptibility (measured by parasite richness) in a wild population. As such, we argue that this type of integrative approach should be widely used to determine the influence of different ecological and social drivers of parasite spread in a given population at different scales. In particular, it allows testing of the relevance of a predicted driver while accounting and controlling for the effects of other factors. Furthermore, it revealed the importance of different factors (*e.g.* temperature and home range use) that are particularly affected by seasonal changes, which could help in explaining the seasonal emergence of many EIDs associated with gastrointestinal parasites.

Table 1. Potential factors influencing host parasite richness in a wild baboon population

Factors are grouped by the scale at which they operate (population, group, and individual). Further information is also provided on the proposed mechanism (whether each factor is more likely to influence parasite richness through the probability of parasite encounter or susceptibility to infection following encounter). The positive effect of a considered factor is noted (+), and a negative effect is noted (-). The last column shows the results reported from this study (Table adapted from Article 1).

Scale	Factor	Hypotheses under test		This study
Population	Rainfall	H1	(+) encounter probability ~ due to the accelerated development, replication or survival of parasites in wetter conditions (Nunn and Altizer, 2006).	(+)
	Temperature	H2	(+) encounter probability ~ due to the accelerated development, replication or survival of parasites in hotter conditions (Nunn and Altizer, 2006).	(+)
Group	Home range productivity (measured by NDVI)	H3	(+) encounter probability ~ because vegetation can be a surrogate measure of environmental moisture and thermal conditions for parasites (Bavia et al., 2001), or can represent a breeding or sheltering site for parasites (Ceccato et al., 2005; Lindsay et al., 1991).	0
	Ranging behavior	H4.a	Home range size: (+) encounter probability ~ due to an increased probability of encounters with parasites in a larger home range (Nunn and Altizer, 2006).	0
		H4.b	Daily travel distance: (+) encounter probability ~ due to an increased probability of encounters with parasites in a more intensively used home range (Nunn and Altizer, 2006; Nunn et al., 2011).	(+)
Individual	Age	H5.a	(+) encounter probability ~ due to an accumulation of parasites in older individuals resulting from a stable probability of encounters with new parasites over time (Nunn and Altizer, 2006)	Polynomial relationship with (+) effect before sexual maturity and a (-) effect for adults
		H5.b	(-) susceptibility to infection ~ due to a reinforced immunity in older individuals following repeated contacts with multiple parasites (Hudson and Dobson, 1997)	
	Sex	H6	(+ males) encounter probability ~ higher parasite richness in males due to greater consumption of food, and thus more opportunity to eat contaminated items (Nunn and Altizer, 2006)	(+) females)
			(+ males) susceptibility to infection ~ higher parasite richness in males (Zuk and McKean, 1996), due to immunosuppression typically resulting from elevated testosterone levels (Roberts et al., 2004)	
	Physical condition	H7.a	(+) exposure probability ~ animals that eat more are in better physical condition, but also have more opportunity to eat contaminated items (Nunn and Altizer, 2006)	(-)
		H7.b	(-) susceptibility to infection ~ due to a better ability to resist infections for animals in good physical condition (Irvine et al., 2006)	
	Social rank ¹	H8.a	(+) encounter probability ~ higher parasite richness in dominant individuals due to higher consumption of food, and thus more opportunity to eat contaminated items (Nunn and Altizer, 2006)	0
		H8.b	(-) susceptibility to infection ~ higher parasite richness in subordinate individuals due to stress compromising immunocompetence (Nunn and Altizer, 2006)	

5. Heterogeneity in host contacts

Contact networks

Another form of host heterogeneity is that not all individuals in the population have the same level of contacts and thus, their possibilities to transmit or contract a disease differ. However, a key assumption of most classical epidemiological models used to predict disease spread is that populations are homogeneous and well-mixed (Anderson & May, 1991). Perhaps the most important contribution from physics into epidemiology over the last decade has been to describe the “contact networks” of a population and to challenge the “well-mixed population” assumption (Bansal, Grenfell & Meyers, 2007; Barabasi & Albert, 1999; Newman, 2002; Watts & Strogatz, 1998). Network theory, grounding in social sciences and graph theory describes host and transmission in terms of “actors” and “relationships”, or “nodes” and “edges” (Keeling & Eames, 2005). Different types of “idealized” networks have been described, going from highly clustered lattices, networks with both clustered and long-range contacts (*e.g.* “small world”), and highly heterogeneous networks governed by some individuals with a very high number of contacts (*e.g.* “scale-free networks”). Examples of these networks are given in Box 2. Biological systems are now being classified according to these types of networks. For example, friendship groups in humans (*e.g.* “who knows whom”) are generally considered as small world networks, whereas human sexual contacts can be described as scale-free networks (Amaral et al., 2000; Schneeberger et al., 2004). See Jeger et al. (2007) gives for a detailed list of examples. The major conclusion resulting from this work is that populations are very rarely randomly and well-mixed, and that network characteristics (*e.g.* topology) have a tremendous impact on disease dynamics (see Bansal et al., 2007; Jeger et al., 2007; Keeling & Eames, 2005) for excellent descriptions of network theory applications in epidemiology). As such, at least three relevant phenomena have been described. First,

when a population behaves as a scale-free network, strategies for disease control targeting “super-spreader” individuals or places (*e.g.* schools and hospitals) are expected to be more efficient than randomly-chosen targets (Lloyd-Smith et al., 2005b; Small, Tse & Walker, 2006).

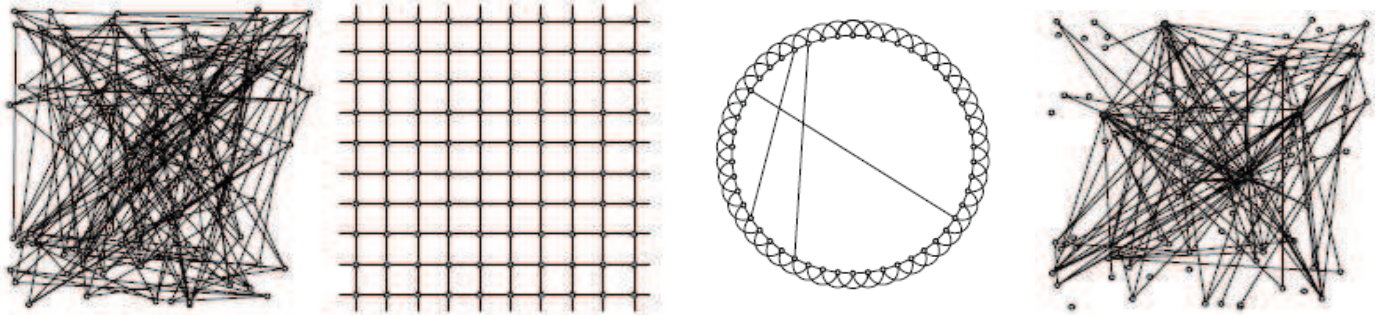


Fig 1: Four networks types. Figure adapted from Keeling & Eames 2005. From left to right: random, lattice, small world and scale-free networks. All graphs contain 100 individuals and the average number of contacts per individual is approximately four.

Second, scale-free networks can have unique properties such as the absence of density thresholds for parasite persistence (Pastor-Satorras & Vespignani, 2001; Small, Walker & Tse, 2007). This could help explain why diseases spreading through the scale-free network of human sexual contacts such as HIV are so difficult to eradicate despite extensive control measures. Finally, the level of a network’s connectivity can generate discrete behaviors that are not captured by the differential equations of classical epidemiological models. For example, clustered populations exhibit a level of connectivity (*i.e.* “percolation” threshold) above which an epidemic will propagate within the entire population (Moore & Newman, 2000). Overall, the importance of these and other phenomena for wild populations needs to be explored by injecting network theory into the study of wildlife epidemiology.

Networks in wild populations

Despite its recent major contribution to the understanding of disease spread in humans, contact network theory has been largely underutilized in wildlife epidemiology (Craft & Caillaud, 2011). This is certainly explained by a combination of both field biologists not familiar with these methods and the difficulty to quantify interactions relevant to transmission in wild populations. Despite these constraints, several examples are showing that contact networks can be estimated and used to understand disease dynamics in wild populations (a detailed list of examples is given by (Tompkins et al., 2010)). For example, Drewe (2010) used a network approach to demonstrate that in meerkats, tuberculosis risk was not associated with the most socially interactive animals but with individuals that groomed others. Similarly, proximity sense radio collars were used to show that all the individuals in a population of Tasmanian devils were connected as a single giant component (Hamede et al., 2009). Finally, Davis et al., (2008) showed how a percolation phenomenon was responsible for the outbreaks of plague in a highly clustered population of great gerbils. The recent development of new technology such as biologging or telemetry will improve our ability to describe networks in wild populations, along with their influence on disease dynamics (Craft & Caillaud, 2011). In humans, relatively simple mechanisms such as the position of airports will ultimately determine the structure of infectious contacts and the spread of disease at a global scale (Colizza et al., 2006). In wildlife, however, the processes shaping the contact-network of a population are very poorly understood. Outside epidemiology, some promising work has linked resources heterogeneity to scale-free movements (*i.e.* levy-walks) of spider monkeys (Boyer et al., 2006). Thus, similar approaches are needed in wildlife epidemiology to comprehend how different ecological and social processes will generate particular contact-networks that will in turn affect the spread of disease within a wild population. In this thesis, we specifically focused on how landscape heterogeneity can affect between-group contacts in a socially-structured population.

Landscape heterogeneity, population's network and disease spread

Even at a very fine scale, host movement and contacts will depend on the physical barriers imposed by the habitat or the distribution of resources across the landscape. In wildlife epidemiology, the dependence of infectious disease spread on these spatial and ecological processes has recently been receiving considerable attention (Real & Biek, 2007). On the one hand, physical barriers such as rivers can slow down the spread of diseases because they impose a physical barrier to the movement of their host (e.g. rabies in racoons: Smith et al., 2002). On the other hand, places that attract several individuals of one or more species, referred to here as “habitat hotspots”, will increase animal contacts and thus the probability of disease transmission. For example, the aggregation of wild boar at watering sites significantly increases the transmission of tuberculosis-like lesions (Vicente-Baños et al., 2007), whereas the distribution of resources is expected to affect the spread of parasites among red colobus monkeys (Bonnell et al., 2010). Despite these examples, our ability to predict the impact of habitat hotspots in wild populations is still very limited, as opposed to the extensive work focusing on this question in humans (Cauchemez et al., 2009; Cauchemez et al., 2008; Paunio et al., 1998; Small et al., 2006). However, this knowledge will be particularly needed to develop strategies of disease mitigation that include the manipulation of habitat hotspots such as waterholes (Wobeser, 2002).

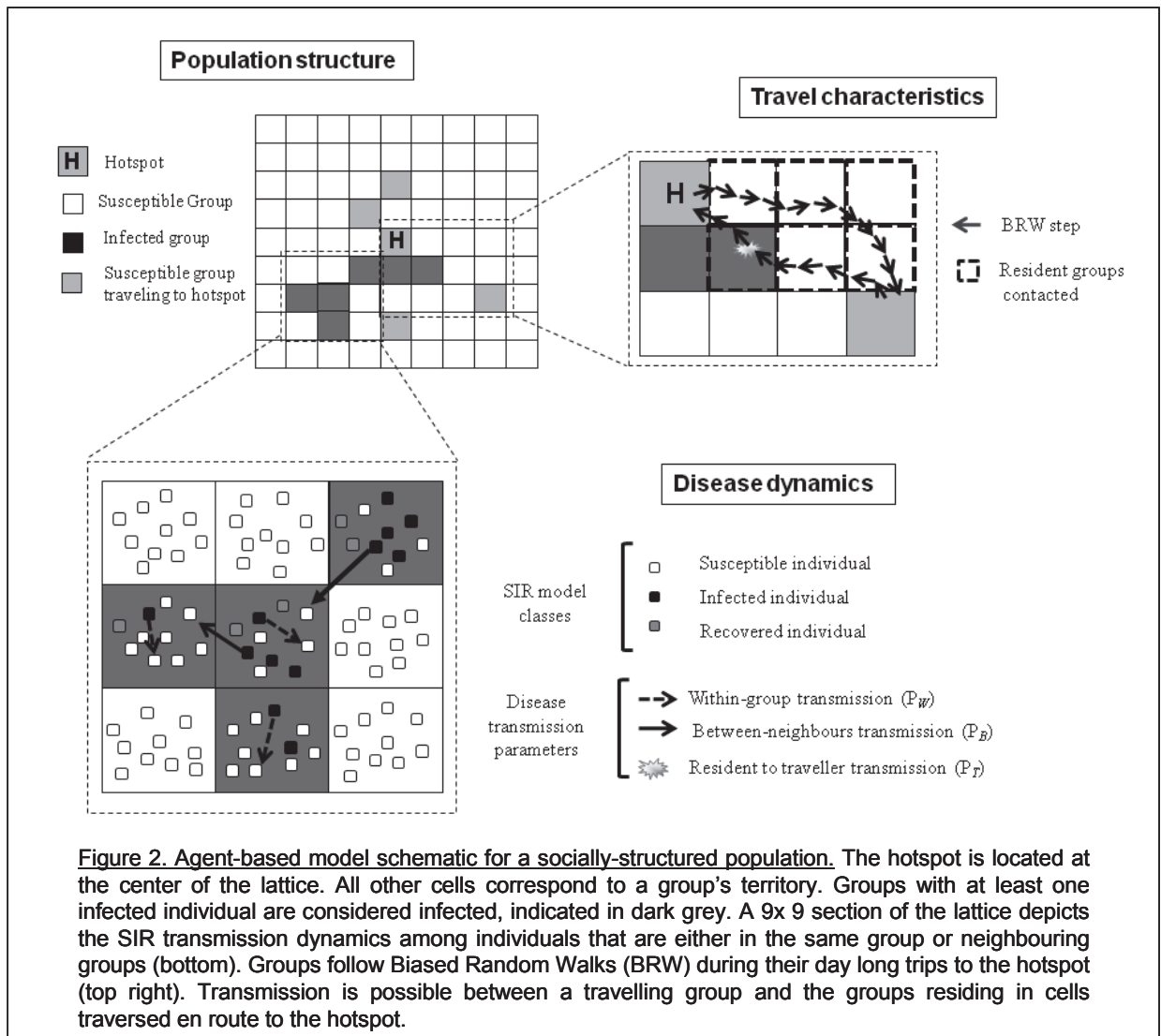
6- Can transmission around habitat hotspots substantially affect disease dynamics? :

Article 2

Until now, research on the importance of habitat hotspots in wildlife epidemiology has primarily focused on how inter-individual contacts occurring at the hotspot itself increase disease transmission. However, inter-individual contacts may not always significantly increase at the hotspot itself. This is for example the case of habitat hotspots that some animal

species only visit occasionally, such as some mineral licks (Blake et al., 2011; Matsubayashi et al., 2007). Also, animals present at the same time at a particularly large hotspot may not be close enough to each other to transmit infectious diseases. This is the case for large forest clearings (Vanleeuwe, Cajani & Gautier-Hion, 1998) or large waterholes. Finally, species such as primates and ungulates might avoid defecating in hotspots of high food resources, limiting the transmission of fecal-oral parasites at hotspots (Ezenwa, 2004b; Gilbert, 1997). Thus, in territorial animals, epidemiologically important contacts may primarily occur as animals cross through territories of conspecifics en route to habitat hotspots. However, this phenomenon has received little attention so far. During this thesis, we developed an agent-based simulation model to explore how group's visits to habitat hotspots influence the dynamics of a disease in a socially-structured population. In complex systems including multiple host characteristics, agent-based models have the ability to capture the realism of host-parasite interactions, while predicting patterns of disease dynamics emerging from these interactions (Roche, Guegan & Bousquet, 2008). Our model, illustrated in Fig. 2, was built to capture the principal characteristics of a socially-structured population where group's territories are spatially clustered in space but groups can also travel over long distances to visit a hotspot. In this study, we investigated the importance of group contacts in the case where infectious individuals keep visiting the hotspots, and in the case where these individuals are unable to travel to the hotspots any longer. These two scenarios were developed to explore the consequences of sickness behavior on disease dynamics, another overlooked phenomenon in wildlife epidemiology. The results demonstrated that even when sick groups stay in their territory, hotspots may increase the size of an epidemic. Surprisingly, when infected animals cease to visit the hotspot, groups ranging at intermediate distances to the hotspot are the most vulnerable (Fig. 4, Article 2). The model also suggests that the epidemiological impact of hotspots extends far beyond the subset of the population that visits

it; even groups having no contact with those visiting the hotspot display elevated risks of infection. Finally, our model predicted that when groups visit their nearest hotspots, the epidemiological impact of hotspots are most severe when the number of hotspots is intermediate. Details on methods and results are given in Article 2. Overall, this model showed the importance of landscape heterogeneity on disease spread, revealing unexpected results regarding the impact of hotspots on disease dynamics. Linking the spatial distribution of resources to the actual between-contact network of the population (Fig. S3, Article 2), our model could be easily adapted to different host-parasite systems where disease dynamics may be driven by inter-group contacts.



II. Impact of human activities on bacterial transmission in wildlife populations

The human-wildlife interface is a key component of the spread and emergence of infectious diseases in wildlife populations (Daszak, 2000; Lloyd-Smith et al., 2009). As the human population continues to grow exponentially, humans overlap more and more with the territory of previously isolated wild populations. Thus, human activities such as agriculture, hunting and even tourism are heavily affecting wild populations and their habitat. Changes in the composition and the environment of wildlife populations induced by human activities are suggested as the main cause for the current emergence of infectious diseases in wildlife (Daszak et al., 2001). However, the mechanisms explaining how and where diseases are jumping from humans to wildlife are still poorly understood. This section focuses on studying the impact of humans and domestic animals in the spread of bacteria among populations of wild non-human primates. First, we explored if humans or domestic animals are spreading bacteria into wildlife populations in both tropical and arid protected areas. Second, we studied the effects of gradients in animal densities on the dynamics of human-introduced bacteria. As highly pathogenic bacteria are rarely found at a high prevalence in wild populations unless an epidemic is occurring, we instead explored these questions using commensal enterobacteria as a potential marker for bacterial transmission.

1. Human and domestic animals as “bacteria polluters”

Several studies have demonstrated that domestic animals are spreading pathogens into wild populations (Daszak et al., 2001; Dobson & Foufopoulos, 2001a; Smith et al., 2009). In fact, over 80% of diseases in domestic animals can also infect wildlife (Cleaveland, Laurenson &

Taylor, 2001; Smith et al., 2009). Given the close phylogenetic relationship between humans and non-human primates, pathogen transmission from humans is a particular risk for wild primate populations (Wolfe et al., 1998). Despite an increased surveillance in areas of human-wildlife overlap, many areas and parasite species representing a high risk of disease transmission from human/domestic animals to wildlife have not been studied. In particular, cross-species exchanges of bacteria, a main cause of emerging infectious disease in humans (Jones et al., 2008), has only been well-documented in a few cases. For example, bovine tuberculosis spread from Europe-imported cattle into buffalo populations in South Africa (Michel et al., 2006). Likewise, outbreaks of pneumonia in the bighorn sheep of California were associated with transmission from domestic sheep (George et al., 2008). However, in several developing countries with a high potential for cross-species bacteria transmission, bacteria spread from humans/domestic animals to wildlife such as primates has been poorly documented (Jones et al., 2008; Pedersen et al., 2005). Therefore, there is an urgent need for generating empirical data that will be used to identify areas with a high risk of human-to-wildlife bacteria transmission.

2. Antibiotic-resistant Enterobacteria as a marker of transmission

In order to estimate the risk of bacterial transmission from humans/domestic animals to wildlife in an area where these populations overlap, we compared the populations of commensal gastrointestinal bacteria among different host populations. To do so, we used a series of phenotypic and genetic markers that can be present or absent among bacteria. The idea behind this approach is that a bacteria present in host B, but transmitted from host A, will share the markers of other bacteria present in host A (Archie, Luikart & Ezenwa, 2009). Here, we focused on describing bacteria strains according to their ability to express antibiotic resistance, because the extensive use of antibiotics in humans and domestic animals has

selected bacteria expressing antibiotic resistance (Khachatourians, 1998; Witte, 2000). Thus, since wildlife populations are rarely treated with antibiotics, the presence of antibiotic-resistant bacteria among wildlife is expected to reflect bacterial transmission from humans or domestic animals (Guenther, Ewers & Wieler, 2011; Skurnik et al., 2006). However, the genetic material conferring antibiotic resistance can also be transmitted between unrelated bacterial strains (Courvalin, 1994; de la Cruz & Davies, 2000; Ochman, Lawrence & Groisman, 2000) and antibiotic resistant can also be selected within the environment (Allen et al., 2010; Goldberg et al., 2007). Thus, the study of antibiotic-resistant bacterial transmission represents a particularly complex challenge in distinguishing whether antibiotic resistant strains originated from human spillover or from endemic circulation generated by environmental selection. Therefore, we mainly focused on one of the most described bacteria of the intestinal flora, *Escherichia coli*, and combined antibiotic-resistant information with genetic markers to infer the phylogenetic group of each strain. This allowed us to confirm or reject if the presence of antibiotic-resistant bacteria among wildlife was explained by the direct transmission of bacteria from humans or domestic animals to wild animals. Using this methodology, we assessed whether antibiotic-resistant bacteria was spread from humans or domestic animals to wild primates in three different locations in Africa.

3. Is bacteria being exchanged at the interface between humans/domestic animals and wildlife in the periphery of protected areas? : Articles 3, 4 and 5

We addressed this question by collecting and analysing samples from three regions of Africa where wild primates interact with local humans and/or their domestic animals: one semi-arid

desert area (Tsaobis Nature Park-Namibia) and two national parks in humid tropical forest (Lope NP-Gabon and Dzanga-Ndoki NP-Central African Republic).

In our first study (Article 3), we aimed to estimate both the prevalence and the source of antibiotic-resistant strains in Enterobacteria from wild chacma baboons (*Papio ursinus*), overlapping foraging areas with livestock in Tsaobis NP. Our results showed a high prevalence of antibiotic-resistant isolates from fecal samples of livestock, wildlife and water samples. Furthermore, we found a strong correlation in both the prevalence and the nature of antibiotic resistance between livestock and baboons across five local areas of interaction. Thus, these findings suggest that antibiotic resistant material may be spreading from livestock to wild animals, possibly through foraging overlap. The presence of antibiotic-resistant bacteria in the water also supports the role water in facilitating the transmission of antibiotic-resistant bacteria transmission within these two populations. Overall, this work suggests that antibiotic resistant material, independent of the bacteria species carrying it, may be spreading by different mechanisms from livestock to wild animals in protected areas where livestock is treated with antibiotics. Furthermore, these findings further suggest that even indirect contacts through territory overlap may be sufficient to generate the transmission of gastro-intestinal bacteria at the wildlife-livestock interface.

Our two other studies focused specifically on the transmission of antibiotic-resistant *Escherichia coli* between humans (and/or domestic animals) and the critically endangered western lowland gorilla (*Gorilla gorilla gorilla*) or other mammals living within the protected areas of Lope NP (Article 4) and Dzanga-Ndoki NP (Article 5). In previous studies, *E. coli* exchanges with humans were observed among wild populations of mountain gorillas and chimpanzees exposed to research and tourism activities in Uganda (Goldberg et al., 2007; Rwego et al., 2008). Our studies extend this work by focusing in areas of Central Africa where human and domestic animal densities are much lower than Uganda.

The study carried out in Lopé NP focused on testing whether the risk of bacterial transmission from humans to gorillas and other wildlife was low in a region with one of the lowest densities of human population in Africa. Thus, we determined the prevalence of *Escherichia coli* antibiotic resistant isolates from western lowland gorilla and other wild mammals, and tested whether the observed pattern could be explained by bacterial transmission from humans and domestic animals into wildlife populations. Our results show a high prevalence of antibiotic resistant bacteria isolates in humans, but low levels in domestic animals, gorillas and other wildlife. Furthermore, we found significant differences in the genetic background of the resistant bacteria isolated from humans (or domestic animals) and gorillas (or other wildlife). This suggests low or non-existing *E. coli* transmission between habitants of the local villages and animals in the forest. This also indicates that the presence of antibiotic resistant strains in wildlife may not necessarily imply direct bacteria transmission from humans. Rather, it might be better explained by other mechanisms for resistance acquisition such as horizontal gene exchange amongst bacteria, or naturally-acquired resistance. Overall, this study suggests that in areas of low human and domestic animal density, human-to-wildlife *E. coli* transmission appears to be low.

Our study in Dzanga-Ndoki focused partially on the same question as the study in Lopé, but its conclusion differs substantially. As in Lopé, we found a high prevalence of antibiotic resistant bacteria isolates in humans, but low levels in gorillas and other wildlife. However, there were no difference in the genetic background of resistant bacteria isolated from humans and gorillas, despite significant differences in the genetic background of non-resistant isolates (Fig. 3). Furthermore, there was a strong correlation in the specific prevalence of resistance to each antibiotic between humans and gorillas. Thus, these results suggest that humans might be spreading antibiotic-resistant strains to gorillas. The difference observed between Dzanga and Lopé in the origin of antibiotic resistance among gorillas may

be explained by the activities of the human populations sampled. In Lopé NP, villagers do not enter into the deep forest often, and levels of hunting are relatively low. In Dzanga, we primarily sampled men from the Ba'Aka ethnic group. This population, previously nomadic within the forest but now forced to live at the periphery of the park, undertake recurrent incursions into the forest for hunting, fishing or collecting different plants. Thus, transmission of bacteria from humans to gorillas in this area may be a consequence of these activities.

Overall, these three studies suggest that if humans or domestic animals living at the periphery of protected areas are locally treated with antibiotics, they represent a source of antibiotic-resistant bacteria. Whether they spread these bacteria into wild populations or not may depend on their level of habitat overlap with wild animals.

4. Can bushmeat hunting affect the dynamics of human-introduced antibiotic resistant bacteria in a wild population by a density-dependent effect? : Article 5

The spatial distribution of antibiotic resistant isolates among gorilla groups collected in Dzanga-Ndoki revealed a rather unexpected result: the level of multiresistance (*i.e.* the number of antibiotics a given isolate was resistant to) in the isolates of a gorilla social group increased with the distance between that group and the borders of the park. In other words, the further gorillas were from human activity, the higher the level of multiresistance among their bacteria isolates. Since antibiotic-resistant isolates found in gorillas at Dzanga are most likely spread from humans, this result was opposed to the expected pattern: the prevalence of an introduced pathogen from humans should decrease with distance to the point of introduction. In Dzanga, the density of gorilla groups is mainly shaped by hunting intensity, and increases with distance from the park boundary. Thus, we suggested that a higher bacterial transmission

rate among groups living in forced closer proximity could explain our result. Therefore, we developed a spatially explicit Lotka-Volterra host-parasite model to test this hypothesis. Our model predicted that a gradient in transmission between groups can explain a higher level of antibiotic resistant prevalence in groups far from the introduction point. Thus, by affecting the density and transmission rates between gorilla groups, our model predicts that bushmeat hunting may be an important driver in the dynamics of a human-introduced pathogen in a wild animal population.

Conclusions and perspectives

Using both empirical data analysis and a modelling approach, this thesis identified a series of ecological, social and individual drivers of parasite spread. Furthermore, the use of commensal bacteria was used as a promising marker to identify areas where bacterial transmission is occurring between humans, domestic animals and wildlife. Finally, we combined both empirical data and a spatially explicit model to show how gradients in host density caused by hunting could modify the prevalence of human-introduced bacteria within the population. Each of the articles of this thesis has raised a series of possibilities for future work. Below, we describe some open questions and methodological developments that would help extend this work to allow a better understanding of the processes responsible for the spread of diseases in wildlife epidemiology.

The first article showed the importance of several factors at different scales driving individual parasite richness. Therefore, it also opened further questions regarding the effects of these factors. For example, the collection of additional data during the wet season, if logistically possible, should help quantifying the total amount of parasite richness variation attributable to seasonal changes in the environment. This will in turn confirm or reject if individual traits are more important for individual parasite richness than environmental factors (*e.g.* temperature or vegetation), as suggested by our study focusing within the dry season. Furthermore, the causality of many of the factors explored in our study needs to be established. In particular, very little is known on whether the correlation between body condition and parasite richness reflects a causality effect. Experiments such as the deworming of the population performed during the animal's trapping in 2006 will certainly help in elucidating this question. Altogether, our study encourages the development of a theoretical

framework combining the effects of all these factors (and others) across different scales on parasite spread. As a first step, detailed individual-based models could be used to capture social and demographic parameters governing the population, while accounting for changes in exposure associated with the environment (Roche et al., 2008). These models could then be used to predict how different social systems or environmental scenarios (*e.g.* global warming or resources decline) may affect the exposure and spread of gastro-intestinal parasites among a wild animal population.

At least two applications for the model developed in Article 2 could be explored. First, control strategies such as vaccination could be easily incorporated into the model, in order to test the efficiency of these strategies under different scenarios of disease spread. Preliminary results on this question show that vaccination focusing in groups ranging at intermediate distances from a habitat hotspot is more effective than vaccination targeting random groups or groups located around the hotspot. Second, this model can be applied to a variety of empirical systems. Specifically, we are using this model to study whether the Lokoué forest clearing accelerated the epidemic of Ebola affecting western lowland gorillas visiting this clearing in the Republic of Congo. This is being achieved by fitting the model to Capture-Mark-Recapture data of gorillas visiting the clearing before, during and after the epidemics. Model fitting is being implemented with the Approximate Bayesian Computation Sequential Markov Chain (ABC SMC) method (Toni et al., 2009).

Our work on bacteria transmission from humans to wildlife focused mainly on testing whether transmission was (or not) occurring in different areas. Thus, in order to fully understand the mechanisms explaining the observed patterns, several questions need to be answered. First, the transmission mechanism explaining the presence of antibiotic-resistant material in wild animals remains to be understood. Testing the presence of genetic material

conferring resistance among isolates such as gene cassettes, transposons or plasmids will help understanding this mechanism. Secondly, where transmission from humans or domestic animals to wild animals is occurring (Dzanga and Tsaobis), the environmental pathway responsible for this transmission needs to be better understood. Our study focusint in Tsaobis supports the role of water in the transmission of resistant material. Therefore, this and other mechanisms need to be studied in more detail. Finally, this work started with the ambition of using commensal enterobacteria as a marker for bacterial transmission. However, the pertinence of this model to predict other bacterial pathogens with similar modes of transmission requires additional work. For example, this could be achieved by simultaneously collecting information on commensal bacteria and other pathogens (*e.g.* *Salmonella*) within the same host populations.

A more detailed analysis of the relationship between bacteria strains across individuals and groups could help in understanding the dynamics of bacteria between social groups of a population. This might be possible with the recent development of techniques such as bacteria genome sequencing, which offer new opportunities to achieve our “holy grail”: tracking the spread of a bacteria by looking at the relationships between different isolates. This would follow previous studies on viruses such as Foot and Mouth disease or influenza (Cottam et al., 2008; Hovmoller et al., 2011; Jombart et al., 2011; Wallace et al., 2007). Using parasite diversity (either genetic or phenotypic) to track epidemiological transmission raises a fundamental theoretical question that will also have important methodological implications: what are the expected patterns of diversity generated by different transmission scenarios? For example, what type of parasite phylogeny is generated by a given contact network? Some pioneering work has linked diversity to networks structure in a neutral scenario(Economo et al., 2008). This has also been conducted for parasites, focusing particularly on the impacts of cross-immunity and different small networks on parasite diversity (Buckee, Danon & Gupta,

2007; Buckee, Gupta & Sintchenko, 2011; Buckee et al., 2004). Future work on this question will require the development of theoretical models including both host features and different processes that can generate diversity at the parasite level, such as mutation rates, selection, between-strains competition and host-parasite immune interactions, among others.

The interdisciplinary nature of this thesis required a long list of collaborators. In particular, I had the opportunity to work directly with local communities, authorities and park managers in Africa. Although public health is a worldwide priority, studying infectious diseases in poor countries where people fight daily for their survival remains a tremendous challenge. Improving the surveillance and control of infectious diseases in these countries will necessarily require better training of local scientists, doctors and veterinary staff, along with improved involvement of local communities. In practice, this is far from being accomplished. In particular, local authorities and community members complained to us that many research projects do not communicate their results to the local community or do not seek to systematically train local students (despite, in some cases, mandatory requirements of the research permits). Finally, as we are dealing with disease, local people are interested in both understanding the problem (our research) but also in finding solutions. For example, in Gabon, many participants of our project complained that we investigated bacteria in the water, but we did not provide them with a solution to access drinking water. Overall, a better interaction between researchers, the local community and different organisations (local universities, authorities and NGOs) should help extending the contributions of our research, and convert our results into practical solutions. This will probably encourage the engagement of local people in future studies and disease control measures.

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Articles

Article 1

From parasite encounter to infection: Multiple-scale drivers of parasite richness in a wild social primate population

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From Parasite Encounter to Infection: Multiple-Scale Drivers of Parasite Richness in a Wild Social Primate Population

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ABSTRACT Host parasite diversity plays a fundamental role in ecological and evolutionary processes, yet the factors that drive it are still poorly understood. A variety of processes, operating across a range of spatial scales, are likely to influence both the probability of parasite encounter and subsequent infection. Here, we explored eight possible determinants of parasite richness, comprising rainfall and temperature at the population level, ranging behavior and home range productivity at the group level, and age, sex, body condition, and social rank at the individual level. We used a unique dataset describing gastrointestinal parasites in a terrestrial subtropical vertebrate (chacma baboons, *Papio ursinus*), comprising 662 fecal samples from 86 individuals representing all age–sex classes across two groups over two dry seasons in a desert population. Three mixed

models were used to identify the most important factor at each of the three spatial scales (population, group, individual); these were then standardized and combined in a single, global, mixed model. Individual age had the strongest influence on parasite richness, in a convex relationship. Parasite richness was also higher in females and animals in poor condition, albeit at a lower order of magnitude than age. Finally, with a further halving of effect size, parasite richness was positively correlated to day range and temperature. These findings indicate that a range of factors influence host parasite richness through both encounter and infection probabilities but that individual-level processes may be more important than those at the group or population level. *Am J Phys Anthropol* 147:52–63, 2012. © 2011 Wiley Periodicals, Inc.

Understanding the forces driving the spread of infectious diseases in wild animal populations is becoming increasingly important, given the primary role that parasites and pathogens may play in driving both population dynamics and evolutionary processes (Anderson and May, 1978; Tompkins, 2001). In population dynamics, wildlife diseases can lead to rapid declines in threatened species (Smith et al., 2009) and pose a growing threat as a source of human zoonoses (Jones et al., 2008). In evolutionary processes, infectious diseases have long been proposed as a significant pressure in the shaping of mating and social systems (Freeland, 1976), partly because frequent contact rates between mates and social partners might greatly facilitate the transmission of pathogens.

Since most animals are infected by several parasite species, and even individually benign infections can have a cumulative pathogenic impact (McCallum, 1994; McCallum and Dobson, 1995), an understanding of the factors that determine the number of parasites an individual carries (i.e., host parasite richness) may be crucial to elucidating patterns of host vulnerability and the wider impacts of parasitism on host ecology and evolution (Bordes and Morand, 2009). Indeed, host para-

site richness has been linked to a diverse range of micro- and macroecological and evolutionary processes,

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such as adult mortality rates (Simkova et al., 2006), the population-level maintenance of polymorphisms in immune genes such as the major histocompatibility complex (mammals: Simkova et al., 2006; Gouy de Bellocq et al., 2008), and species diversification (e.g., primates: Nunn et al., 2004). Parasite richness is also becoming an increasingly important metric for understanding the impacts of anthropogenic disturbance on threatened taxa (e.g., primates: Chapman et al., 2005b; Gillespie et al., 2005; Valdespino et al., 2010).

Despite its importance, we know surprisingly little about the determinants of host parasite richness. Indeed, theoretical progress in this area is constrained by the dearth of empirical research—and this is particularly true for field data—and a lack of information necessary for modeling (Tompkins et al., 2011). Within species, a variety of forces can potentially interact with host susceptibilities to shape parasite transmission across a range of ecological scales, from populations to individuals (Tompkins et al., 2011). At the population level, seasonal environmental factors, such as increasing rainfall and temperature, are expected to increase parasite richness (Nunn and Altizer, 2006), along with intrinsic factors such as population size and density, number of groups (for social species), and degree of population fragmentation (Morand and Poulin, 1998; Chapman et al., 2005b; Nunn and Altizer, 2006). At the group level (in socially structured populations), the group size, daily travel distance, and area and productivity of the home range, might all affect parasite richness (Vitone et al., 2004; Nunn and Altizer, 2006) (but see also Snaith et al., 2008; Bordes et al., 2009). Finally, at the individual level, a wide range of traits might influence parasite richness including body mass, sex, age, social rank, reproductive state, hormone levels, immune status, and genetic constitution (for reviews, see: Nunn and Altizer, 2006; Tompkins et al., 2011). However, identifying independent, contemporaneous, effects of such myriad factors across spatial scales, and assessing their relative importance, remains a substantial challenge—especially when the complexity of factors involved necessitates an integrative approach, using concurrent monitoring of individuals and their environment through a longitudinal, rather than cross-sectional, design (Tompkins et al., 2011).

Here we investigate the relative importance of a range of factors that might influence host parasite richness. We structure our analysis to recognize the multiple spatial scales over which these factors operate (i.e., the population, group, and individual), and specify whether their mode of action is most likely to affect parasite richness through the probability of encounter with parasites or the susceptibility to infection following encounter (*sensu* Nunn and Altizer, 2006). Our analysis focuses on patterns of gastrointestinal parasite richness in a wild social primate population of chacma baboons (*Papio ursinus*). Although individually based parasite studies in wild primates are uncommon (Nunn and Altizer, 2006), they are of particular interest for at least three reasons. First, wild primates are perhaps the most serious wild source of cross-species disease transmission to humans, with sometimes catastrophic consequences for public health, e.g., SIV-HIV (Keele et al., 2006) and malaria (Liu et al., 2010). Second, primates are a taxon of high conservation concern, with disease posing a serious threat in some populations (Chapman et al., 2005a). Finally, studying a social species will contribute to our

understanding of the dynamics of parasite transmission in group-living organisms that may be especially vulnerable to infectious diseases (Altizer et al., 2003).

In Table 1, we have detailed the eight hypotheses tested. At the population level, our hypotheses predicted that parasite richness would increase with wet (H1) or hot (H2) conditions. At the group level, we predicted that parasite richness would be higher in more productive home ranges (H3), or in association with more extensive ranging behavior (H4). At the individual level, we predicted that parasite richness would be influenced by age (H5), sex (H6), physical condition (H7), and social rank (H8). Finally, we investigated the relative magnitude of the effects of all those factors found to influence parasite richness, across spatial scales, in a single global model.

MATERIALS AND METHODS

Study system

Our study was carried out on wild chacma baboons on the edge of the Namib Desert, in central Namibia, at Tsaobis Leopard Park (22°23'S 15°45'W). Tsaobis is characterized by mountains and rocky foothills that descend to rolling gravel and alluvial plains. Vegetation is sparse, comprising grasses, herbs, shrubs and dwarf trees, although patches of aquifer-dependent woodland grow along the ephemeral Swakop River bordering Tsaobis to the north. The landscape is arid and strongly seasonal: annual rainfall is low (mean $\pm$ SD: 123 $\pm$ 77 mm, n = 68 years) and falls only during the austral summer, primarily between December and April. The altitudinal range is 683–1,445 m. Shade temperatures can approach zero on winter nights, but exceed 40°C on summer days.

Data were collected during two field seasons (June to December 2005, May to October 2006) on two social groups. These comprised, in October 2006, 9 adult or subadult males, 16 adult females, and 32 juveniles for the larger group (Troop J) and 7 adult or subadult males, 9 females and 16 juveniles for the smaller group (Troop L). All subjects were fully habituated and individually identifiable.

Fecal sampling and analysis

A total of 662 fecal samples were collected immediately after defecation from 86 individuals. The feces were homogenized and a portion (mean $\pm$ SEM: 0.73 $\pm$ 4.10⁻³ g) was weighed and stored in 4 ml of 10% buffered formalin solution immediately after collection, at room temperature. A mean of 8.1 samples per individual (SD = 6.40, median = 7, range: 1–37), and 53.4 samples per month (SD = 27.8, median = 61, range: 17–104), were collected through the study period. Fecal analysis was carried out using the modified formol-ether sedimentation technique (Allen and Ridley, 1970), using merthiolate-formalin as a stain. Parasitic eggs, larvae, trophs, and cysts were recorded by species or morphotype, with measurements made to the nearest 0.1 mm using an ocular micrometer fitted to a compound microscope (for further details on parasite identification, see Appleton et al., 1986, 1991). Because of difficulties in identifying rounded-up trophozoites or precystic stages within small-sized amoebae, *Entamoeba hartmanni*, *Endolimax nana*, and *Dientamoeba fragilis* were pooled together into a morphotype designated as “small amoebae” (Fiennes, 1972). Similarly, *Entamoeba chattoni*, *Entamoeba histolytica*, *Entamoeba dispar*, and *Iodamoeba buetchlii* were pooled as “medium amoebae.” Host

TABLE 1. Potential factors influencing host parasite richness explored in this study

Scale	Factor	Hypotheses under test
Population	Rainfall	H1 (+) Encounter probability—due to the accelerated development, replication, or survival of parasites in wetter conditions (Nunn and Altizer, 2006)
	Temperature	H2 (+) Encounter probability—due to the accelerated development, replication, or survival of parasites in hotter conditions (Nunn and Altizer, 2006)
Group	Home range productivity	H3 (+) Encounter probability—because vegetation can be a surrogate measure of environmental moisture and thermal conditions for parasites (Bavia et al., 2001) or can represent a breeding or sheltering site for parasites (Ceccato et al., 2005; Lindsay et al., 1991)
	Ranging behavior	H4.a Home range size: (+) Encounter probability—due to an increased probability of encounters with parasites in a larger home range (Nunn and Altizer, 2006)
		H4.b Daily travel distance: (+) Encounter probability—due to an increased probability of encounters with parasites in a more intensively used home range (Nunn and Altizer, 2006; Nunn et al., 2011)
	Age	H5.a (+) Encounter probability—due to an accumulation of parasites in older individuals resulting from a stable probability of encounters with new parasites over time (Nunn and Altizer, 2006)
Individual	Sex	H5.b (–) Susceptibility to infection—due to a reinforced immunity in older individuals following repeated contacts with multiple parasites (Hudson and Dobson, 1997)
		H6 (+ males) Encounter probability—higher parasite richness in males due to higher consumption of food and thus more opportunity to eat contaminated items (Nunn and Altizer, 2006)
	Physical condition	(+ males) Susceptibility to infection—higher parasite richness in males (Zuk and McKean, 1996), due to immunosuppression typically resulting from elevated testosterone levels (Roberts et al., 2004)
		H7.a (+) Encounter probability—animals that eat more are in better physical condition but also have more opportunity to eat contaminated items (Nunn and Altizer, 2006)
	Social rank ^a	H7.b (–) Susceptibility to infection—due to a better ability to resist infections for animals in good physical condition (Irvine et al., 2006)
		H8.a (+) Encounter probability—higher parasite richness in dominant individuals due to higher consumption of food and thus more opportunity to eat contaminated items (Nunn and Altizer, 2006)
		H8.b (–) Susceptibility to infection—higher parasite richness in subordinate individuals due to stress compromising immunocompetence (Nunn and Altizer, 2006)

Factors are grouped by the scale at which they operate (population, group, and individual). Further information is also provided on the proposed mechanism (whether each factor is more likely to influence parasite richness through the probability of parasite encounter or susceptibility to infection following encounter). The positive effect of a considered factor is noted (+), and a negative effect is noted (–).

^a Here, high social rank indicates dominant individuals and low social rank, subordinates.

parasite richness was estimated for each fecal sample by the number of different species/morphotypes recorded. We assumed that when species/morphotypes were present we were able to detect them, but some false negatives may have occurred if a species was harder to detect when its intensity of infection or reproductive output were lower.

Population-level environmental conditions (H1–H2)

Rainfall was monitored on a daily basis. Similarly, maximal temperatures ($T_{\max}$) in the shade were recorded on a daily basis and were available for 179 days (78% of the study period). $T_{\max}$ varied across the study period (maximal mean $\pm$ SD = 31.9 ± 4.8 , range = [20–41]), with maximal values during summer (December) and minimal values in winter (July) in both years. Minimal and maximal daily temperatures were strongly correlated (Pearson's correlations: $\rho = 0.71$, $n = 283$, $P < 0.001$), so only $T_{\max}$ was used here.

Group-level range productivity and ranging behavior (H3–H4)

Group location waypoints were taken at half-hour intervals over at least 100 full-day follows for each group conducted between May and November, thus covering

periods of both high and low plant productivity in the late austral summer and winter. Minimum convex polygons (Heupel et al., 2004), were constructed around these waypoints in ArcMap Version 9.3 using Hawth'sTools extension package (<http://www.spatialecology.com/htools/>) to provide a simple estimation of the home range boundaries over the study period. Within the home ranges, plant production was estimated using the Normalized Difference Vegetation Index (NDVI: Pettorelli et al., 2011): a satellite-based vegetation index based on the information collected by the Satellite Pour l'Observation de la Terre-Vegetation (SPOT VGT). NDVI is derived from the red to the near-infrared reflectance ratio [$\text{NDVI} = (\text{NIR} - \text{RED})/(\text{NIR} + \text{RED})$], where NIR and RED are the amounts of near-infrared and red light, respectively, reflected by the vegetation and captured by the satellite sensor (Jensen, 2006). We use a spatial resolution of $1 \times 1 \text{ km}^2$ available at 10-day intervals in each troop's home range (J and L). The home range boundaries were also used to determine the monthly home range size for each group (J : mean $\pm$ SD = $12.3 \pm 6.5 \text{ km}^2$, range = [5–27], L : $26.8 \pm 13.5 \text{ km}^2$, [8–49]), while the waypoint locations were also converted into paths to measure daily travel distance for 208 days (92% of the study period) (J : mean $\pm$ SD = $5.9 \pm 0.8 \text{ km}$, range = [5.2–8.0], L : $5.9 \pm 1.5 \text{ km}$, [2.6–7.3]). In the analyses, we use the mean daily travel distance per month.

Individual traits (H5–H8)

We investigated the influence of age, sex, body condition, and dominance rank on parasite richness. Age and condition were determined through individual inspection during troop captures: in *J* troop, 42 individuals (of 52) were captured in July 2005 and 55 (of 57) in October 2006, in *L* troop, 32 individuals (of 32) were captured in October 2006. Briefly, troops were captured using individual cages baited with corn cobs and set-up at dusk. The baboons were captured at dawn, anesthetized using tiletamine-zolazepam, and all processed within a day to be released together the following morning when fully awake. Age was estimated through dentition: tooth eruption schedules for wild baboons were used to assign age up to the eruption of the molars, while age beyond this point was estimated on the basis of molar wear (Huchard et al., 2009a). Body size was estimated by crown-rump length, measured during capture. Physical condition was measured through morphometric data. Because there is no consensus on the best way to index condition (Lukaski, 1987; Green, 2001), we used three different measures: (1) body mass, (2) mean skinfold thickness (MST), averaged across the triceps, abdominal, and subscapular regions, and (3) mid upper-arm fat (MUAF), a combination of the triceps skinfold thickness and the mid upper-arm circumference:

$$\text{MUAF} = \frac{SC}{2} - \frac{\pi S^2}{4}$$

where S = triceps skinfold thickness and C = upper-arm circumference (Gibson, 2005). To summarize these three measures into one general index, we conducted a principal component analysis (PCA). This analysis included all data from all individuals across 2005 and 2006 for which the three indices were available (49 of 51 individuals). The contribution of each measure to the first component (estimated through PCA square cosinus) was 0.71, 0.75, and 0.91 for MUAF, MST, and body mass, respectively. The first principal component of the PCA accounted for 81% of the total condition variation, and was then used as the body condition variable in our analyses. The mean time difference between our assessment of parasite richness (i.e., a given fecal sample) and the closest estimate of age/condition (at capture) was 73.4 ± 46.9 days.

Sex was determined by visual inspection. To establish dominance ranks, agonistic and approach/avoid interactions (following Smuts, 1985) were collected using *ad libitum* and focal observations across the study period (for details see: Huchard et al., 2009b). To control for differences in troop size, an animal's absolute rank is divided by the total number of individuals in the group—thus alpha animals have the smallest rank. Ranks were estimated for sexually mature individuals (females reach sexual maturity around 4 years of age and males around 5 years of age; e.g., Altmann and Alberts, 2003).

In summary, the data available for each individual variable were as follows: 86 individuals (662 samples) for sex, 76 individuals (613 samples) for age, 73 individuals (456 samples) for body condition, and 44 individuals (298 samples) for dominance rank.

Statistical analyses

To test the influence of socio-ecological factors on individual parasite richness, we ran five sets of linear mixed models (LMMs) with parasite richness as the response

variable. Although our response variable was discrete, we used LMMs rather than generalized linear mixed models (GLMMs) due to the need to incorporate temporal autocorrelation in the analysis (see below) which is so far only possible using LMMs fitted with a Gaussian distribution (Pinheiro and Bates, 2000). The residuals of all models were constant and normally distributed as checked by Q–Q plots and Shapiro–Wilk normality tests ($P > 0.05$ in all models). However, we also ran our models using GLMMs with a Poisson distribution (but without the autocorrelation term) and obtained the same results. All models tested include “baboon identity” nested in “troop membership” as random effects, to account for the nonindependence of multiple data collected from the same individual within a troop. Because estimations of parasite richness can increase (in a non-linear way) with fecal sample weight (Walther et al., 1995), we also controlled in each model for a potential effect of sample weight by introducing it as a fixed factor at the third polynomial degree. This degree was selected using an information theoretic approach: briefly, for each of the five models presented below, we initially compared the Akaike Information Criterion (AIC) scores of three alternative models with fecal sample weight fitted at the first, second and third order, and found that the latter consistently performed best (i.e., presented an AIC score at least two points lower than the alternative models). This third-order relationship was further confirmed graphically by an asymptotic curve linking parasite richness to fecal sample weight. As a final statistical control, we also included the year of sample collection as a fixed effect. However, this was not found to be significant in any model examined and was therefore excluded in the final set of analyses, for simplicity.

The first set of analyses occurred in three successive stages, exploring the effects of the different variables at each considered scale (population, group, and individual). The first model was designed to investigate the effect of a population-level factor on host parasite richness, specifically the effects of the maximum daily temperature ($T_{\max}$) averaged over the 7-day period during which the individual was sampled (Hypothesis H2; the effects of rainfall, Hypothesis H1, were tested independently due to the limited number of rainfall events: see below). We further explored if $T_{\max}$ collected before the time of fecal collection predicted parasite richness better than contemporary measures by using an additional subset of lagged models for $T_{\max}$. These models included maximum daily temperature averaged across the 7-day period occurring one, two, three, 4 or 5 weeks before the sampling date, compared by AIC and the $T_{\max}$ p-value. The model including $T_{\max}$ averaged 4 weeks before sampling performed best (see Supporting Information Table S1), and was therefore used in further analyses (the global model).

The second model was designed to investigate the group-level effects of home range productivity (H3) and ranging behavior (H4.a,b) on host parasite richness. Therefore, it included home range NDVI, home range area, and daily travel distances as fixed effects. As for $T_{\max}$, we also tested the NDVI measure lagged for 10, 20, and 30 days before sampling (as NDVI data were available for 10 days intervals), but found that contemporary NDVI performed best (see Supporting Information Table S2).

The third model was designed to investigate the first three of our four individual-level effects, namely age (H5),

TABLE 2. Individual patterns of parasite infection (662 samples, 86 individuals), with species/morphotypes ordered by prevalence

Species	Median	Range	Prevalence (%)	Parasite phylum
<i>Streptopharagus pigmentatus</i>	100.0	0.0–5154	77.5	Nematode
<i>Entamoeba coli</i>	1	0.0–4.0	77.1	Amoeboid
<i>Balantidium coli</i>	1	0.0–4.0	66.6	Ciliate
Small-sized amoeba	0	0.0–4.0	30.3	Amoeboid
<i>Chilomastix mesnili</i>	0	0.0–5	23.1	Flagellate
Medium-sized amoeba	0	0.0–4.0	21.9	Amoeboid
<i>Physaloptera caucasia</i>	0.0	0.0–2787.0	14.6	Nematode
Unidentified species (Egg1)	0.0	0.0–176.0	5.8	Nematode
<i>Ascaris</i> sp.	0.0	0.0–81	0.02	Nematode
<i>Subulura</i> sp.	0.0	0.0–98	0.01	Nematode
<i>Macracanthorhynchus hirudinaceus</i>	0.0	0.0–1	0.01	Acanthocephalan

The “medium amoeba” category includes *E. chattoni*, *E. histolytica*, *E. dispar*, and *I. buetchlii*. The “small amoeba” category includes *E. hartmanni*, *E. nana*, and *D. fragilis*. “Egg 1” corresponds to an unidentified nematode species. For nematodes, the median and range of intensity of infection is expressed in egg per gram. For protozoans, the intensity of infection is expressed as a score on a 5-point semiquantitative scale (0–4). Parasite prevalence is expressed as the number of individuals infected by a given parasite species (or category in the case of medium and small amoeba) divided by the total number of individuals and is given as a percentage.

sex (H6), and body condition (H7), on host parasite richness. These variables were all included as fixed effects in the same model. Age was introduced at the third polynomial degree to account for a potential nonlinear effect, which was suggested by graphical exploration of the raw data and by a model AIC score 2 points lower than the alternative models (i.e., with first or second polynomial degrees). Crown-rump length was additionally introduced in the model, to control for body size when investigating condition effects (Jakob et al., 1996). To investigate the effects of our fourth individual-level factor on host parasite richness, namely social rank (H8), we ran the individual-level model again for the subset of animals for whom social ranks were available ($N = 44$ adults), adding social rank as a fixed effect. We also included a sex*rank interaction term to account for profound sex differences in the acquisition of rank in this species (stable and heritable ranks among females; fluctuating ranks determined by fighting ability among males).

Following our analyses of the factors determining host parasite richness at each of the three spatial scales, we ran a final model to integrate our findings and to explore the relative importance of each of these factors across scales. This global model included all the variables that were found to be significant in the single-scale models, and was run using the full sample (juveniles and adults). To compare the effect sizes of each variable in this global model, all variables were standardized to have a mean of zero and a standard deviation of one.

In each model, we controlled for the temporal dependence of observations (i.e., temporal autocorrelation) by including a temporal correlation structure of the residuals. We compared the AIC of models having an autoregressive structure of order 1–7 (i.e., 1–7 lags of dependence between observations). Among all these models, the one with an autoregressive structure of order 5 obtained the lowest AIC (more than two points lower than the alternative models) and was therefore selected. This was implemented using the correlation structure corARMA (Pinheiro and Bates, 2000) in the nlme package of R 2.8.0 (R Development Core Team, 2003). The significance of fixed effects was evaluated using F-tests according to the principle of marginality, testing each fixed effect coefficient when all other fixed

effects are present in the model. Statistical significance is reported for full models (i.e., inferences were drawn with all predictors present) throughout (Whittingham et al., 2006; Mundry and Nunn, 2009). The significance of random effects was tested by performing likelihood ratio tests (following a χ^2 distribution) comparing two models differing only in the presence of this effect. In all the models, the random effect “troop identity” did not significantly affect individual parasite richness (Likelihood Ratio Test, $P > 0.05$) whereas “baboon identity” always had a significant effect (Likelihood Ratio Test, $P < 0.001$).

RESULTS

A total of 11 species or morphotypes of intestinal parasites including five nematodes, one acanthocephalan and nine protozoan, were found in the feces of *P. ursinus* at Tsaobis (Table 2). One type of nematode egg, that occurred in 6% of individuals, could not be identified further (named Egg 1 hereafter). Based on species/morphotype, the median individual parasite richness was 3.00 (range = 0–8, mean $\pm$ SD = 3.2 ± 1.3).

Population-level environmental determinants of parasite richness (H1–H2)

Host parasite richness decreased across the dry season (Fig. 1). However, a peak was observed in November 2005 (median = 4.0; mean $\pm$ SD = 3.8 ± 1.3), 10 days after the first and only rain recorded in the study period (16 mm, October 29, 2005). During November, average individual values of parasite richness were significantly higher than in October 2005 (median = 3.0, mean $\pm$ SD = 2.9 ± 1.1 , Mann–Whitney test: $W = 1345.5$, $n = 86$ individuals, $P = 0.002$), supporting our hypothesis that parasite richness increases after rainfall (H1). This difference was driven by protozoans (analyses excluding protozoans: $W = 3435$, $n = 86$, $P = 0.25$).

The best temperature predictor was $T_{\max}$ averaged across the fourth week preceding the sampling date (Table 3, Supporting Information Table S1), suggesting a lagged response of parasite richness. Thus, host parasite richness was higher following hot weather 4 weeks earlier (Fig. 2).

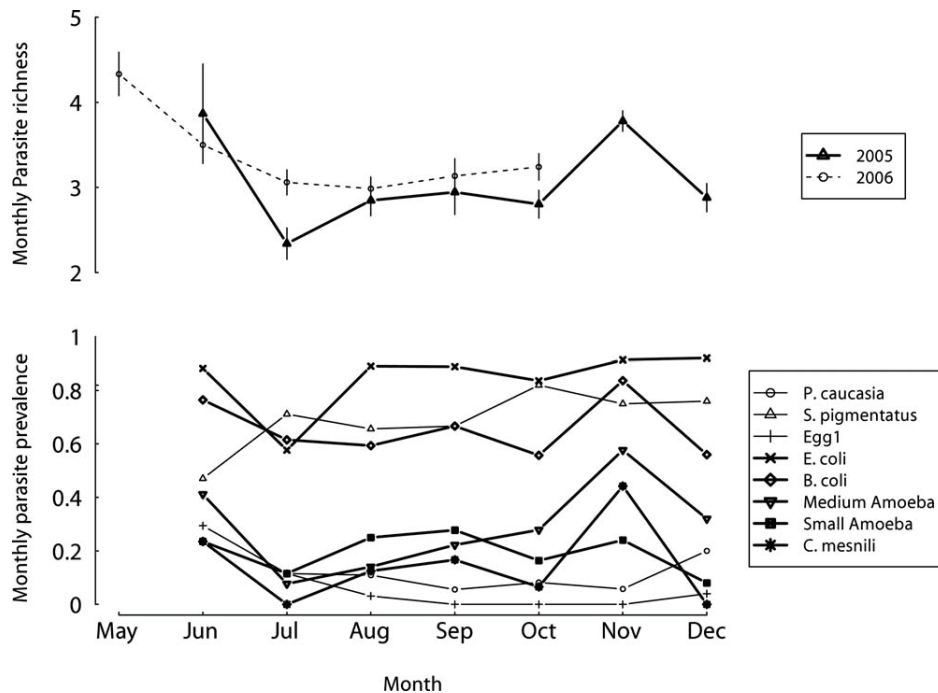


Fig. 1. Temporal variation of parasite richness during the study period. Monthly variation in parasite richness for the 2005 and 2006 study periods (means and standard errors) are displayed in the top panel. Monthly parasite prevalence (expressed as a fraction of total individuals) for each parasite species or morphotype for the 2005 study period are displayed on the bottom panel.

TABLE 3. Influence of environmental factors, ranging behavior, and individual traits on individual baboon parasite richness

Model	Variables	Estimate	SE	F-value	df	P-value
Population level	Sample weight ³	2.06	1.17	3.16	3	0.03
	$T_{\max}^a$	4.83	1.73	7.75	1	<0.01
Group level	Sample weight ³	2.20	1.19	3.02	3	0.03
	Home range NDVI	3.31	4.28	0.6	1	0.44
	Home range area	-0.01	0.01	1.44	1	0.23
	Travel distance	0.28	0.07	15.95	1	<0.001
Individual level	Sample weight ³	3.30	1.06	4.32	3	<0.01
	Age ³	3.45	1.58	2.85	3	0.03
	Sex ^b	-0.53	0.19	7.5	1	<0.01
	Body condition	-0.18	-0.08	5.08	1	0.02
	Body size	0.01	0.01	4.45	1	0.04

Each model represents a different scale: population-level factors (524 samples, 82 individuals, AIC = 1685.28), group-level factors (599 samples, 86 individuals, AIC = 1955.47), and individual-level factors (456 samples, 73 individuals, AIC = 1437.07).

^a Daily maximum temperature is averaged over the 7-days occurring 4 weeks before sample collection (see Results section).

^b The reference category for sex is female.

Group-level ranging determinants of parasite richness (H3–H4)

Host parasite richness increased when groups travelled further, as predicted by hypothesis H4.b. In contrast, there was no effect of home range NDVI (H3, for either contemporary or lagged measures, Supporting Information Table S2) or home range area (H4.a) (Table 3).

Individual-level trait determinants of parasite richness (H5–H8)

Across all individuals, host age, sex, and body condition (together with body size, included as a control variable for condition) influenced host parasite richness

(Table 3). Host parasite richness initially increases with age (supporting H5.a), but then peaks around sexual maturity, following which it declines (supporting H5.b) (Fig. 3). The sex effect indicated that parasite richness was higher in females than in males (contrary to H6), while the condition effect suggested that animals in better condition exhibited lower parasite richness (in support of H7.b) (Table 3, Fig. 4). Among adults only, we found no evidence that dominance rank affected parasite richness (failing to support H8), while the effects of age and sex were no longer significant ($P > 0.05$ in each case). The age effect remained nonsignificant when age was included at the first or second order (instead of the third) in this last model (after sexual maturity, the relationship between age and parasite richness appears roughly

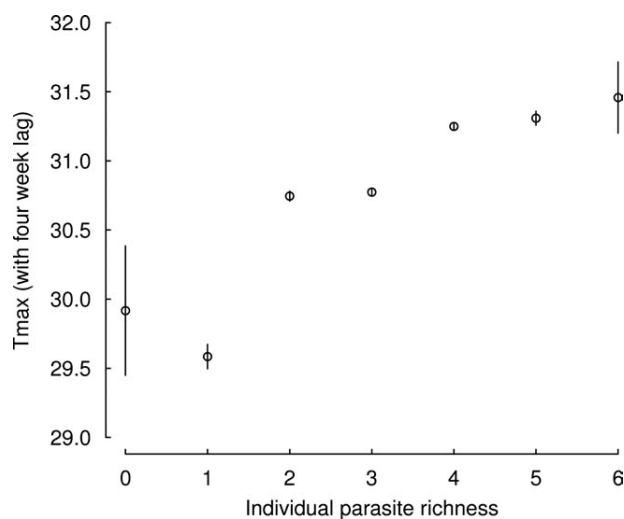


Fig. 2. Relationship between host parasite richness and daily maximum temperature ($T_{\max}$), averaged over the 7 days occurring 4 weeks before sample collection. The means and standard errors of $T_{\max}$ for each parasite richness score are shown.

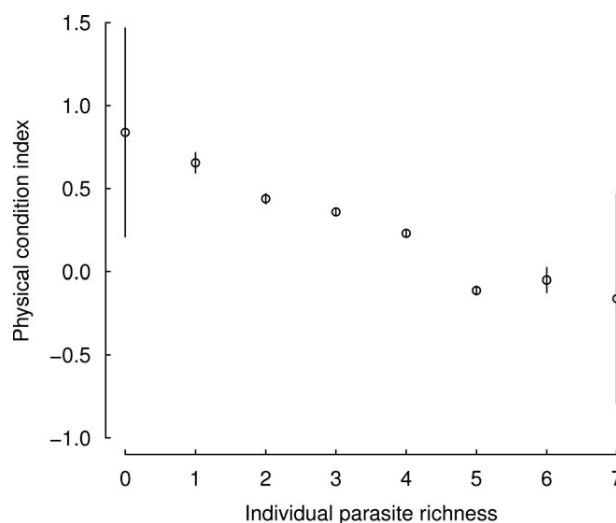


Fig. 4. Relationship between host parasite richness and physical condition. The means and standard errors of physical condition for each parasite richness score are shown.

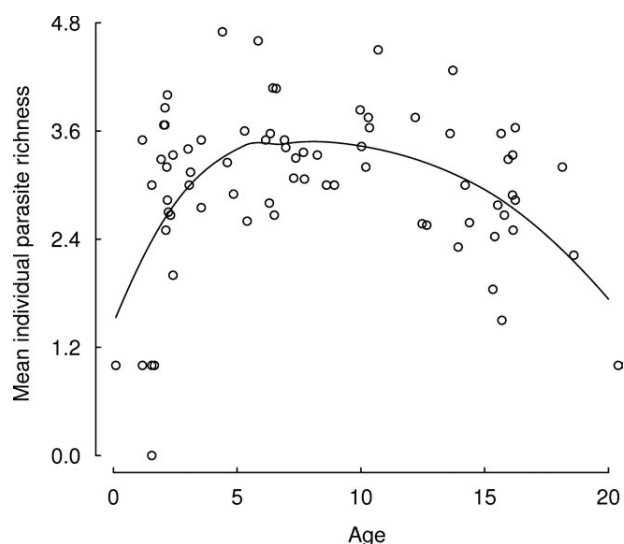


Fig. 3. Relationship between host parasite richness and age. Circles represent the mean parasite richness for an individual. The fitted line was drawn using a locally weighted polynomial regression (Cleveland, 1979) with the lowest command in R 2.8.0 (R Development Core Team, 2003).

linear, Fig. 3). However, adults in better condition still exhibited lower parasite richness than those in poor condition ($F = 4.43$, $P = 0.03$).

Integrated global model for multiple-scale effects on parasite richness

The integrated model corroborated the single-scale models (Table 4). A comparison of the effect sizes indicates that age had by far the strongest influence on parasite richness (effect size = 3.92 ± 1.46) followed by sample weight (effect size = 2.75 ± 1.03). Sex and body condition (together with body size) had comparable effect

TABLE 4. Multiple-scale influences on baboon parasite richness (386 samples, 72 individuals, AIC = 1170.02)

Variables	Estimate	SE	F-value	df	P-value
Age ³	3.92	1.46	4.41	3	<0.01
Sample weight ³	2.75	1.03	5.79	3	<0.001
Body size	0.48	0.19	6.24	1	0.01
Sex ^a	-0.47	0.19	5.97	1	0.02
Body condition	-0.43	0.13	11.22	1	<0.001
$T_{\max}$ (with 4-week lag) ^b	0.22	1.07	5.79	1	<0.01
Travel distance	0.21	0.06	10.56	1	0.001

All variables were standardized (mean of zero, standard deviation of one) and are ordered by their effect size.

^a The reference category for sex is female.

^b Daily maximum temperature is averaged over the 7-days occurring 4 weeks before sample collection.

sizes, which were almost an order of magnitude smaller than the age effect (effect size mean $\pm$ SD = -0.47 ± 0.19 and -0.43 ± 0.13 , respectively). Finally, travel distance and lagged $T_{\max}$ had the smallest effects (effect size = 0.21 ± 0.06 and 0.22 ± 1.07 , respectively).

DISCUSSION

Identifying the determinants of multiple parasite infections in wild animals is crucial for both fundamental and applied, conservation-based, reasons, since they may represent important drivers of both evolutionary change and population dynamics. However, there are surprisingly few studies of the drivers of parasite richness in wild populations (Tompkins et al., 2011), and most of these have worked at a single spatial scale. In this study, we found that gut parasite richness in a wild primate population increases with higher rainfall and maximum daily temperature at the population level, and with longer daily travel distances at the group level, as well as showing more complex covariation with age, sex, and body condition at the individual level. These findings, and how they compare to previous studies on parasite richness in wild populations, are summarized in Table 5. Finally, integrating our analyses across the

TABLE 5. Evidence from previous empirical studies and the findings of the present study

Scale	Factor	Evidence from previous empirical studies	This study
Population	Rainfall	(+) Comparative studies: bacteria across human populations (Guernier et al., 2004); gamasid mites across small mammals (Krasnov et al., 2008) (0) Field study of helminths in red foxes (Barbosa et al., 2005)	(+)
	Temperature	(+) Comparative study of fungi in French forest (Vacher et al., 2008); field study of helminths in red-legged partridge (Calvete, 2003) (0) Comparative studies: all parasite types in humans at large geographical scale (Guernier et al., 2004); endo- and ectoparasites in fish (Rohde and Heap, 1998); field study of helminths in red foxes (Barbosa et al., 2005)	(+)
Group	Home range productivity	No previous studies	0
	Home range size	(+) Comparative study of all parasite types in carnivores (Lindfors et al., 2007) (0) Comparative study of gut parasites in mammals (Watve and Sukumar, 1995) (-) Comparative study of helminths in mammals (Bordes et al., 2009)	0
	Daily travel distance	(+) Comparative study of helminths in primates (Nunn and Dokey, 2006); field study of chigger infections in California meadow mice (Mohr and Stumpf, 1964)	(+)
Individual	Age	(+) Longevity: comparative studies of Protozoans across primates (Nunn et al., 2003); ectoparasites across Pericidae fish (Ranta, 1992); helminths across freshwater fish (Bell and Burt, 1991); field study of endo- and ectoparasites in coral-reef fish (Lo et al., 1998) (0) Longevity: comparative study of ectoparasites across cyprinid fish (Simkova et al., 2006); field studies of gut parasites: red-fronted lemurs (Clough et al., 2010); mandrills (Setchell et al., 2007); chimpanzees (Muehlenbein, 2005) (-) Longevity: comparative study of helminths across mammals (Morand and Harvey, 2000)	Polynomial relationship with (+) effect before sexual maturity and a (-) effect afterwards
	Sex	(+ males) Comparative study of ectoparasites in small mammals (Morand et al., 2004); field study of fleas in desert rodents (Krasnov et al., 2005) (+ females) Field studies: fleas in rodent <i>Acromys russatus</i> (Krasnov et al., 2005); lice in Neotropical birds (Clayton et al., 1992) (0) Field study of gut parasites in red-fronted lemurs (Clough et al., 2010)	(+ females)
	Physical condition	(-) Field study of helminths in wild rabbit (Lello et al., 2005)	(-)
	Social rank ^a	(+) Field study of gut parasites in chimpanzees (Muehlenbein, 2005) but analyses not shown. (0) Field studies: gut parasites in ursine colobus (Teichroeb et al., 2009); red-fronted lemurs (Clough et al., 2010); mandrills (Setchell et al., 2007)	0

Previous studies in captivity, or studies examining other parasitic measures such as prevalence or load, are not reported here. The positive effect of a considered factor is indicated by (+), a negative effect (-), and no effect (0).

^a Here, high social rank indicates dominant individuals and low social rank, subordinates.

three scales of population, group, and individual, suggests that host age is the primary predictor of parasite richness.

At the population level, we observed significant effects of rainfall and maximum daily temperature on host parasite richness, indicating an important influence of climatic conditions on parasite encounter rates. The increase in water-borne protozoan parasites associated with rainfall represents a preliminary result since it is

based on only a single rainfall event. Nevertheless, it provides circumstantial evidence that precipitation can increase parasite richness on a short timescale (H1). Parasite richness also increased following a period of hot weather but with a 4-week lag (H2). The mechanisms linking temperature to gastro-intestinal parasite prevalence have been extensively studied, with several species of helminths requiring a minimum temperature for development (Boag, 1985), having shorter generation times

at relatively high temperatures, and/or producing more intermediate stages in their life cycle when temperature increases (Pietroock and Marcogliese, 2003). Protozoan taxa are similarly affected, commonly displaying higher reproductive rates at higher temperatures (Rodríguez-Zaragoza, 1994). The lagged response most likely reflects the cumulative time required by the free-living stages of parasites to react to environmental variation and for the host to be exposed to, and contaminated by, the growing populations of infectious parasitic forms.

At the group level, we found that longer daily travel distances (H4.b) but not larger home ranges (H4.a) were associated with higher host parasite richness. This supports the idea that more intensive movement patterns within a relatively stable home range, rather than variation in the home range area itself, are associated with increased parasite exposure and subsequent infection with parasites that accumulate in the environment and mature in the host to produce ova (Nunn and Altizer, 2006). The lack of association between home range NDVI and parasite richness (H3) further suggests that group-level changes in parasite encounter rates primarily result from the group's behavioral response to environmental variation rather than fluctuations in the density of infectious parasite stages, i.e., the baboons encounter more parasites because their groups are travelling further, not because there are more parasites to encounter per unit distance travelled.

At the individual level, we found covariation between parasite richness and age, sex, and body condition. Previous research on the age-parasite richness relationship (H5) has produced inconsistent results when assuming a linear pattern (Table 5). Our finding of a nonlinear relationship, positive before sexual maturity but negative afterward, might help to explain these inconsistencies—and reflect a combined effect of both encounter and infection probabilities. In the first case, the positive part of the curve might reflect cumulative exposure to parasites if the probability of encountering new parasite species is constant over time (Nunn and Altizer, 2006). This would suggest a relatively slow rate of acquisition of new infections by young animals in this population. In the second case, the negative part of the curve, exhibiting a weaker slope, might reflect an improved adaptive immune response following repeated exposures to parasites (Hudson and Dobson, 1997) and/or better survivorship of those individuals possessing stronger immune defenses against multiple infections. This latter hypothesis is supported by a recent study in this same population, where MHC heterozygotes (class II *Mhc*-DRB region) appeared to show higher survivorship (Huchard et al., 2010). Heterozygosity at MHC class II loci has also been found to mediate individual parasite richness in natural populations (Goüy de Bellocq et al., 2008; Oliver et al., 2009). Multiple infections might thus constitute the selective pressure increasing the mortality rate of individuals with low MHC diversity, if they display limited ability to fight multiple parasites, as previously found in fish hosts (Simkova et al., 2006). Notably, a recent comparative primate study found that parasitic nematode richness associated positively with the nonsynonymous nucleotide substitution rate at the functional part of the MHC molecule, but not with MHC allelic diversity (Garamszegi and Nunn, 2011). It is also possible that the weaker relationship linking age to parasite richness after sexual maturity might at least partially reflect the stabilization of individual parasite communities when they have reached

a given threshold, mediated through competitive interactions among multiple coinfecting parasites (decreasing the probability of subsequent infection by additional parasite species) (Graham, 2008).

We also found that females harbor more parasite species than males (H6). Although males are generally found to be more susceptible to parasitism than females (e.g., Klein, 2004), results from primate field studies have been less consistent, with several reported cases of female-biased parasitism (e.g., Clough et al., 2010; reviewed in Nunn and Altizer, 2006). In this case, there is no reason to expect female baboons to have a higher probability of encounter with parasites than males, so the most likely explanation for this difference is that females have a higher susceptibility to infection. One possibility is a social effect, given that all adult males outrank all adult females, but the lack of a sex*rank interaction does not support this. Alternative explanations may relate to the costs of reproduction in females, including the production of exaggerated sexual swellings when cycling and the nutritional stress associated with pregnancy and lactation, or to complex interactions between sex hormones and immune status. A recent field study in lemurs reported immune-enhancing effects of testosterone on parasite species richness, suggesting that differences in immune responses due to sex steroids might potentially lead to female-biased parasitism, at least in the case of host parasite richness (Clough et al., 2010).

Parasite richness was higher in poor-condition animals (H7.b), but there was no evidence that dominant animals carried more or fewer parasite taxa (H8). Our findings for the effects of physical condition corroborate the results of the one previous study to date that has also explored this relationship (Lello et al., 2005). The negative association between body condition and parasite richness suggests a role of infection rather than encounter probability, but the direction of the causal arrow remains uncertain: while poor condition might reflect a host's weak capacity to fight parasites on the one hand, it is also possible that the deleterious effects of multiple infections could lead to poor condition on the other. In the latter case, although most of the parasites reported here are not thought to be highly pathogenic, some might still impact baboon health (Ruch, 1959). The amoeba *E. histolytica* can cause severe diarrheal and dysenteric diseases, and affect the liver, lungs, brain, and other areas; whereas others like *B. coli* can become pathogenic if the host's natural resistance is depleted by a poor diet (Ruch, 1959). Whatever the causal direction, the observed association may help to explain why females in better condition in this population display a higher reproductive success (Huchard et al., 2009b). Our lack of rank effect was in contrast to theoretical expectations but consistent with most previous empirical studies in primates (Table 5), and may reflect confounding covariation between rank and condition.

When focusing solely on adults, body condition remained the only individual trait influencing parasite richness. In comparison with the full model including juveniles, the disappearance of both age and sex effects reflects either decreased statistical power arising from a smaller sample, or a weaker influence of such traits after sexual maturity. The latter hypothesis is plausible in the case of age, since the slope of the relationship linking age to parasite richness weakens in adulthood (Fig. 3), but seems counter-intuitive in the effect of sex, which is

usually reinforced among sexually mature individuals. Given that sex ratios are relatively balanced in both our full and restricted sample, the disappearance of this effect among adults might reflect a genuine pattern. Post-hoc interpretation is necessarily speculative, but could involve parental investment or maternal effects preferentially biased toward male offspring, which might translate into improved parasite resistance in early life (Hayward et al., 2010)—although the hypothesis of sex-biased maternal investment has not been strongly supported by empirical studies of non-human primates so far (Brown, 2001; Bercovitch, 2002).

The final global model integrating variables across scales largely confirmed the results obtained within scales (all variables previously found to be significant in their respective single-scale models remained significant in the multiscale model), but also emphasized the importance of working at multiple ecological scales. Comparing the effect sizes of each variable in the global model suggests that individual-level factors have a higher influence on patterns of variation in parasite richness than population- or group-level factors. In fact, age had by far the biggest effect on parasite richness, followed by sex and body condition, and finally by maximal daily temperature and daily travel distance. As such, the global model suggests that, while variation in encounter probability at both the population and group level do influence host parasite richness, the strongest effects are related to both encounter and infection probabilities at the individual level. Two areas of uncertainty in this interpretation should be highlighted. First, due to the difficulties involved in working at wider spatial scales with large social vertebrates, our sample of groups and populations is necessarily small. Similarly, we only sample one season (the dry winter season) over two years, and it is possible that in other seasons and/or years different patterns would be obtained. Extrapolation of our conclusion (that individual-level processes play the predominant role) beyond the sample and conditions investigated here should therefore be made with caution. Second, estimation of the relative importance of encounter and infection probabilities at the individual level is challenging. On the one hand, the effects of body condition (and probably sex) emphasize the importance of susceptibility to infection at the individual level. On the other, the age effect includes both encounter and susceptibility to infection, with the former having the strongest effect (since the negative relationship between age and parasite richness after maturity is relatively weak). Although neither of these uncertainties can be fully resolved here, they do help to highlight those areas that might be prioritized for further research.

In conclusion, these findings demonstrate that host parasite richness in animal populations may be associated with a range of factors operating on multiple scales. In this case, parasite richness is highest in poor-condition females at the time of sexual maturity, when their social group is travelling longer daily distances, and when environmental conditions are characterized by high rainfall and temperature. This study also suggests that individual traits, acting through both encounter and infection rates, can have a higher impact on parasite richness than group- or population-level factors acting through encounter rates alone. Our results emphasize the value of integrative approaches based on the longitudinal sampling of known animals in well-documented ecological contexts, and suggest that such a design can

provide unique insights into the relative importance of different factors shaping host parasite richness and its impact in wild populations.

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Supplementary Information

Table S1. Comparison of models for different measures of maximal temperature, Tmax: mean weekly Tmax around the sampling day, and the mean weekly Tmax across the preceding week before the sampling day, two weeks before (i.e. 14 to 8 days before sampling), three weeks before (i.e. 21 to 15 days before sampling), four weeks before (i.e. 28 to 22 days before sampling) and five weeks before (i.e. 35 to 29 days before sampling). Each model includes the same fixed effects (sample weight and Tmax) as well as the same random effects (“baboon identity” nested in “troop identity”). The following table shows the AIC for each model as well as the estimate and *P*-value of the temperature measure included. The same analysis was performed for the six, seven and eight week lags before sampling date, with the results remaining unchanged. All models were compared with the same number of observations, in order to avoid possible effects of sample number on the model AIC. The model including Tmax four weeks before the sampling date was identified as the best model, on the basis that its AIC score was more than two points lower than any of the competing models.

Variables	Estimate	SE	F-value	<i>P</i> -value	AIC
Mean weekly Tmax around sampling date	0.05	0.02	5.67	0.02	922.34
One week before sampling date	0.05	0.02	5.57	0.02	922.33
Two weeks before sampling date	0.08	0.02	13.70	<0.001	914.90
Three weeks before sampling date	0.05	0.02	5.21	0.02	922.78
Four weeks before sampling date	0.10	0.02	20.71	<0.0001	909.40
Five weeks before sampling date	0.10	0.02	3.59	0.06	924.02

Table S2. Comparison of models for different measures of home-range NDVI: the NDVI score on the sampling day, plus the NDVI scores lagged by 10, 20 and 30 days before the sampling day, respectively. Each model includes the same fixed effects (sample weight, NDVI, home range area and daily travel distance) as well as the same random effects (“baboon identity” nested in “troop identity”). The following table shows the AIC for each model as well as the estimate and *P*-value of the NDVI measure included. The model including NDVI at the sampling date was identified as the best model, on the basis that its AIC score was more than two points lower than any of the competing models.

Variables	Estimate	SE	F-value	<i>P</i> -value	AIC
NDVI at sampling date	3.31	4.28	9.98	0.43	1955.48
10 days lagged NDVI	4.95	3.28	2.27	0.13	1973.27
20 days lagged NDVI	2.06	2.74	0.56	0.45	1975.31
30 days lagged NDVI	0.76	2.34	0.10	0.74	1976.08

Article 2

Transmission of infectious diseases en route to habitat hotspots

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Transmission of Infectious Diseases En Route to Habitat Hotspots

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Abstract

Background: The spread of infectious diseases in wildlife populations is influenced by patterns of between-host contacts. Habitat “hotspots” - places attracting a large numbers of individuals or social groups - can significantly alter contact patterns and, hence, disease propagation. Research on the importance of habitat hotspots in wildlife epidemiology has primarily focused on how inter-individual contacts occurring at the hotspot itself increase disease transmission. However, in territorial animals, epidemiologically important contacts may primarily occur as animals cross through territories of conspecifics en route to habitat hotspots. So far, the phenomenon has received little attention. Here, we investigate the importance of these contacts in the case where infectious individuals keep visiting the hotspots and in the case where these individuals are not able to travel to the hotspot any more.

Methodology and Principal Findings: We developed a simulation epidemiological model to investigate both cases in a scenario when transmission at the hotspot does not occur. We find that (i) hotspots still exacerbate epidemics, (ii) when infectious individuals do not travel to the hotspot, the most vulnerable individuals are those residing at intermediate distances from the hotspot rather than nearby, and (iii) the epidemiological vulnerability of a population is the highest when the number of hotspots is intermediate.

Conclusions and Significance: By altering animal movements in their vicinity, habitat hotspots can thus strongly increase the spread of infectious diseases, even when disease transmission does not occur at the hotspot itself. Interestingly, when animals only visit the nearest hotspot, creating additional artificial hotspots, rather than reducing their number, may be an efficient disease control measure.

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Introduction

The spread of infectious diseases strongly depends on how habitat characteristics shape patterns of between-host interactions [1,2]. In particular, habitat heterogeneity influences patterns of between-individual contacts and hence, disease dynamics [1,3]. For example, “habitat hotspots”, sites that attract individuals or social groups over long distances, can be visited by a large subset of a population. Around hotspots, between-individual contact rates often increase in frequency, which amplifies disease transmission. In humans, schools and working places are typical examples of hotspots and have been shown to accelerate the spread of measles, influenza and SARS [4,5,6]. Thus, limiting transmission at hotspots has become a promising strategy for mitigating epidemics (e.g., influenza [7]) although the efficiency of such strategies also depends on the role hotspots plays relative to other sources of local transmission (e.g., influenza [6,7]).

In wild animal populations, high quality feeding spots (e.g., fruit trees), breeding sites, waterholes or sleeping sites can exacerbate

direct physical contacts. Empirical and theoretical studies on the epidemiological importance of habitat hotspots have mainly focused on how the spatial aggregation of animals favors disease transmission at the hotspot itself [8,9]. For example, the aggregation of wild boar at watering sites significantly increases the transmission of tuberculosis-like lesions [8]. However, inter-individual contacts may not always significantly increase at the hotspot itself. This is for example the case of habitat hotspots that some animal species only visited occasionally, such as some mineral licks [10,11]. Also, animals present at the same time at a particularly large hotspot may not be close enough to each other to transmit infectious diseases. This is the case of large forest clearings [12,13] or large waterholes. Finally, species such as primates and ungulates might avoid defecating in hotspots of high food resources, limiting the transmission of fecal-oral parasites at hotspots [14,15].

When disease transmission does not occur at the hotspot, it can still occur at a certain distance from the hotspot. This phenomenon has received little attention so far. Specifically,

infective contacts may be observed when infectious individuals travel to the hotspot and cross the territory of susceptible individuals and, reversely, when susceptible individuals cross the territory of infectious individuals. This second type of transmission may be prominent when the disease reduces the mobility of sick individuals (*i.e.*, sickness behavior [16,17,18]). For example, in humans, sick individuals often stay home, which alters disease dynamics [19,20]. Sick wild animals also commonly reduce their rate of search for food or water [21]. Such transmission may particularly apply to parasites that can survive in the environment (*e.g.*, gastrointestinal parasites) for which the spatial overlap of the home ranges of sympatric hosts favors transmission [22].

To investigate these transmission mechanisms, we developed an agent-based model exploring patterns of disease spread in a large closed population composed of territorial social groups, in which one or more hotspots influence group movement patterns, but where direct disease transmission at the hotspot itself is negligible. Our hypothesis is that terrestrial animals necessarily cross conspecifics' home ranges on their way to a hotspot, which modifies the contact network of the population and may subsequently alter disease transmission. We assumed that between-group disease transmission can occur both between groups having neighbouring territories and between groups travelling to a hotspot and groups whose territories are crossed en route. We also assumed that only groups whose territory lies within a certain distance from the hotspot (further referred as "radius of attraction") can visit it, and that their visitation rate decreases as this distance increases.

The relationship between the radius of attraction and the disease dynamics was then investigated under two scenarios: i) when groups including sick individuals do not travel to the hotspot, and ii) when these groups still travel to the hotspot. The first scenario corresponds to the case of virulent parasites that can strongly decrease the mobility of infected individuals, such as Ebola virus in western lowland gorillas [23], whereas the second scenario applies to pathogens that do not strongly modify the behavior of their host, such as some gastro-intestinal macro-parasites and bacteria [24]. Under both scenarios, we investigated the relationship between the disease attack rate and the hotspot radius of attraction, identified the groups in the population that have the highest risk of infection and explored the relationship between the number of hotspots and the magnitude of an epidemic.

Methods

General characteristics

The model has a 51×51 lattice structure, where each cell of the lattice corresponds to a group's territory. We assumed disease transmission can occur between each group and its eight neighbours (Fig. 1). We use N_i to denote the list of indices of the eight neighbours of group i . Initially, a single habitat hotspot is placed at the center of the lattice. All groups are assumed to include ten individuals. At each daily time step, each group either visits the hotspot or stays in its territory. The probability $P_{visit}(i)$ of a visit by group i is a decreasing function of the Euclidean distance, d_i , between the group's territory and the hotspot. We assume that all groups gain the same benefit from visiting the hotspot and that the travel cost is proportional to d_i , leading to:

$$P_{visit}(i) = \max \left[0, P_{max} \times \left(1 - \frac{d_i}{R} \right) \right]$$

where P_{max} is the probability of a visit for the eight groups directly

neighbouring the hotspot, and R is the hotspot radius of attraction. Groups occupying cells that are farther from the hotspot than R never visit it.

When a group visits the hotspot, it follows a Biased Random Walk from its home cell to the hotspot (BRW [25]) and returns to its home cell on the same day. The length of each step of the BRW (denoted S) is held constant and the direction of the step is consistently biased towards the hotspot during the approach to the hotspot and towards the group's home cell during the return from the hotspot. Each turning angle is randomly drawn from a normal distribution $N(0, \sigma^2)$, where σ is a standard deviation parameter. The list of groups residing in cells encountered along each BRW to the hotspot is recorded. Groups travel to the hotspot and come back within a single time step.

At each time step, each group interacts with (i) groups occupying neighbouring cells (neighbour-neighbour contact), and (ii) if the group travels to the hotspot, all of the groups it encounters along the BRW (traveler-resident contact).

Disease dynamics

We model infectious disease dynamics using a simple stochastic susceptible-infectious-removed (SIR) epidemic model, with one-day time steps. Each individual moves independently through the three states: Susceptible (at risk of contracting the disease), Infectious (capable of transmitting the disease), or Removed (recovered or dead). Susceptible individuals can be infected by infected individuals from either its own group or other groups. The latter can occur during neighbour-neighbour contacts or traveler-resident contacts.

The local transmission probability P_i is defined as the per-time-step probability that a susceptible individual in group i is infected by an infectious individual from its own group or a neighbouring group. Let I_i denote the number of infectious individuals in group i . The probability that the focal individual is infected by at least one of the infectious individuals in its own group is $1 - (1 - P_w)^{I_i}$, where P_w is the within-group transmission probability from an infected individual to a susceptible individual of the same group. Likewise, the probability of a susceptible individual being infected by an infectious individual from one of the eight neighbouring groups is $1 - \prod_{j \in N_i} (1 - P_B)^{I_j}$, where P_B is the between-group transmission probability from an infected individual to a susceptible individual of a neighbouring group. Combining these two sources of infection, the local transmission probability is given by

$$P_i = 1 - \left[(1 - P_w)^{I_i} \times \prod_{j \in N_i} (1 - P_B)^{I_j} \right]$$

The per-time-step probability of becoming infected during transit to a hotspot P_{H_i} depends on the number of infectious individuals I_c in each group c encountered en route and the P_T "travelling" probability of transmission during one of these transient contacts between a resident and traveling group. Specifically, during a one-day trip to a hotspot, the probability that a susceptible individual in the group is infected along the way is given by

$$P_{H_i} = 1 - \prod_{c \in BRW_i} (1 - P_T)^{I_c}$$

where BRW_i denotes the list of indices of the groups encountered by group i as it travels to and from the hotspot.

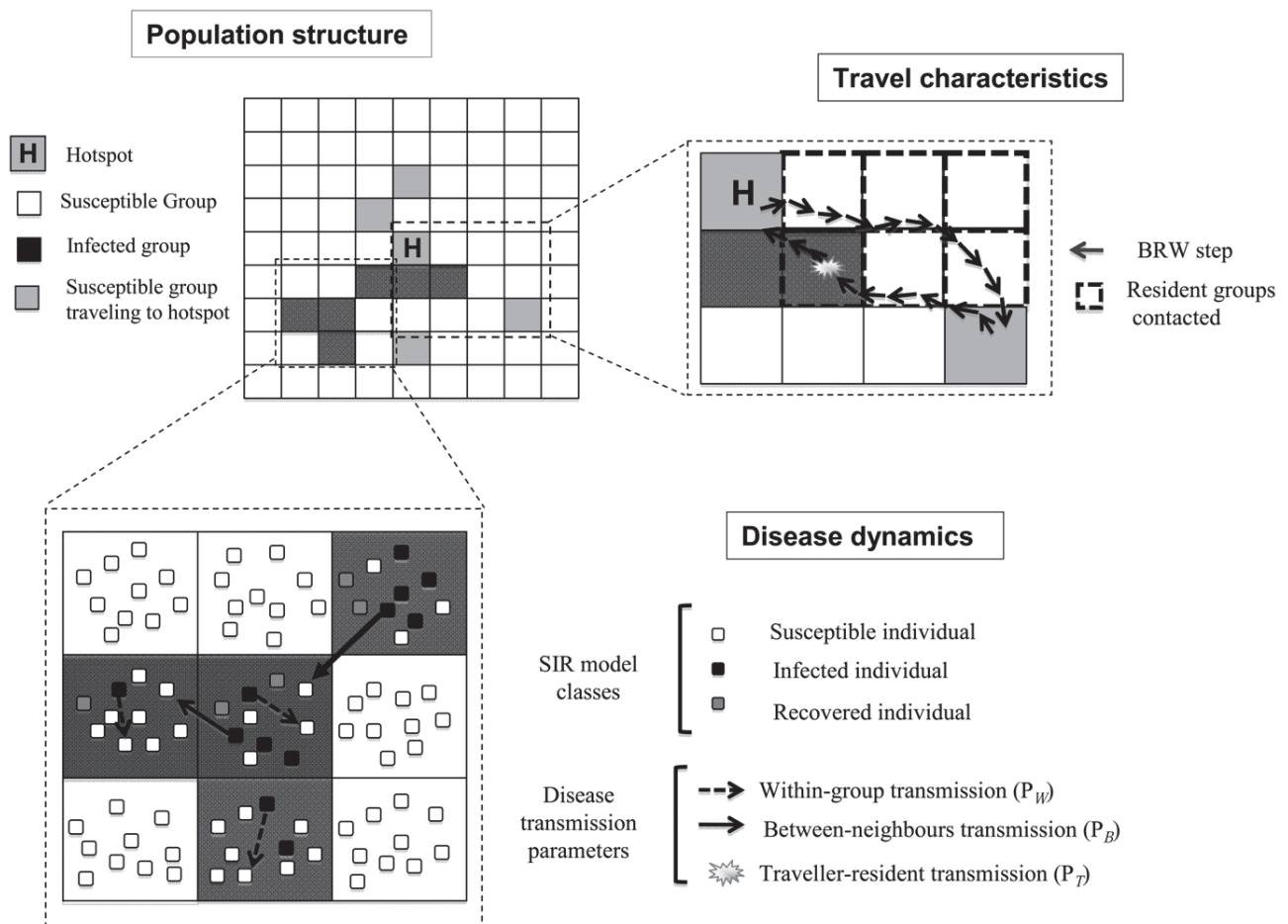


Figure 1. Model schematic. The hotspot is located at the center of a 51×51 lattice. All other cells correspond to a group's territory. Groups with at least one infected individual are considered infected, indicated in dark grey. A 9×9 section of the lattice depicts the SIR transmission dynamics among individuals that are either in the same group or neighbouring groups (bottom). Groups follow Biased Random Walks (BRW) during their daylong trips to the hotspot (top right). Transmission is possible between a travelling group and the groups residing in cells traversed en route to the hotspot. doi:10.1371/journal.pone.0031290.g001

In a second version of the model, infected groups are assumed to travel to the hotspot. Transmission from infected travelers to susceptible residents encountered en route can then occur. In this case, the per day probability that a susceptible individual in group i is infected by a traveler depends on the numbers of infected individuals in each of the groups that travels through the territory of i en route to the hotspot, and is given by

$$P_{H_2} = 1 - \prod_{m \in PT_i} (1 - P_T)^{I_m}$$

where PT_i denotes the set of groups passing through i 's territory.

At the end of each time step, each infected individual is removed with probability γ . We assume that no transmission occurs between groups travelling to the hotspot simultaneously.

Model versions

We explored two versions of the transmission model. In the "Sick-stay" model, groups that included at least one infected individual – infected groups – were assumed to stop travelling to the hotspot; in the "Sick-travel" model, infected groups continue to travel to the hotspot as if uninfected. In the Sick-stay model, disease transmission between travelers and residents can only

occur from an infected resident to a susceptible traveler, while in the Sick-travel model, transmission can be bi-directional.

We also considered models with multiple hotspots. A specified number of hotspots are randomly placed on the lattice, and groups visit only their nearest hotspot (according to P_{visib} , described above).

Initial conditions

At the beginning of each simulation, all individuals were susceptible. Epidemics were started with a single infected individual. Unless stated otherwise, the first case was introduced into one of the eight groups adjacent to the hotspot. At each time step, the number of infected and removed individuals (and groups) was recorded until no individual in the population was infected, which indicated the end of the epidemic. For each parameter combination, we ran 1000 simulations.

For all simulations, the recovery rate γ was set to 0.1, the maximum probability of a visit to the hotspot, P_{max} , was set to 0.1, and the BRW step length S was set to 0.25 (*i.e.*, 1/4 of the distance between the center of neighbouring group's territories). We also assumed that the within-group transmission probability, P_W , was at least ten times higher than the between-group transmission probabilities (P_B and P_T).

Table 1. Overview of processes and parameters of the model.

Parameter	Value
Population structure	
Number of groups	2601
Group size	10
Hotspot travel characteristics	
R : Hotspot radius of attraction	0–35
P_{max} : Visit probability for groups located next to the hotspot	0.1
S : Step length of the Biased Random Walk (BRW)	0.25
σ : Standard deviation for the BRW deviation angle	2
Disease dynamics	
γ : Recovery rate (days ⁻¹)	0.1
P_W : Within-group transmission probability	0.02–0.06
P_B : Between-neighbours transmission probability	4e-04 – 16e-04
P_T : Traveler-resident transmission probability	1e-06 – 1e-03

doi:10.1371/journal.pone.0031290.t001

The model was implemented in Delphi 7 (Borland Software Corporation, 2002). Table 1 summarizes parameter definition and values, and a sample run of the model is shown in Video S1.

Results

Epidemiological impacts of transmission rates and location of first case

The attack rate (proportion of groups becoming infected following a single disease introduction) generally increases with the hotspot radius of attraction R , and the traveler-resident transmission probability P_T (Fig. 2). This occurs whether or not infected groups are assumed to travel during infection (Fig. 2 and Fig. S1 in supplemental materials). As predicted by percolation theory [26], the attack rate also increases with both the within-group transmission parameter P_W and the between-group transmission parameter P_B . Interestingly, the highest impact of the hotspot radius of attraction on the attack rate was observed for intermediate values of P_W and P_B . For low values of P_W and P_B , inter-group disease transmission primarily occurred between travelling and resident groups and was often not sufficient to sustain an epidemic. For high values of P_W and P_B , the disease always percolated, even in the absence of the hotspot. For intermediate values of P_W and P_B , groups infected en route to the hotspot then stochastically triggered small outbreaks around their territories. The epidemiological impact of the hotspot was amplified by this interaction between traveler-resident transmission and neighbour-neighbour transmission.

In both models, the attack rate decreased as the distance between the hotspot and the point of disease introduction increased (Fig. 3). The greater this distance, the lower the probability that a group visiting the hotspot encountered the group initially infected. The Sick-travel model yields higher attack rates than the Sick-stay model, particularly for groups ranging at intermediate distances between the location of the first disease case and the hotspot.

Group-specific epidemiological risk

In the Sick-travel model, groups ranging closer to the hotspot exhibited higher probabilities of infection (Fig. 4 and Fig. S2 in supplemental materials), since their territories are crossed by large

numbers of infected groups travelling to the hotspot (Fig. S4a). The relationship is more complex in the Sick-stay model: groups ranging at intermediate distances from the hotspot experience the highest risks of infection (Fig. 4 and Fig. S3 in supplemental materials). In this case, hotspot-mediated infection occurs only from infected residents to susceptible travelers. Groups ranging close to the hotspot travelled more often, but encountered only a small number of potentially infected groups. Groups ranging far from the hotspot encountered larger numbers of groups when visiting the hotspot, but did so only rarely. Thus groups ranging at intermediate distances experienced the greatest number of potentially infective contacts with resident groups encountered en route to the hotspot (Fig. S4b). When epidemics occur, the difference in spatial pattern of disease spread observed between the models is insensitive to the parameter values (Fig. S2 and Fig. S3).

The fact that groups ranging at an intermediate distance from the hotspot display a higher number of potentially infective contacts can easily be understood using a simple mathematical model. Indeed, the expected number of groups encountered by a group i visiting the hotspot, per time unit, can be assumed to be approximately proportional to P_{visit} and to the territory-hotspot distance d_i :

$$E_H(i) \propto P_{visit} d_i \\ \propto P_{max} \left(d_i - \frac{d_i^2}{R} \right).$$

This approximation holds as long as d_i is large or the turning angle is low. The derivative of this second-order polynomial has a maximum in $R/2$.

Second, we assessed the epidemiological impact of the hotspot on groups that never visit it because they range at a distance larger than R from the hotspot. For both models, when P_B is high enough to allow some between-neighbour transmission, the attack rate for these groups was found to be larger than expected under a model with no hotspot ($R=0$) (Fig. 4). Stochastic, local between-group transmission events allow the spread of the disease beyond the radius of attraction. Groups that never visit the hotspot are thus indirectly impacted by the hotspot.

Finally, outside of the radius of attraction, disease spreads as expected for a lattice model [27] whereas inside the radius of attraction, disease spread rapidly among the groups, with no apparent spatial structure.

Number of hotspots

For both models, the relationship between the number of hotspots and the attack rate is bell-shaped (Fig. 5). For a low number of hotspots, adding new hotspots increases the fraction of the population ranging within the radius of attraction of these hotspots and, thereby, increases the overall attack rate. Beyond a certain number of hotspots, however, all groups are already attracted by at least one hotspot on the landscape. Under the assumption that these groups travel exclusively to the nearest hotspot, adding more hotspots decreases the distance travelled by the groups and the number of infective contacts they can have en route, and thereby lowers the attack rate.

Discussion

Spatial features of the landscape such as habitat hotspots can profoundly influence the spread of infectious diseases [28,29]. Our model extends previous studies focusing on transmission at the

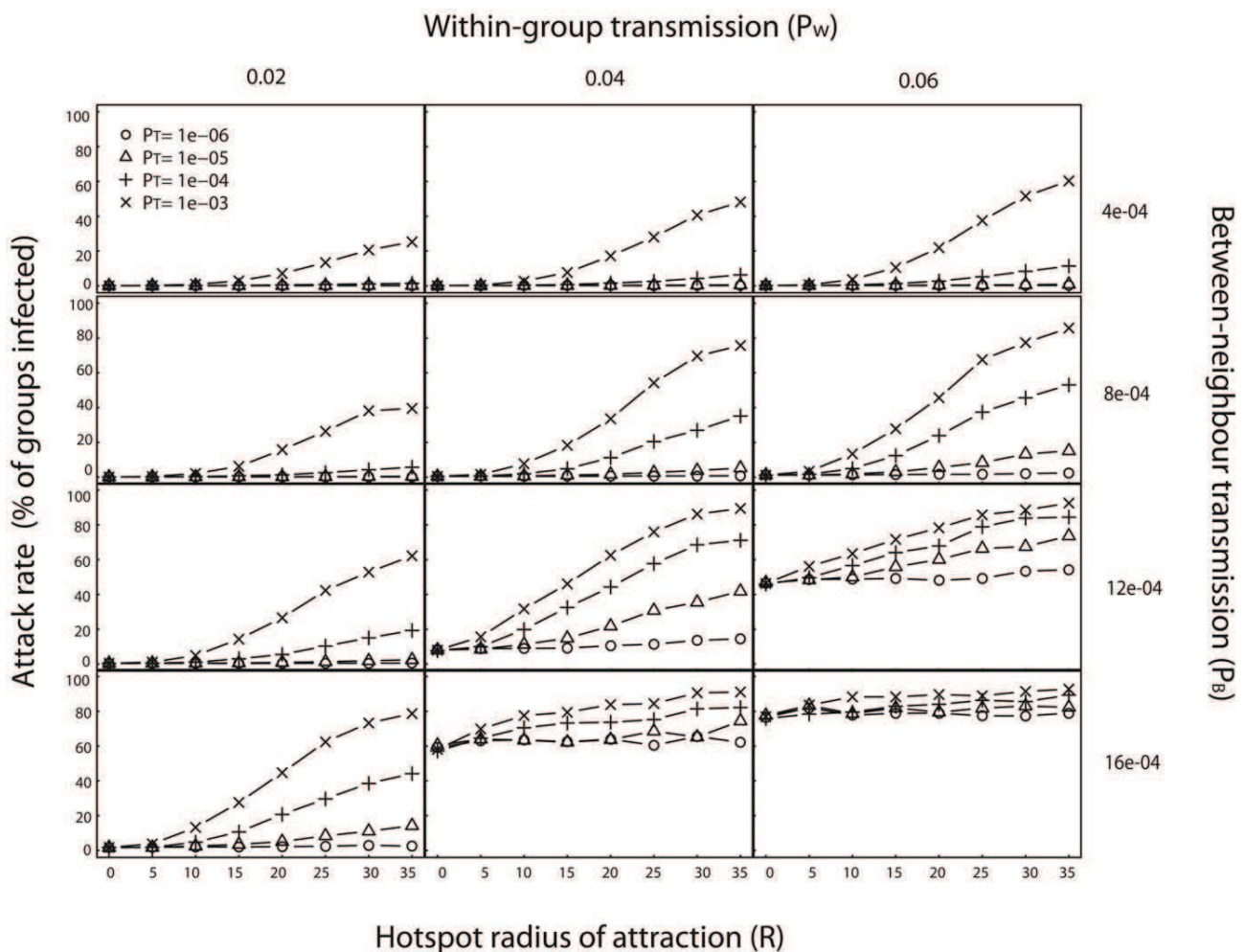


Figure 2. Influence of multiple model parameters on attack rate, when infected groups do not travel (Sick-stay model). The fraction of groups infected increases with the hotspot radius of attraction, but varies with the traveler-resident transmission probability P_T (four lines in each graph), within-group transmission probability P_w (three different columns of graphs), and between-neighbour transmission probability P_b (four different rows of graphs). Each value is based on 1000 simulations in which disease was introduced randomly in one of the eight groups adjacent to the hotspot.

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hotspot, and reveals that hotspots can also strongly alter disease transmission by generating infective contacts between animals travelling towards or from the hotspot and animals whose territories are traversed. Our results show that even when sick groups stay in their territory, hotspots may increase the size of an epidemic. When infected animals cease to visit the hotspot, groups ranging at intermediate distances to the hotspot are the most vulnerable. We also found that the epidemiological impact of hotspots extends far beyond the subset of the population that visits it; even groups having no contact with those visiting the hotspot display elevated risks of infection. Finally, our model predicts that when groups visit their nearest hotspots, the epidemiological impact of hotspots is most severe when the number of hotspots is intermediate.

Hotspots impact disease transmission via a combination of both local between-neighbour and long-range traveler-resident transmissions, which is characteristic of a small-world network [30]. Disease dynamics in our model resemble those in a classic small-world network in several aspects. First, the attack rate increases with long-distance interactions, determined by the hotspot radius

of attraction (Fig. 2). Second, new foci of infection established by long-distance traveler-resident contacts only spread when the local transmission rate, between neighbours, is sufficiently high. This phenomenon extends the influence of the hotspot beyond the radius of attraction (Fig. 4). Finally, as in small-world networks [31], all groups within the hotspot radius of attraction were infected almost at the same time. Thus, habitat hotspots potentially play a significant role in fuelling disease outbreaks, much like other natural mechanisms that generate small-world networks, such as the movement of vectors between plants [32,33].

We find that hotspots are expected to influence disease dynamics significantly, even when infected groups do not travel to the hotspot at all. However, in this case, the hotspot effect strongly decreases as the distance between disease introduction and the hotspot increases. The reduction of mobility in infected groups also generates an unexpected spatio-temporal pattern: groups ranging at intermediate distance from the hotspot have the highest risk of infection, even if the disease is introduced immediately next to the hotspot. This counterintuitive result highlights the importance of understanding the behavioral effects

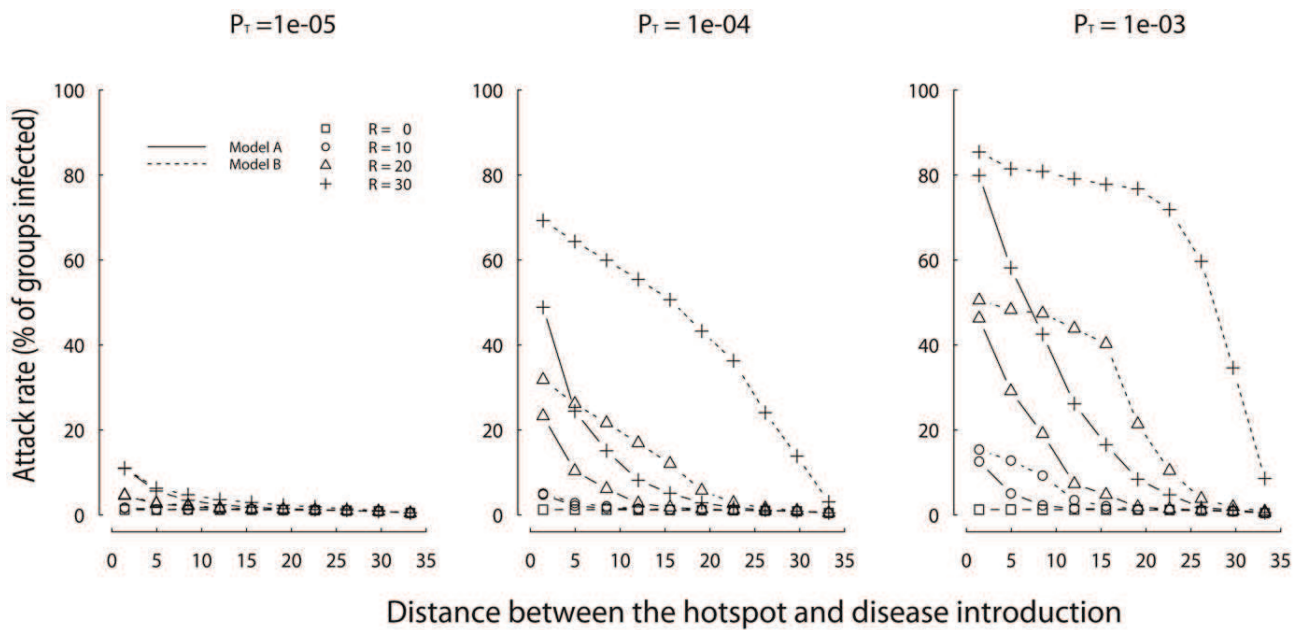


Figure 3. Attack rate decreases with the distance between the hotspot and point of disease introduction. Sick-stay model (solid lines) and Sick-travel model (dashed lines) are compared for different values of the hotspot radius of attraction (R). Each graph presents a different value of the traveler-resident transmission rate (P_T). Each value is averaged over 1000 stochastic simulations, assuming $P_B = 8\text{e-}04$ and $P_w = 0.06$. doi:10.1371/journal.pone.0031290.g003

of disease in wild animal populations. For example, as in humans, predicting the impact of hotspots on disease dynamics will strongly depend on understanding whether infectious individuals still travel to hotspots because disease symptoms appear after an infectious state (e.g., influenza H1N1 [6,19]), or whether infectious individuals do not visit hotspots because disease symptoms appear

before the infectious state (e.g., SARS [34,35]). Furthermore, our results suggest that when transmission does not directly occur at hotspots, disease control measures targeting groups residing around the hotspot might not necessarily be the most efficient ones. Further simulation work is needed to identify optimal disease control measures.

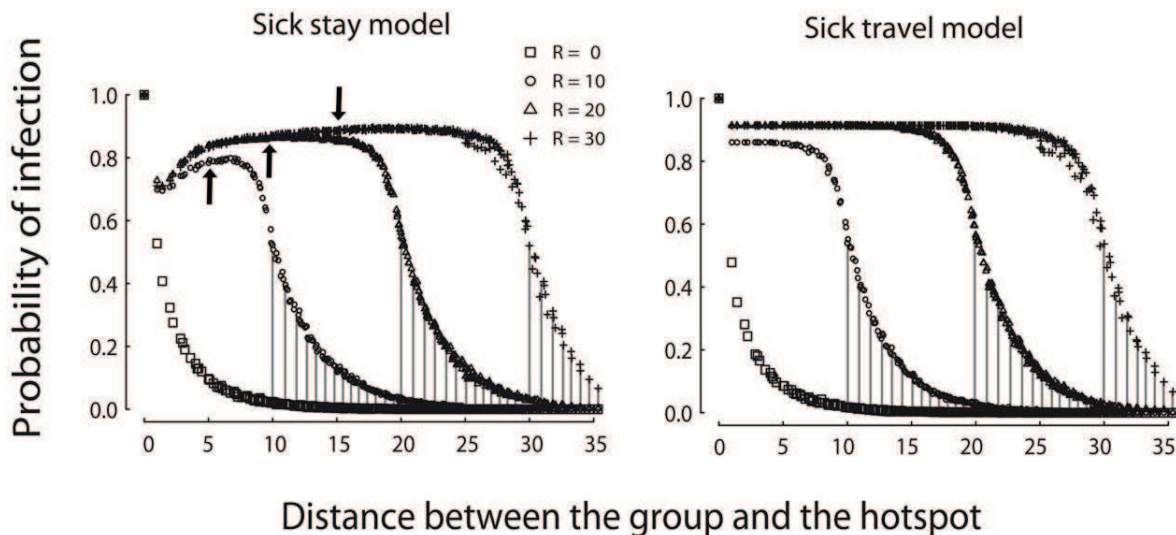


Figure 4. Probability of infection depends on distance to hotspot. The relationship is presented for different values of the hotspot radius of attraction (R) in Sick-stay model (left) and Sick-travel model (right). Vertical lines compare the probability of infection when there is no hotspot ($R = 0$) to the probability of infection when there is a hotspot ($R > 0$), for groups residing beyond the radius of attraction (distance to hotspot greater than R). This quantifies the indirect epidemiological impact of the hotspot on groups that never travel themselves or encounter travelers en route to the hotspot. Black arrows show the analytical prediction of the most vulnerable group to disease for $R = 10, 20$ and 30 respectively. Parameter values are $P_B = 8\text{e-}04$, $P_T = 0.001$ and $P_w = 0.06$. Each value is based on 1000 stochastic simulations in which disease was introduced randomly in one of the eight groups adjacent to the hotspot. Results for other parameter values are shown in Fig. S2 and S3. doi:10.1371/journal.pone.0031290.g004

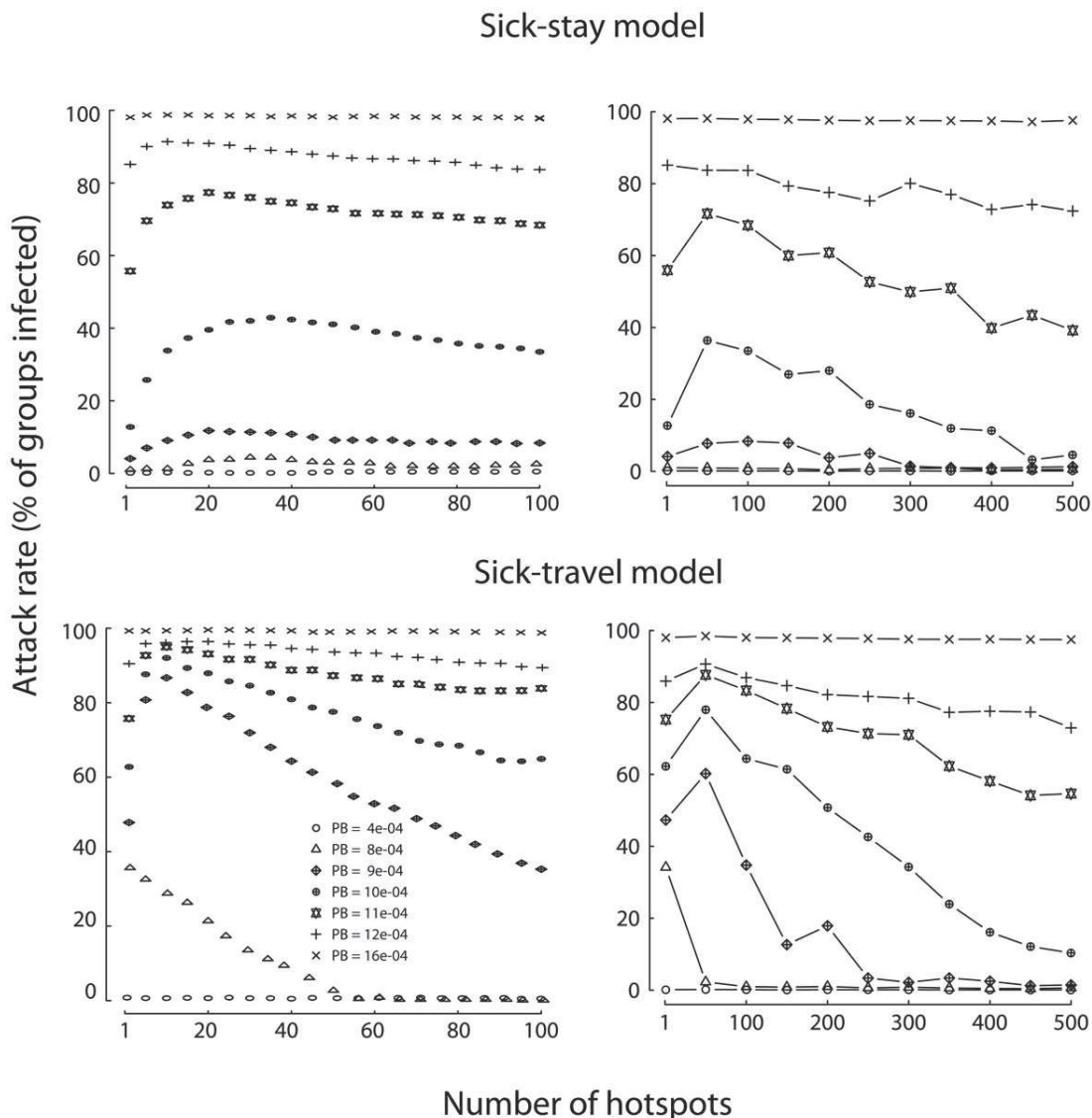


Figure 5. Number of hotspots. Each line graphs the change in attack rate as a function of the number of hotspots, for a different value of P_B (from $4e-04$ to $16e-04$). Results are presented for hotspots ranging from 1–100 (left) and 1–500 (right) in the Sick-stay model (top) and the Sick-travel model (bottom). Each value is averaged over 1000 stochastic simulations assuming $R = 30$, $P_w = 0.06$, $P_T = 4e-04$. Each hotspot was located randomly in the population, and disease was introduced into the group ranging in the middle of the habitat.
doi:10.1371/journal.pone.0031290.g005

The habitat of wild animal populations often includes more than one hotspot. For example, the habitat of terrestrial mammals can include a small number of high-value hotspots attracting dozens of groups (*e.g.*, salt licks or forest clearings) and more numerous low-value hotspots attracting only a few groups (*e.g.*, fruiting trees). Our model reveals that, when groups are assumed to travel to their nearest hotspot, the impact of disease outbreaks is a bell-shaped function of the number of hotspots (Fig. 5). This result challenges the hypothesis that the number of hotspots and disease prevalence will correlate positively [8], and could be used to optimize strategies for controlling disease in wild animal populations. Thus, wildlife managers may consider increasing, rather than decreasing [36], the number of water holes in order to reduce the number of highly-connected individuals or social groups, and hence the impact of an outbreak. However, additional studies are needed to

determine if our result still holds when each animal visits more than one hotspot.

The values of the parameters of our model can be estimated from empirical data. The relationship between the distance from a group's territory and the hotspot visitation rate can be estimated using capture-mark recapture and telemetric data, between-group contact rates can be estimated from direct observation or telemetric data, and plausible distributions of disease transmission rates can be found in the literature. The step length of the biased random walk is assumed to have a fixed value (here, 0.25 times the size of a territory). This parameter does not need to be estimated accurately since it is redundant with another parameter, the traveler-resident contact rate, which is allowed to vary. Thus, the model can be applied to a broad range of host-parasite systems, from primate groups travelling to waterholes on a daily basis [37,38] to large mammals visiting every few weeks mineral-rich

areas [12,13,39]. In our model, the impact of the hotspot is particularly sensitive to the ratio between the local and the traveler-resident between-group transmissions. When the local between-neighbour transmission is high compared to the traveler-resident transmission, the impact of the hotspot is minimal.

We considered two discrete transmission scenarios, the Sick-travel and the Sick-stay scenarios. However, intermediate scenarios are also possible. For example, infected groups may fission such that only healthy individuals travel to the hotspot. In this case, we expect that although the overall disease transmission will increase compared to the pure Sick-stay scenario, the spatial pattern of the disease impact will be qualitatively similar to that observed for the Sick-stay model.

In this study, we have shown how transmission occurring around habitat hotspots influences disease transmission patterns, while previous studies have focused on disease transmission occurring at the hotspot itself. In some ecological systems, both transmission modes may coexist. For example, some fecal-orally transmitted parasites can infect both the soil and waterholes, and spore-forming bacteria such as *Bacillus anthracis* can persist for extended periods of time in animal carcasses, water and soil [40]. Additional works are needed to understand such epidemiological systems.

Supporting Information

Figure S1 Influence of multiple model parameters on attack rate, when infected groups travel (Sick-travel model). The fraction of groups infected increases with the hotspot radius of attraction, but varies with the traveler-resident transmission probability P_T (four lines in each graph), within-group transmission probability P_w (three different columns of graphs), and between-neighbour transmission probability P_B (four different rows of graphs). Each value is based on 1000 simulations in which disease was introduced randomly in one of the eight groups adjacent to the hotspot.
(TIF)

Figure S2 Group's probability of infection in relation to the distance to the hotspot, predicted by the Sick-travel model. The relationship is presented for different values of the hotspot radius of attraction (R). Each graph represents a combination of the between-neighbour (P_B) and the traveler-resident (P_T) transmission. The disease was introduced randomly

in one of the eight groups adjacent to the hotspot. For all simulations, $P_w = 0.06$.
(TIF)

Figure S3 Group's probability of infection in relation to the distance to the hotspot, predicted by the Sick-stay model. The relationship is presented for different values of the hotspot radius of attraction (R). Each graph represents a combination of the between-neighbour (P_B) and the traveler-resident (P_T) transmission. The disease was introduced randomly in one of the eight groups adjacent to the hotspot. For all simulations, $P_w = 0.06$.
(TIF)

Figure S4 Traveler-resident contact patterns. Each graph shows the relationship between the distance of a group from the hotspot and (a) the number of other groups that travel through its territory when travelling to and from the hotspot, (b) the number of resident groups it encounters when travelling to and from the hotspot. Values are based on encounters occurring during 100 time steps, in the absence of disease transmission.
(TIF)

Video S1 Model dynamics. The model shows one simulation run corresponding to the Sick-travel model. White, red and black squares represent susceptible, infected and removed groups, respectively. Blue squares represent groups travelling to the hotspot at each time step. The hotspot, in green, is in the middle of the lattice. The disease is introduced at the periphery.
(WMV)

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Author Contributions

Conceived and designed the experiments: JB DC PW LM MR. Performed the experiments: JB. Analyzed the data: JB DC LM PW. Wrote the paper: JB DC PW LM.

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SUPPLEMENTARY MATERIAL

Figure S1. Influence of model parameter values on disease attack rate in the Sick-travel model.

The relationship between the percentage of infected groups and the hotspot radius of attraction is presented for four values of traveler-resident transmission probability (P_T). Each graph represents a combination of within (P_w) and between-neighbour (P_B) transmission. Disease was introduced randomly in one of the eighth groups adjacent to the hotspot.

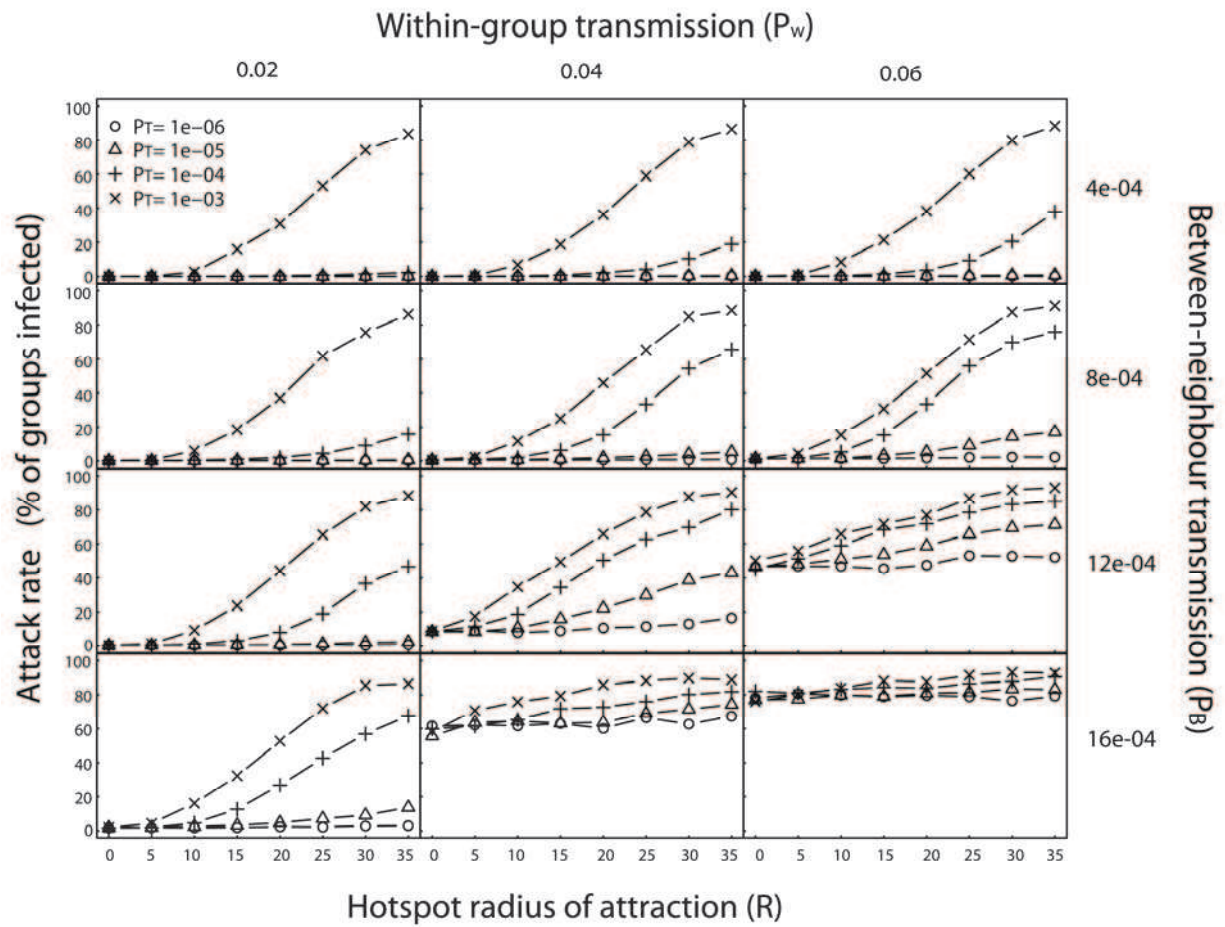


Figure S2. Group's probability of infection in relation to the distance to the hotspot, predicted by the Sick-travel model. The relationship is presented for different values of the hotspot radius of attraction (R). Each graph represents a combination of the between-neighbour (P_B) and the traveler-resident (P_T) transmission. The disease was introduced randomly in one of the eight groups adjacent to the hotspot. For all simulations, $P_w = 0.06$.

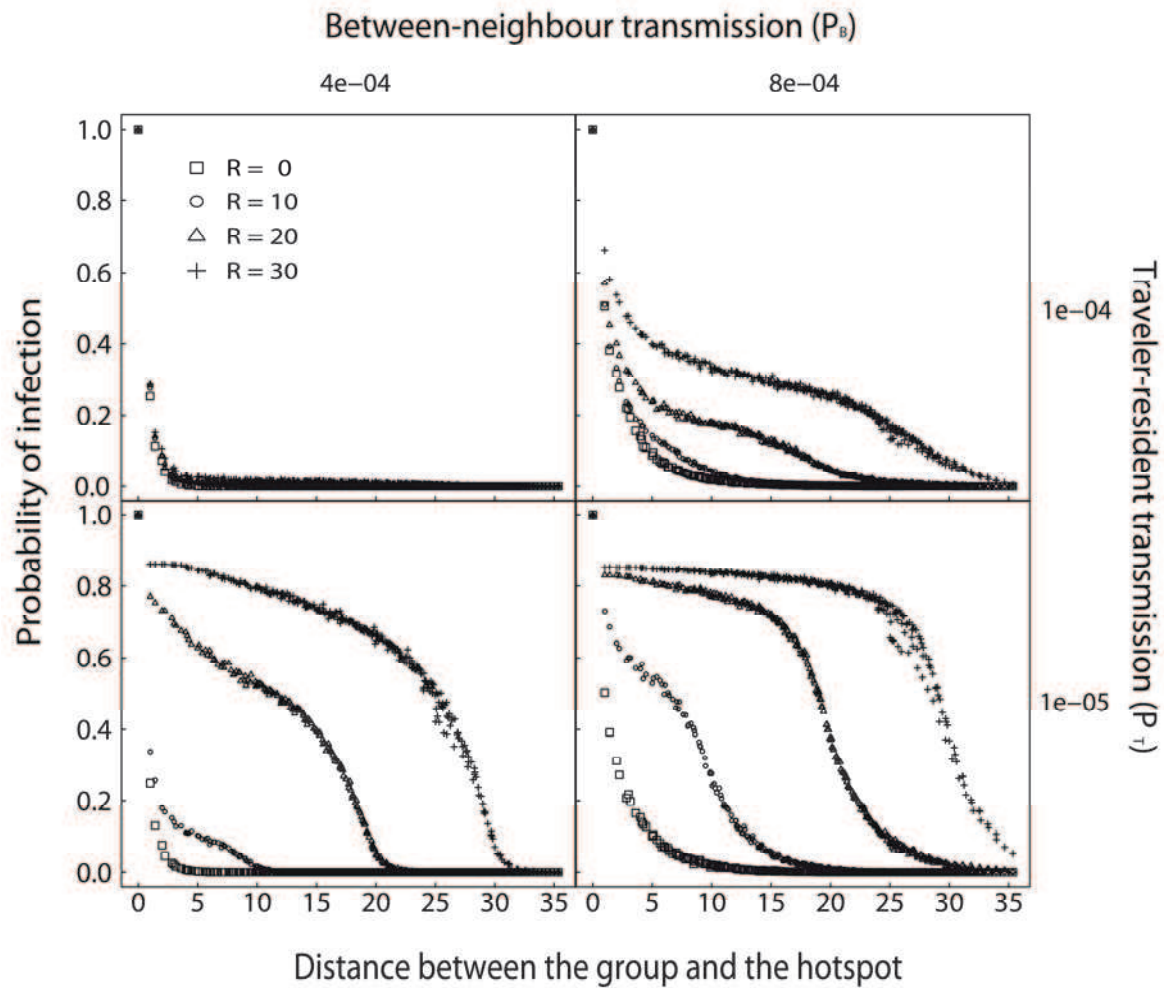


Figure S3. Group's probability of infection in relation to the distance to the hotspot, predicted by the Sick-stay model. The relationship is presented for different values of the hotspot radius of attraction (R). Each graph represents a combination of the between-neighbour (P_B) and the traveler-resident (P_T) transmission. The disease was introduced randomly in one of the eight groups adjacent to the hotspot. For all simulations, $P_w = 0.06$.

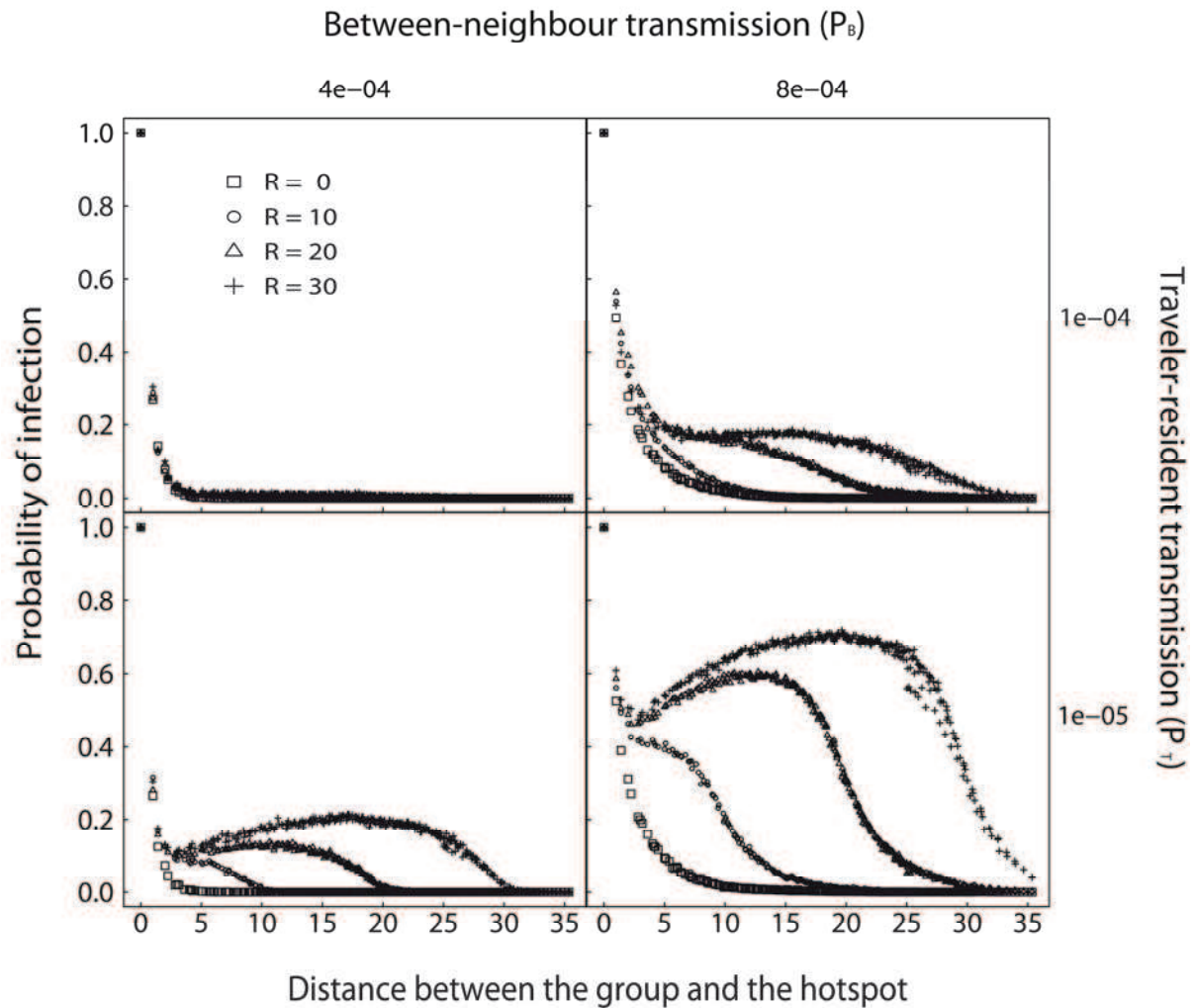
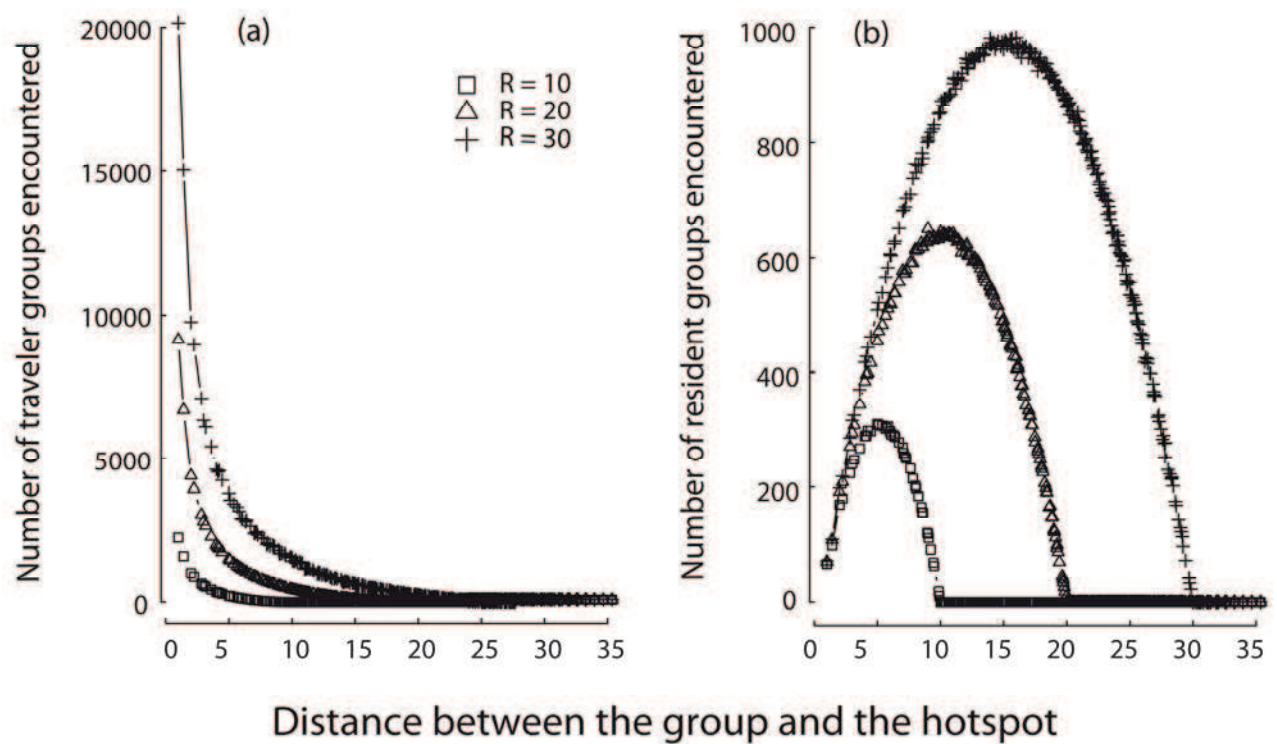


Figure S4. Traveler-resident contact patterns. Each graph shows the relationship between the distance of a group from the hotspot and (a) the number of other groups that travel through its territory when travelling to and from the hotspot, (b) the number of resident groups it encounters when travelling to and from the hotspot. Values are based on encounters occurring during 100 time steps, in the absence of disease transmission.



Video S1. Model dynamics. The model shows one simulation run corresponding to the Sick-travel model. White, red and black squares represent susceptible, infected and removed groups, respectively. Blue squares represent groups travelling to the hotspot at each time step. The hotspot, in green, is in the middle of the lattice. The disease is introduced at the periphery.

Article 3

No evidence for transmission of *Escherichia coli* antibiotic resistant strains from humans to wild western lowland gorillas in Lopé National Park, Gabon.

Authors: Benavides JA, Godreuil S, Bodenham R, Ratiarison S, Devos C, Petretto MO, Raymond, Escobar-Paramo P.

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22 **Keywords:** *Escherichia coli*, western lowland gorillas, antibiotic resistance, genetic structure

23

24 **Abstract**

25 The intensification of human activities within the habitats of wild animals is increasing the
26 risk of interspecies disease transmission. This risk is particularly important for great apes,
27 given their close phylogenetic relationship with humans. Areas of high human density or
28 intense research and ecotourism activities expose apes to a high risk of disease spillover from
29 humans. But is this risk lower in areas of low human density? We determined the prevalence
30 of *Escherichia coli* antibiotic resistant isolates in a population of the critically endangered
31 western lowland gorilla (*Gorilla gorilla gorilla*) and other wild mammals in Lopé National
32 Park (LNP)-Gabon, and tested whether the observed pattern could be explained by bacterial
33 transmission from humans and domestic animals into wildlife populations. Our results show
34 high prevalence of antibiotic resistant bacterial isolates in humans, and low levels in gorillas
35 and other wildlife. The significant differences in the genetic background of the resistant
36 bacteria isolated from humans and gorillas suggest that transmission is low, or does not occur
37 between these two species. These findings indicate that the presence of antibiotic resistant
38 strains in wildlife may not necessarily imply direct bacteria transmission from humans. Thus,
39 in areas of low human density, human-wildlife *E. coli* transmission seems to be low. The
40 presence of antibiotic resistant isolates in gorillas may be better explained by other
41 mechanisms for resistance acquisition such as horizontal gene exchange amongst bacteria, or
42 naturally-acquired resistance.

43

44 INTRODUCTION

45 The intensification of human activities within habitats of previously isolated wild animals is a
46 key factor in the emergence of infectious diseases (7, 18). Although major focus has been
47 given to the spread of zoonotic diseases into human populations (23, 43, 45), anthropogenic
48 activities also cause the emergence of disease in wildlife populations (7, 8). In particular, the
49 close phylogenetic relationship between great apes and humans exposes apes to a high risk of
50 disease spillover from humans (44). In the last two decades, bushmeat hunting, forest
51 encroachment, ecotourism and research activities are increasing the levels of contact between
52 humans and great apes. This in turn has resulted in several confirmed cases of human
53 pathogen transmission to apes (*e.g.* human respiratory virus in chimpanzees (19) and
54 mountain gorillas (28) amongst other examples (12)). *Escherichia coli* exchanges between
55 humans, domestic animals and great apes have been reported in densely human-populated
56 areas of western Uganda. Within Uganda, habituated groups of wild apes are visited daily by
57 researchers and tourists (*e.g.* chimpanzees at Kibale (13) and mountain gorillas at Bwindi
58 Impenetrable National Park (33)). However, there has been little attention directed towards
59 the quantification of bacterial transmission in areas with low human impact, which could
60 serve to elucidate whether the pattern of pathogen prevalence observed in wildlife is
61 explained by transmission from humans.

62 Ubiquitous and commensal bacteria such as *E. coli* represent a convenient model to
63 study patterns of bacterial transmission between humans, domestic and wild animals (13, 33).
64 In particular, the presence of antibiotic resistant strains in untreated wild animals has been
65 suggested to reflect bacterial exchange with humans or domestic animals, in which treatment
66 with antibiotics actively selects antibiotic resistant strains (1, 6, 9, 22, 32, 34). Furthermore,
67 several molecular techniques are now available to compare the genetic structure of *E.*

68 *coli* strains from different populations (4, 37), thus contributing to the understanding of the
69 subjacent transmission process.

70 In this study, we determined the pattern of *E. coli* antibiotic resistant isolates derived
71 from feces of a population of the critically endangered western lowland gorilla (*Gorilla*
72 *gorilla gorilla*) and other wild mammals, and assessed if the observed pattern may have
73 occurred as a result of bacterial transmission from humans. This study was conducted in Lopé
74 National Park (LNP) in central Gabon (Fig. 1), which hosts important populations of western
75 lowland gorillas and other wild mammals (38, 39). Lopé is surrounded by a human and
76 domestic population density that is one of the lowest around the vicinity of any African
77 protected area (17). In addition, the use of antibiotics in Lopé is almost entirely restricted to
78 the treatment of human infectious diseases. Thus, the frequency of human-wildlife
79 transmission of resistant strains is expected to be low.

80 Our results show high levels of antibiotic resistance in *E. coli* isolates from human
81 feces, and low levels in those from domestic animals, gorillas and other wildlife. However,
82 the significant difference in the genetic structure of *E. coli* resistant isolates derived from
83 human-domestic animals and those from gorillas does not support direct bacterial
84 transmission between these two populations. These findings suggest that the presence of
85 antibiotic resistant strains in wildlife may not necessarily imply direct bacterial transmission
86 from humans, and that in areas of low human density, human-wildlife *E. coli* transmission
87 seems to be low.

88

89 **MATERIALS AND METHODS**

90 **Sample collection**

91 *Wild animals*

92 Collection of wildlife samples was conducted in an area of 42 km² around the Mikongo
93 Conservation Centre (MCC), located in the North-East region of LNP (0,30651°S
94 11,70189°E) (Fig. 1). Since 2001, the MCC has been carrying out research and tourism
95 activities on western lowland gorillas, but without full gorilla habituation. Fresh fecal
96 samples (less than 24 hours after defecation) were collected during a two week census
97 designed to study gorilla population density surrounding MCC in February 2010. Each day
98 four teams composed of three experienced trackers and one researcher walked predetermined
99 forest transects designed to optimize the sampling area and to avoid duplicating the sampling
100 of the same gorilla group (16). When a gorilla trace was found, the team tracked the traces in
101 order to locate the nest built by the gorilla group the previous night. A total of 119 gorilla
102 fecal samples were collected from 14 different gorilla nest sites. Only one fecal sample was
103 collected per nest in each site. Samples from other mammals were also collected
104 opportunistically, including 6 chimpanzees (*Pan troglodytes troglodytes*), 5 mandrills
105 (*Mandrillus sphinx*), 5 monkeys (including black colobus, *Colobus satanas* and the grey-
106 cheeked mangabey, *Lophocebus albigena*), 12 duikers of several species, 5 river hogs
107 (*Potamochoerus porcus*), 8 forest buffalos (*Syncerus caffer nanus*), and 7 African elephants
108 (*Loxodonta africana cyclotis*) (Table 1). Approximately 2 g of fecal material sample was
109 collected from the interior of the fresh fecal stool and placed in a 1.5 ml Eppendorf tube
110 containing 1 ml of sterile and isotonic buffered solution composed of NaCl at 0.9%.

111 *Humans and domestic animals*

112 Samples from 25 adult humans that agreed to participate in this study and 34 domestic
113 animals were collected in the five villages bordering the park located close to the area of
114 wildlife sampling: Lopé (number of inhabitants $n \approx 300$), Makoghé ($n \approx 50$), Badondé ($n \approx$

50), Mikongo ($n \approx 100$) and Massenghelani ($n \approx 70$) (see Fig. 1 for location). Each village had a small number of livestock (between 10 and 50 animals) composed essentially of sheep and goat, and several households possessed companion animals (*i.e.*, cats and dogs). Incursion of wild animals such as elephants, buffalos or monkeys into crop fields and streams used by the villagers for water consumption or bathing was common in the villages. Humans were given a 150 ml sterile Falcon (BD) tube and were asked to put a small fecal swab in the tube using provided sterile gloves and/or a sterile cocktail stick. Tubes containing samples were collected the next day and filled with buffer solution. None of the samples presented signs of diarrhoea. Domestic animal samples included 21 sheep (*Ovis aries*), 4 goats (*Capra aegagrus hircus*), 8 dogs (*Canis lupus familiaris*) and 1 cat (*Felis catus*). Livestock animals were followed until defecation occurred, whereas samples from companion animals were collected rectally. The collection of human (and domestic animal) samples was approved by a written statement obtained from volunteers (or livestock owners), the village chief, and the Centre National de Recherche Scientifique et Technologique du Gabon (CENAREST, permit number: AR0026/09).

Water and soil samples

Twenty water samples were collected from five different streams encountered in the forest during the surveys, and from the fifteen water sources available within the five villages. These sources included 2 main rivers (Offoué and Ogooué), 12 streams and 1 open well. Water samples were collected in a 50-ml syringe, and were systematically filtered with a high flow syringe containing a 0.45 μm poly-ether sulfone membrane filter (Sartorius Stedim Biotech), which retained bacteria (29). The membrane filter was then placed on top of a MacConkey (MC) Agar plate (VWR) no more than 12 hours after collection. Ten soil

samples were collected in proximity to ten gorilla nests sampled. Soil samples were stored in buffer solution for further bacterial isolation.

Bacterial isolation

Bacteria from fecal samples were isolated in MacConkey (MC) selective media following the methods described in Quinn (1994) as soon as possible within the 48 hours following their collection (29). Previous studies have shown that short-term storage of samples (up to 2 weeks) does not affect the phylogenetic structure, abundance and diversity of microbial communities (20). After collection each fecal, soil and water membrane sample was streaked on a MacConkey (MC) agar plate (VWR) and incubated at 37°C for 24-48 hours. Five isolated pink lactose positive colonies, visually resembling *E. coli*, were transferred into a 200 µl 96 wells microtiter plate containing MC agar. Microtiter plates were then kept at 4°C and shipped to France for further analyses. Once in France, colonies were re-isolated and identified by biochemical testing (API 20E gallery or bioMerieux Vitek). Only one identified *E. coli* isolated per fecal sample was saved in 20% glycerol and stored at -80°C for further analysis. Unbalanced sampling per individual and pseudo-replication was therefore avoided. For descriptive purposes, all different morphotypes present within the five isolates belonging to water samples were identified (Table 3).

Analyses

Antibiotic resistance

Antibiotic susceptibility was tested using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (5). Ten antibiotics were selected according to their availability in Lope and Gabon. Ampicillin, chloramphenicol and tetracycline represent the most common antibiotics used in Gabon (27), and were available in the only local

pharmacy of Lopé. Ciprofloxacin and neomycin were rarely available in the Lope area. Doxycycline, sulfamethoxazole, rifampicin and streptomycin were not available at Lope, but are used in Gabon for the treatment of malaria (26), *Shigella* (27) or tuberculosis (25). Ceftiofur, a third-generation cephalosporin antibiotic, was never available at Lope and it is also very rarely used in Gabon. Two-fold dilutions of the antibiotics were added in order to obtain the final concentrations superior to critical concentration according to breakpoints provided by the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Resistance to antibiotics was tested at the following concentrations: ampicillin ($> 8 \mu\text{g/ml}$), ceftiofur ($> 8 \mu\text{g/ml}$), ciprofloxacin ($> 2 \mu\text{g/ml}$), tetracycline ($> 8 \mu\text{g/ml}$), chloramphenicol ($> 8 \mu\text{g/ml}$), doxycycline ($> 8 \mu\text{g/ml}$), neomycin ($> 4 \mu\text{g/ml}$), rifampicin ($> 16 \mu\text{g/ml}$), streptomycin ($> 16 \mu\text{g/ml}$) and sulfamethoxazole ($> 256 \mu\text{g/ml}$). Inocula were prepared in saline solution to achieve 10^8 cfu/ml (0.5 McFarland standard), then diluted 1 in 10 using sterile saline solution. A multipoint inoculator was used to dispense $1 \mu\text{l}$ of diluted inoculum to achieve 10^4 cfu/ml per plate, with and without antibiotics. Plates were incubated in air at 37°C for 18 h. A fully susceptible *E. coli* (ATCC 25922) was used as a control. An isolate was considered resistant if it grew in the presence of the antibiotic and formed a homogenized colony similar to the control colony grown in medium without antibiotic. A replicate was systematically run to confirm each resistant case. To evaluate the reproducibility of the method, independent tests were performed for *E. coli* ATCC 25922, and 10 clinical strains (two tests each).

E. coli phylogenetic groups

Each *E. coli* isolate was assigned to one of the four major phylogenetic groups (A, B1, B2 and D) (21), using a multiplex PCR assay which creates the simultaneous amplification of the *chuA* and *yjaA* genes and a DNA fragment TSPE4.C2 (4). The presence of the three markers

in the same strain denotes the subgroup B2₃. This method has been widely used for the genetic description of different *E. coli* populations given its rapidity and simplicity (4, 10, 37), and the equivalence to other more sophisticated methods such as multilocus sequence typing (14). Primers used in this study were those from Clermont et al. (2000).

Statistical analyses

Fisher exact tests were used to compare the prevalence of resistant isolates and the proportion of the different phylogenetic groups of the *E. coli* isolates derived from different host populations. This test is recommended for the analysis of contingency tables with small samples sizes, including the distribution of genetic characteristics (11, 30). The number of resistant isolates to each antibiotic was compared by means of a Spearman correlation test, which is a non-parametric test suitable for a small number of data points (ten in our case) (35).

RESULTS

Patterns of antibiotic resistance

A total of 236 *E. coli* isolates were considered in this study of which 226 came from fecal samples (one isolate per each stool of the 25 humans, the 34 domestic animals, the 119 gorillas and the other 48 wild animals sampled) and 10 from water (Table 1). Although all water samples harbored a variety of enterobacteria species (Table 3), *E. coli* was present in only 10 out of the 20 samples. No enterobacteria were isolated from the ten soil samples. Thirty of the 226 fecal derived isolates were resistant to at least one antibiotic. In humans, 48.0 % (12 out of 25) of the *E. coli* isolates were resistant to at least one antibiotic, compared to 14.7 % (5 out of 34) in domestic animals, 10.4 % (5 out of 48) in wild animals and 6.7 % (8 out of 119) in gorillas (Fig. 1). Resistance to at least one antibiotic was significantly higher in

E. coli isolates from stools collected in the villages (*i.e.* humans and domestic animals), compared to those collected in the forest (*i.e.* gorillas and other wildlife) (2x2 Fisher's exact test, odds ratio = 4.79, $P < 0.001$). However, this difference seems to be driven by the high prevalence of antibiotic resistant isolates in humans, because no significant differences were found in isolates from domestic animals, gorillas and other wildlife (2x3 Fisher's exact test, $P = 0.69$). *E. coli* isolates derived from human stools were resistant to seven out of the ten antibiotics tested, whereas those from wild animals, gorillas and domestic animals were resistant to six, five and four antibiotics respectively (Table 1). None of the *E. coli* isolates from water were resistant to any of the antibiotics tested in this study (Fig. 1).

Overall, 7.6 % of all isolates were resistant to ampicillin, 6.4 % to sulfamethoxazole, 5.9% to streptomycin and 5.5 % to tetracycline. No resistance was observed for neomycin, ciprofloxacin and ceftiofur. Multidrug resistance was detected in 17 (56%) of the 30 resistant isolates. Multidrug resistance averaged (mean $\pm$ SD) 3.8 ± 1.8 antibiotics in isolates from humans, 1.4 ± 0.9 in isolates from domestic animals, 2.0 ± 1.4 in isolates from wild animals, and 1.6 ± 0.7 in isolates from gorillas. In addition, 88.2 % of multidrug resistant combinations included ampicillin and 70.6 % included tetracycline, streptomycin, or sulfamethoxazole. There was a large diversity of antibiotic phenotypes, with 18 isolates having a unique phenotype (Table 2).

The number of resistant strains to each antibiotic was correlated between isolates from the stools of humans and gorillas (Spearman correlation test, $\rho = 0.75$, $P = 0.01$). Thus, when the number of resistant isolates to an antibiotic was high in humans, it was also high in gorillas; and if the number was low in humans, it was also low in gorillas. This correlation was also observed between gorillas and other wildlife (Spearman, $\rho = 0.71$, $P = 0.02$), but not between gorillas and domestic animals (Spearman, $\rho = 0.61$, $P > 0.05$).

***E. coli* population structure**

The majority of *E. coli* isolates belonged to groups D (54.7 %) and B2 (32.2 %) and only a few belonged to groups B1 (7.6 %) and A (5.5 %) (Fig. 3). Isolates collected from water samples belonged mostly to groups D and B2. The stools of gorillas and other wild animals exhibited similar *E. coli* composition (2x4 Fisher exact test, $P > 0.05$), with high prevalence in isolates from groups D and B2. The stools of human and domestic animals harbored similar *E. coli* composition (2x4 Fisher exact test, $P > 0.05$) with *E. coli* isolates from all phylogenetic groups (Fig. 3). However, the *E. coli* composition in gorillas (or other wild animals) and humans (or domestic animals) stool samples were significantly different (2x4 Fisher exact test, $P < 0.001$). The patterns of phylogenetic groups of resistant isolates reflected that of non-resistant isolates (Fisher exact test, $P > 0.1$) (Fig. 3). Thus, all antibiotic-resistant isolates found in gorillas belonged to group B2 or D, which are also the most prevalent groups among non-resistant isolates. Combining both genetic and antibiotic phenotype profiles revealed that resistant *E. coli* isolates present among gorillas or other wildlife do not match the ones observed among humans or domestic animals (Table 2). Moreover, 6 out of the 8 antibiotic-resistant isolates found in gorillas belong to the subgroup B2₃, which was not found in any of the antibiotic resistant samples detected in humans or domestic animals.

DISCUSSION

Humans as reservoirs of antibiotic resistant *E. coli*

A high percentage of *E. coli* isolates found in human stools were resistant to at least one antibiotic (Fig 1). This result confirms that central African regions share the worldwide trend in increasing antimicrobial resistance (41), and suggests that human populations may be the main reservoir for antibiotic resistant strains in the study area. Resistance was particularly observed in antibiotics commonly used in Lopé, such as ampicillin and tetracycline (Table 1). The lack of resistance to ceftiofur, which is not available in the area, and to ciprofloxacin and neomycin, which are rarely used in Lopé, suggests that the high number of resistant isolates seems to be mostly generated by the selective pressures of antibiotics prescribed to humans within the study area. However, the presence of resistance to other antibiotics such as sulfamethoxazole and streptomycin (available in Gabon but not in Lopé) also suggests that humans in Lopé may be receiving resistant strains from humans living in other areas of Gabon, where these antibiotics are used. Levels of antibiotic resistance were low in isolates from domestic animals, which are almost never treated with antibiotics in this area. However, similarities in *E. coli* phylogenetic groups of resistant and non-resistant isolates in humans and domestic animals may suggest that transmission between these two populations is occurring.

Transmission of antibiotic resistant *E. coli* from humans to wildlife appears very low

E. coli antibiotic resistant isolates were found in the stools of gorillas and other wild animals at a low level compared to humans. But where are these resistant *E. coli* isolates coming from? Previous studies have proposed that contact and subsequent transmission of antibiotic resistant bacterial strains from highly resistant sources such as humans or livestock could account for the presence of antibiotic resistance in wild animals (9, 22, 32, 34). In particular, the presence of multi-resistant strains amongst gorillas has been previously used as evidence

of resistant bacterial transmission from a source such as humans, which are subject to high selection pressure from antibiotics (e.g. 33). However, in our study, we found significant differences in the genetic background of resistant and non-resistant *E. coli* isolates derived from human-domestic animals and gorillas-other wildlife (Fig. 3). In particular, *E. coli* resistant isolates in gorillas mainly belong to group B2₃, which was not a phylogenetic group observed for any resistant isolates from humans. Furthermore, the combination of both antibiotic resistant phenotypes and phylogenetic group information showed that no isolates from gorillas have identical characteristics to human isolates (Table 2). All of these results favor the idea that direct transmission of *E. coli* resistant strains from humans to wild animals is not occurring. Furthermore, although water may be acting as an indirect transmission pathway for resistant strains, we did not find resistant strains in water samples. However, a larger sample size is required to draw definitive conclusions. This could be achieved by repeated sampling of the same source, higher volumes of filtered water or by using an enrichment medium prior to bacteria isolation.

Other mechanisms explaining the observed resistance in wildlife

Mechanisms other than direct transmission of strains may account for the low prevalence of antibiotic resistant *E. coli* isolates found in gorillas and other wildlife stools. These include both the genetic exchange of resistant material by horizontal gene transfer between unrelated strains (e.g., tetracycline (31) and ampicillin (36)), or the independent selection of resistance in wild animals that confers protection, for example, against naturally occurring antibiotics and heavy metals (1). Although specific antibiotic resistant phenotypes between gorillas and humans did not match in our sample, our results did show a significant positive correlation in the specific prevalence of resistance to each antibiotic between these two species. This could have been generated from high transmission rates of resistant material, rather than the bacteria

itself. The physiological cost of resistance in the absence of antibiotic pressure (2, 24) may have subsequently caused the loss of some of this genetic material. This loss could explain the low prevalence of resistance (and multi-resistance) observed in wild animals, and the differences in antibiotic resistant phenotypes between the isolates coming from humans and wild animals. Further analysis on the genetic component of antibiotic phenotypes reported here, such as the amplification of transposons, integrons or plasmids, could help elucidating the origin and mechanisms of antibiotic resistant spread among wild animals.

Microbial exchanges through common food sources

This study reveals that *E. coli* strains from wild gorillas and other wildlife are genetically similar and belong almost exclusively to groups B2 and D. High prevalence of group B2 strains has been previously associated with herbivorous and omnivorous animals (15), and suggests that diet is a major determinant of the composition of *E. coli* flora in animals. In our study, this trend is reinforced as we sampled during a small dry season where ripe fruit is available but is less abundant than in the wet season, and wild animals tend to forage within the same available trees (40, 42). Interestingly, *E. coli* strains from groups B2 and D are not associated with the presence of virulent factors responsible for enteropathogenic *E. coli* causing diarrhoea (10). Thus, it is unlikely that in Lopé, wild animals are the reservoirs for this disease.

Conclusion

The purpose of this study was to infer patterns of *E. coli* antibiotic resistance in wild animals in a region where human density is low, and to assess whether the observed pattern can be explained by transmission from humans or domestic animals. Direct transmission can be suspected if the genotype of the transmitted bacteria to a receiving host is a subset of the

genotypes within the transmitting host (3). The genetic background of resistant strains from both gorillas and other wildlife was different from the background observed in humans and domestic animals. Thus, our study indicates that the observed antibiotic resistance in wild animals may not be caused by the direct acquisition of human bacterial strains. Rather, amongst wild animals, sharing the same local environment such as common foraging places may be the major determinant of the similarities in population structure of *E. coli*. Therefore, our study predicts that in areas of low human density where contact opportunities with wild animals are rare, the potential for *E. coli* transmission is low.

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350 **FIGURE LEGENDS**

351 Figure 1: Study site

352 Figure 2: Antibiotic resistance per sample origin. Bars represent the proportion of isolates
353 resistant to at least one antibiotic (given in percentages) for each source. Error bars were
354 calculated as Standard Errors (SE) for a proportion, given by $SE = \sqrt{p \times (1 - p) / n}$, where p is
355 the proportion of isolates resistant and n is the total number of isolates. SE were multiplied by
356 100 to scale bars given in percentages.

357 Figure 3: *E. coli* phylogenetic group pattern for each sample origin. Each bar represents the
358 percentage of *E. coli* isolates identified in each group. Within a bar, each color represents the
359 percentage of resistant and non-resistant strains.

360

361

TABLES

Table 1 Summary of the distribution of resistant *E. coli* isolates according to each antibiotic and collected source. The number (N) of resistant isolates to each antibiotic is given for each host (in parentheses the percentage from N).

Antibiotic	Gorillas N = 119	Other Wildlife N = 48	Humans N = 25	Domestic animals N = 34	% of resistance from total fecal isolates, N=226	Water N = 10
Ampicillin*	4 (3.4)	4 (8.3)	9 (36.0)	1 (2.9)	8	0
Ceftiofur****	0	0	0	0	0	0
Chloramphenicol*	0	1 (2.1)	1 (4.0)	0	0.9	0
Tetracycline*	3 (2.5)	2 (4.2)	8 (32.0)	0	5.8	0
Ciprofloxacin**	0	0	0	0	0	0
Doxycycline***	0	1 (2.1)	6 (24.0)	0	3	0
Neomycin**	0	0	0	0	0	0
Rifampicin***	2 (1.7)	1 (2.1)	2 (8.0)	2 (5.9)	3.1	0
Streptomycin***	3 (2.5)	1 (2.1)	9 (36.0)	1 (2.9)	6.2	0
Sulfamethoxazole***	1 (0.8)	0	11 (44.0)	3 (8.8)	6.6	0

* Most commonly used (in Lopé), ** Rarely used, *** Not used in Lope but available in Gabon, **** Not used in Lopé and very rarely used in Gabon.

371 **Table 2 Antibiotic resistant phenotype and phylogenetic group for each resistant**
372 ***E. coli* isolate.** Antibiotic resistant phenotypes represent the antibiotics to which an
373 isolate was resistant. Codes for antibiotics are Amp: Ampicillin, Tet: Tetracycline, Chl:
374 Chloramphenicol, Dox: Doxycycline, Rif: Rifampicin, Set: Streptomycin and Sul:
375 Sulfamethoxazole.
376

No. of isolates	Antibiotic resistant phenotype	<i>E. coli</i> phylogenetic group
Gorillas		
1	Tet-Amp-Rif	B2 ₃
1	Amp-Str	B2 ₃
1	Tet-Amp	D
1	Amp-Sul	B2 ₃
2	Str	B2 ₃ , B2 ₃
1	Rif	D
1	Tet	B2 ₃
Wildlife		
1	Tet-Chl-Dox-Amp	D
1	Tet-Amp-Str	A
2	Amp	D, D
1	Rif	B2
Domestic animals		
1	Amp-Str-Sulf	B1
2	Rif	D, A
2	Sul	A, D
Humans		
1	Tet-Chl-Dox-Amp-Str-Sul	D
1	Tet-Dox-Amp-Str-Sul-Rif	A
3	Tet-Dox-Amp-Str-Sul	D,B1,A
1	Dox-Amp-Str-Sul-Rif	A
1	Tet-Amp-Str-Sul	A
1	Tet-Dox-Str-Sul	D
1	Amp-Sul	B1
1	Str-Sul	D
1	Amp	B1
1	Sul	D

377
378

379 **Table 3 Bacteria species recovered from water samples.** Each morphologically
 380 distinct isolate (out of five isolates per sample) was identified to the species level for
 381 each water sample. The number of isolates belonging to each bacteria species is given
 382 by N.
 383
 384
 385

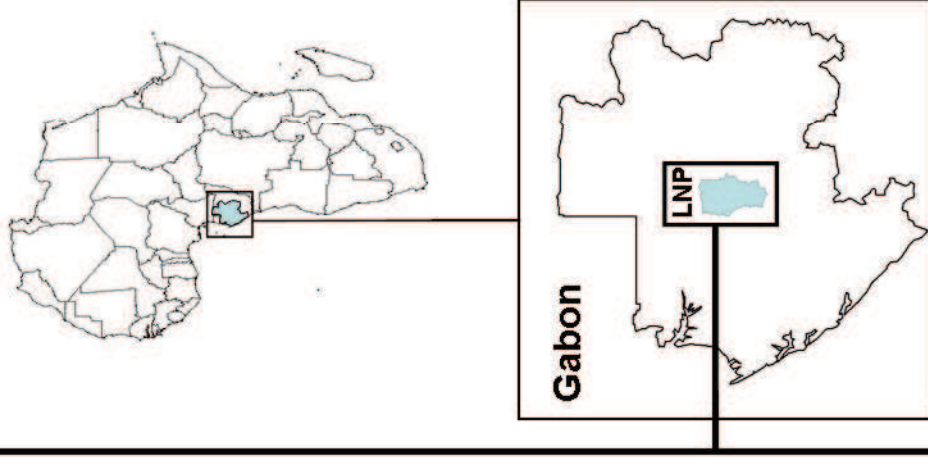
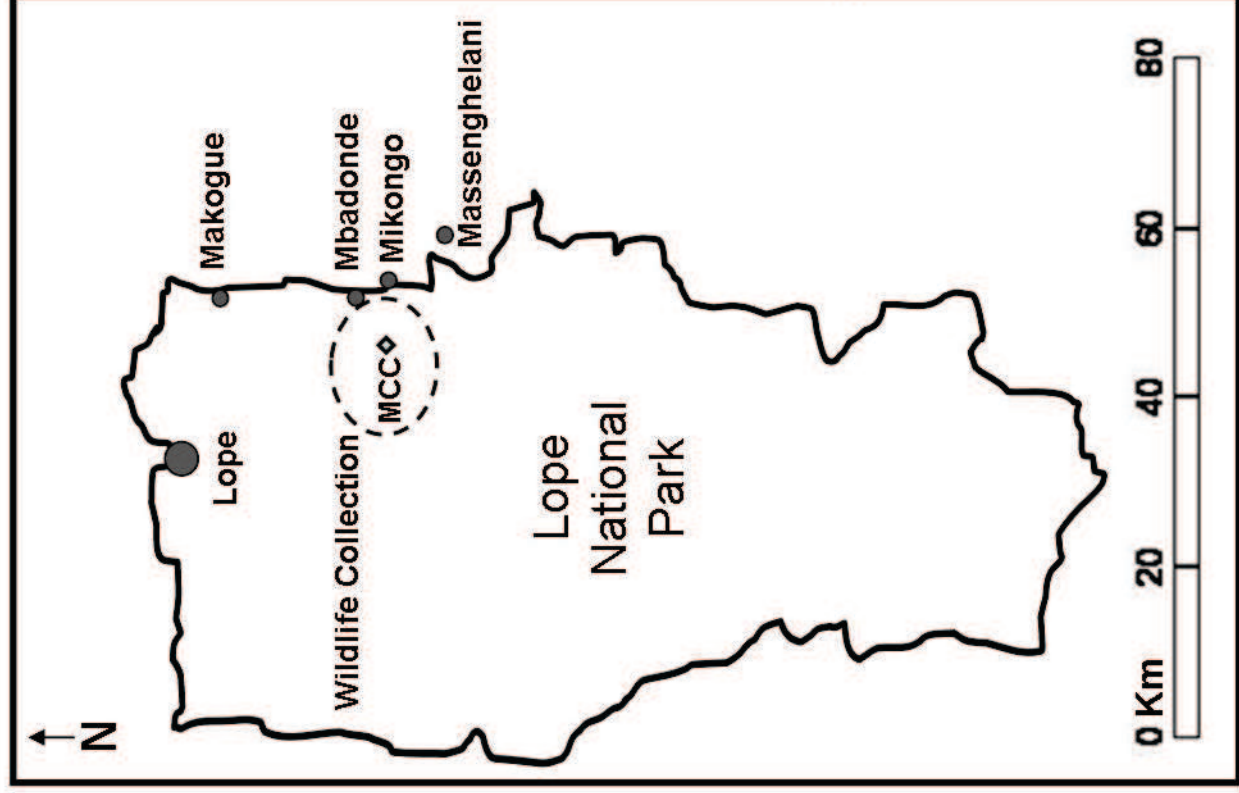
Origin	Number of water sources	Number of lac+ isolates	Species found (N)	Number of Lac- isolates	Species found (N)
Forest streams	5	14	<i>Serratia marescence</i> (7) <i>Escherichia coli</i> (5) <i>Klebsiella pneumonia</i> (1) <i>Klebsiella oxytoca</i> (1)	3	<i>Proteus mirabilis</i> (2) <i>Proteus vulgaris</i> (1)
Villages	15	49	<i>Serratia marescence</i> (33) <i>Escherichia coli</i> (5) <i>Enterobacter sp.</i> (5) <i>Klebsiella pneumonia</i> (3) <i>Klebsiella oxytoca</i> (2) <i>Klebsiella terringeri</i> (1)	8	<i>Proteus mirabilis</i> (3) <i>Morganella morganii</i> (3) <i>Proteus vulgaris</i> (2)

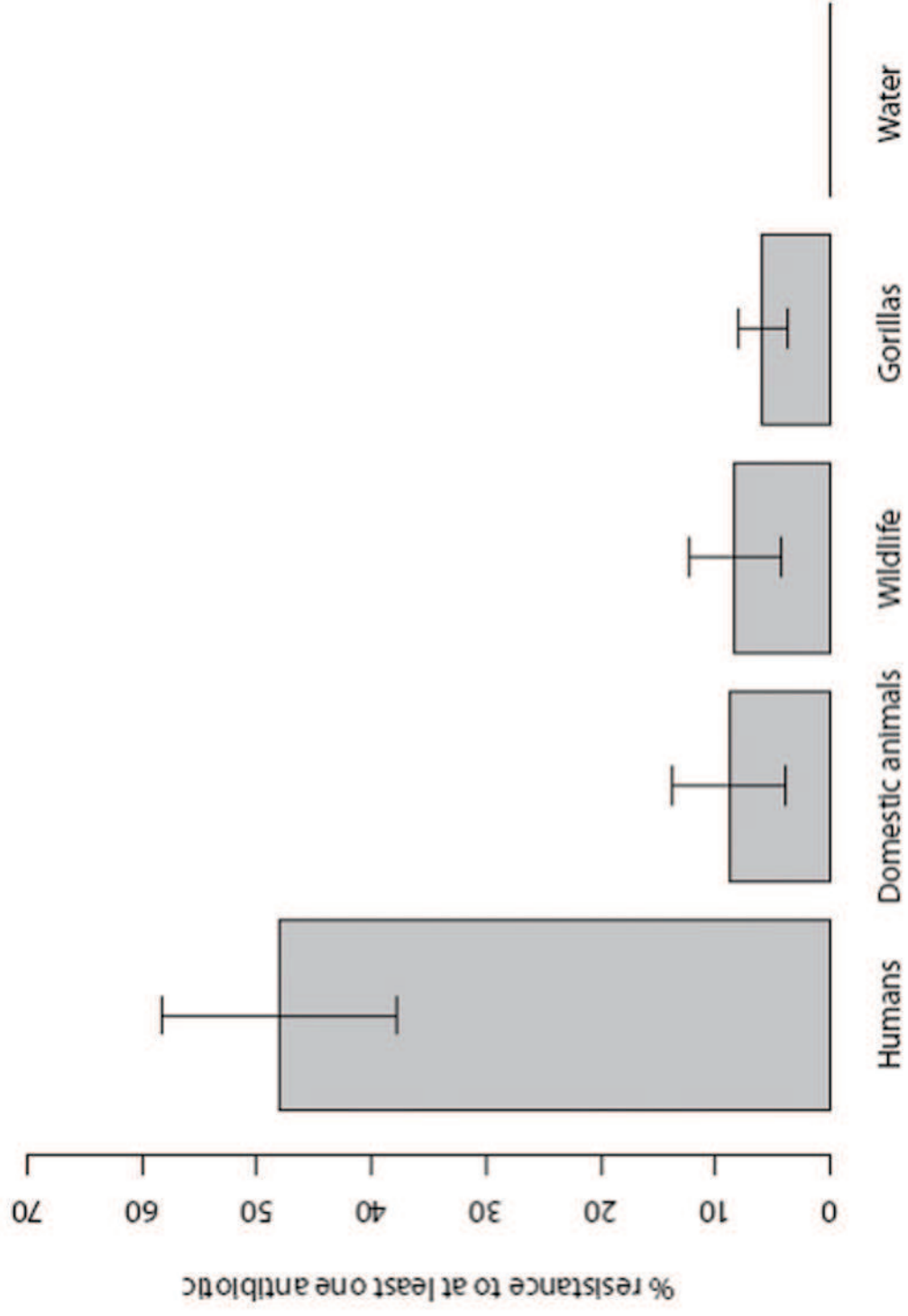
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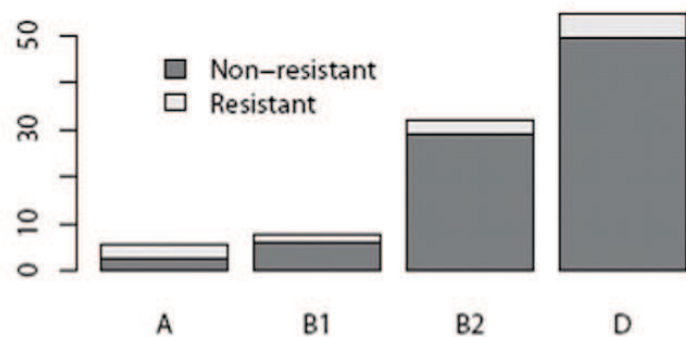
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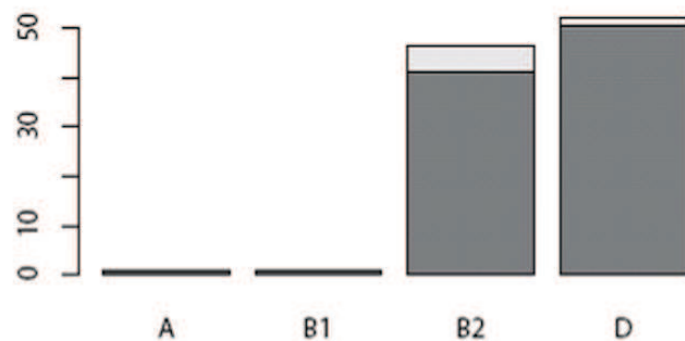




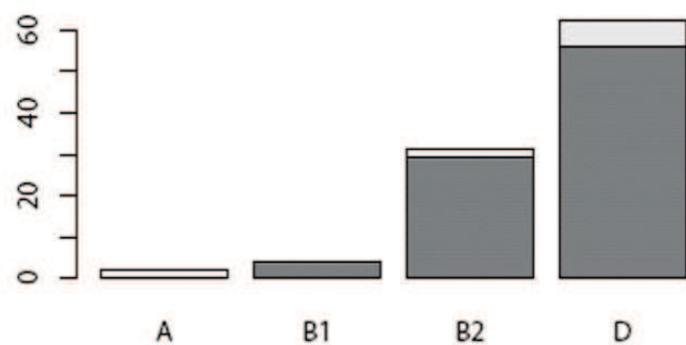
All faecal samples



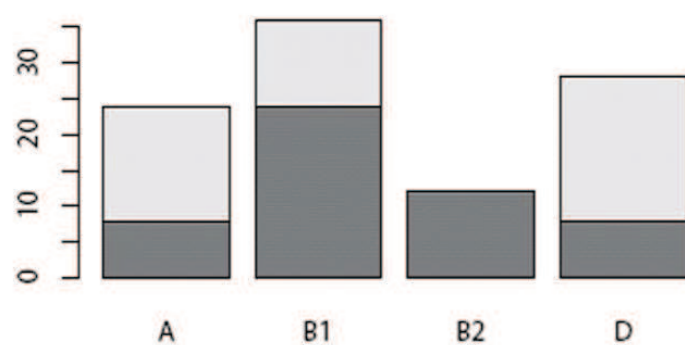
Gorillas



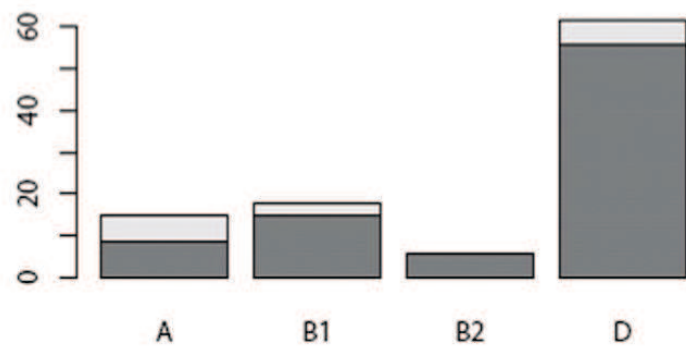
Wildlife



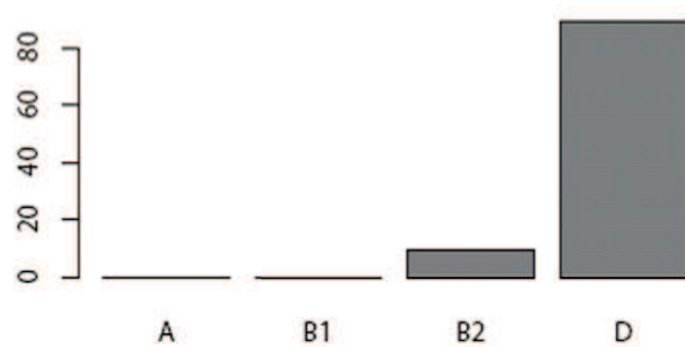
Humans



Livestock



Water



Article 4

Territory overlapping mediates antibiotic resistance transmission between livestock and wild baboons in Namibia

Authors: Benavides JA, Cowlshaw G, Alout H, Godreuil S, Escobar-Paramo P, Raymond M.

In preparation

Title: Territory overlapping mediates antibiotic resistance transmission between livestock and wild baboons in Namibia

Authors: Benavides JA, Cowlishaw G, Alout H, Godreuil S, Escobar-Paramo P, Raymond M.

Abstract:

The presence of livestock in wildlife areas represents a clear risk for disease transmission to wild animals and a threat to endangered species. The high prevalence of antibiotic resistant bacterial strains in livestock allows the study of the dynamics of bacterial transmission into wildlife, and to identify areas of high risk for disease spillover. The purpose of this study was to estimate the prevalence of enterobacteria antibiotic-resistant strains in wild chacma baboons (*Papio ursinus*) in Tsaobis Nature Park Namibia, and to assess whether water and livestock are implicated in the observed pattern. Our results show high levels of resistance in enterobacteria isolates from livestock, wildlife and water samples. A strong correlation in the prevalence and nature of antibiotic resistance between isolates from livestock and baboons across local areas of interaction suggests that antibiotic resistant material may be spreading from livestock to wild animals, possibly through overlapping of home ranges. Furthermore, the presence of antibiotic resistant bacteria in water samples suggests that water may be facilitating the transmission of antibiotic resistant bacteria within wild and domestic animals. Overall, our findings suggest that territory overlapping may be sufficient to trigger wildlife-livestock bacteria transmission, and that water should be considered as an important reservoir for the transmission of antibiotic resistant bacteria within wild and domestic animals.

26 INTRODUCTION

27 The intensification of human activities in the habitat of previously isolated wild
28 animals is a key factor in the emergence of infectious diseases (Daszak, Cunningham &
29 Hyatt, 2001; Jones et al., 2008). In particular, the extension of agricultural activities into
30 wildlife territory is generating an increasing risk in parasite transmission from domestic
31 animals to wild populations (Daszak et al., 2001; Morgan et al., 2004). Although several cases
32 of livestock-to-wildlife transmission have been reported (Daszak, 2000; George et al., 2008;
33 Gortázar et al., 2007; Michel et al., 2006) there is still much uncertainty on how inter-species
34 interactions across the landscape affect the levels of parasite spillover into wildlife. Within
35 wild populations, “habitat hotspots” such as food patches or waterholes are expected to
36 increase the chances of inter-individual disease transmission (Bonnell et al., 2010). For
37 example, water sites can increase the transmission of tuberculosis-like lesions among wild
38 boar (Vicente-Baños et al., 2007). However, little is known on whether the indirect contact
39 through territory overlapping between livestock and wildlife can trigger the transmission of
40 faecal-oral parasites (Morgan et al., 2004). In particular, it is unclear whether the defecation
41 of livestock into the home range of wild animals is sufficient to generate bacteria spillover into
42 wildlife.

43 In this study, we estimated and compared the antibiotic resistance patterns of faecal
44 Enterobacteria collected from several wild baboon troops, livestock, and both natural and
45 artificial waterholes in the semi-desert environment of the Tsaobis Nature Park, Namibia. The
46 presence of antibiotic resistance in fecal coliforms of untreated wild animals has been
47 suggested to reflect bacterial exchange with humans or domestic animals, in which treatment
48 with antibiotics is actively selecting for antibiotic resistant strains (Allen et al., 2010; Blanco
49 et al., 2007; Cole et al., 2005; Dolejska, Cizek & Literak, 2007; Goldberg et al., 2007; Literak

et al., 2010; Rwego et al., 2008; Skurnik et al., 2006). In Tsaobis, livestock are widely treated with tetracycline and other antibiotics, representing a clear source for selection of antibiotic resistant strains. Because livestock forage and defecate into the baboon's territory, foraging overlapping may be generating the transmission of resistant strains between these populations.

In humans, the fecal contamination of water is often described as a major environmental driver of bacterial transmission (Burnes, 2003; Edberg et al., 2000; Wiggins, 1996). However, the role of water in the transmission of antibiotic resistance among wild animals remains poorly understood, because the majority of studies have not focused in isolating bacteria from surrounding water sources (Goldberg et al., 2007; Literak et al., 2010; Rwego et al., 2008). In Tsaobis, livestock drink from artificial waterholes provided at farms, while baboons drink from natural waterholes located mostly within the hills at the beginning of the dry season. Furthermore, water is often contaminated by fecal material in both artificial and natural waterholes. Therefore, water may be acting as a source of antibiotic resistant bacteria for animals drinking from that water.

The purpose of the study was to estimate the prevalence and nature of antibiotic resistance across five different local areas where livestock interact with baboons, in order to assess whether the territory overlap between these populations is triggering the spillover of antibiotic resistant bacteria into the wildlife. Furthermore, we tested the presence of antibiotic resistant bacteria in the water drunk by both wild and domestic animals. Our results suggest a strong correlation in the prevalence and nature of antibiotic resistance between livestock and baboons across the five local areas. Additionally, bacteria in the water show high levels of antibiotic resistance, confirming its role as a reservoir for antibiotic resistant bacteria transmission between animals.

MATERIALS AND METHODS

74 **Study Site**

75 Samples were collected in May 2009 in the Tsaobis Leopard Park (22°23'S 15°45'W) in
76 Namibia, and in areas adjacent to the east side of the park, where both farm and human
77 settlements are established along the Swakop River (Fig. 1). Tsaobis is characterised by
78 mountains and rocky foothills that descend to rolling gravel and alluvial plains. Vegetation is
79 sparse, comprising grasses and herbs, shrubs and dwarf trees, although patches of aquifer-
80 dependent woodland grow along the ephemeral Swakop River bordering Tsaobis to the north.
81 The landscape is arid and during our study period, baboons mainly drunk water from isolated
82 natural waterholes formed during the rainy season (ending in April), which mostly dry-out
83 towards the end of the dry season (November). Five local areas of livestock-baboon
84 interaction were identified (referred in Fig. 1 as Tsaobis, Okarudu 1, Okarundu 2, Karabib and
85 Otjibimwe). Each area was established and delimited by livestock moving patterns, by the
86 presence of fresh livestock faecal samples and tracks found in proximity to baboons, and by
87 interviews with local farmers, who indicated the location of baboon troops that may interact
88 with their livestock (and that were subsequently sampled).

89 **Samples collection**

90 *Wild baboons*

91 A total of 154 fresh faecal samples, each from a different individual, were collected less than
92 1 hour after defecation. Samples correspond to 15 different baboon troops (mean $\pm$ SD = 10.3
93 $\pm$ 13.3 samples/group, range= 4-57), including two habituated troops (Huchard et al., 2009).
94 Each sampled troop was followed during one day and identified by different characteristics
95 such as group number (assessed with video filming during riverbed crossing), number of
96 swelling females and male barking. To avoid sampling the same troop twice, troops outside

Tsaobis (that have not been previously identified) were simultaneously sampled the same day by different collectors. Baboons were followed until the entire troop rest on a cliff or a waterhole and defecate, which avoided sampling the same individual twice. Approximately 2g of faecal material were taken from the interior of the faecal sample and collected in a 1.5 ml Eppendorf tube containing 1 ml of a sterile isotonic buffered solution composed of NaCl at 0.9%. Fecal samples were subsequently isolated for bacteria colonies no more than 12 hours after collection.

Livestock

The faecal samples of 78 livestock animals were collected within each of the seven farms considered in the study. The samples included 22 goats, 13 sheep, 30 cows, 9 donkeys and 4 horses. Samples were collected from feces found in the farms and also by following the animals until defecation. Occasionally, livestock such as donkeys and horses were also collected when ranging freely near a baboon troop. The collection of domestic animal samples was approved by each livestock owner, who had been previously informed and agreed on the project objectives.

Water samples

A total of 13 natural waterholes visited by baboons and 7 artificial waterholes (one per sampled farm) were sampled. Waterholes visited by baboon troops were assessed by observations during troop's tracking, by camera traps posted at waterholes, and by local farmers and team observations. Water samples were collected in a 50 ml syringe and were systematically filtered with a high flow syringe containing a 0.45 µm poly ether sulfone membrane filter (Sartorius Stedium), which retain the bacteria (Quinn, 1994). The membranes

were saved within a sterile plastic container and used for bacterial isolation no more than 6 hours after collection.

Bacterial isolation

After collection, each diluted faecal sample was streaked onto a MacConkey (MC) agar plate (VWR) using a sterile toothpick and water membranes were placed over a MC agar plate. Plates were subsequently incubated at 37° C for 24 hours. For each MC agar plate, we selected one pink lactose-positive colony representing a unique morphotype present on the plate. Thus, up to seven isolated colonies (with a minimum of four) were selected per plate and transferred into a 96-well plate containing MC agar. Well plates were then kept at 4°C and shipped to the laboratory in France for further analyses. Once in the laboratory, bacterial strains were re-isolated and stored in 20% glycerol at -80°C. All isolates from water samples were identified to the genus or species level by biochemical testing (API 20E gallery or bioMerieux Vitek Inc), along with a subset of 100 randomly selected isolates from collected fecal samples.

Antibiotic susceptibility test

The antibiotic susceptibility of each isolate was performed for antibiotics with different uses within the study area. Ampicillin, amoxicillin, chloramphenicol, ciprofloxacin, doxycycline, streptomycin, and chlortetracycline were available for human treatment, although ampicillin was the most commonly used antibiotic in humans within the study area (as reported by the local health administration). Neomycin was very rarely used and ceftiofur was not available in the study area. Oxytetracycline was the most commonly available and used antibiotic to treat livestock, as reported by farmers and also veterinary and agricultural suppliers within the area. Antibiotic susceptibility testing was performed by the agar dilution method (Clinical and

Laboratory Standards Institute, 2006). Twofold dilutions of the antibiotics were added in order to obtain the final concentrations superior to critical concentration according to breakpoints provided by the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The antibiotics and dosage levels used were ampicillin ($> 8 \mu\text{g/ml}$), ceftiofur ($> 8 \mu\text{g/ml}$), ciprofloxacin ($> 2 \mu\text{g/ml}$), oxytetracycline ($> 8 \mu\text{g/ml}$), chloramphenicol ($> 8 \mu\text{g/ml}$), doxycycline ($> 8 \mu\text{g/ml}$), neomycin ($> 4 \mu\text{g/ml}$) and streptomycin ($> 16 \mu\text{g/ml}$). Amoxicillin and chlortetracycline were also tested but generated the same results as ampicillin and oxytetracycline respectively, and were therefore not included in further analyses. Inocula were prepared in saline solutions to achieve 10^8 cfu/mL (0.5 McFarland standard), then diluted 1 in 10 using sterile saline. A multipoint inoculator was used to dispense $1 \mu\text{L}$ of diluted inoculum to achieve 10^4 cfu/mL per plate with and without antibiotic. Plates were incubated in air at 37°C for 18 h. A fully susceptible *E. coli* (ATCC 25922) was used as the control. An isolate was considered resistant if it grew in the presence of the antibiotic and formed a homogenized colony similar to a control colony grown in medium without antibiotic. A replicate was systematically run to confirm each resistant case. To evaluate the reproducibility using the method, independent tests were performed for *E. coli* ATCC 25922, and 5 clinical strains (tested twice each).

Analyses

Resistance to at least one antibiotic was compared between sources using a Proportion's test. Correlations of the specific resistance to each antibiotic were tested using Spearman's correlation tests. To evaluate the effects of sympatric overlapping, we tested the effects of both local area and species identity in the resistance to at least one antibiotic among animals. This was performed by comparing the Akaike Information Criterion (AIC) of different Binomial Generalized Linear Mixed Models (GLMMs), including resistance to at least one

antibiotic as the response variable. The model also included “individual identity” as a random effect along with “local area identity” and/or “species identity” as fixed effects (one model for each of the three possibilities with this two variables). AIC selection was chosen in this case since other conventional tests are not yet implemented for GLMMs with qualitative fixed effects (Pinheiro & Bates, 2000). Finally, we compared resistant phenotypes profiles (*i.e.*, absence/presence of resistant to each antibiotic) for each isolate in baboons, livestock and water using a Correspondence Analysis. All statistical analyses were performed in R 2.13.1 (R Development Core Team, 2003). GLMMs were implemented using the lme4 package (Pinheiro & Bates, 2000) and Correspondence Analysis was implemented using ade4 package (Dray & Dufour, 2007)

RESULTS

Antibiotic resistance prevalence

A total of 878 bacterial colonies were isolated from wild baboons, 366 from livestock and 111 from water samples. Enterobacteria isolates from all sampled sources exhibited high levels of resistance to at least one antibiotic (Fig. 2). The bacteria isolates from natural waterholes showed the highest proportion of resistance. Resistance was slightly but significantly higher in livestock than baboons (Proportion test, ratio= 1.12, $P = 0.01$). Isolates from livestock were resistant on average to $1.3 (\pm 1.1)$ antibiotics, isolates from baboons to $0.95 (\pm 0.93)$, isolates from natural waterholes to $2.51 (\pm 1.89)$ and isolates from artificial waterholes to $0.93 (\pm 0.88)$ antibiotics. The highest levels of resistance in animals were associated with ampicillin (91% in baboons and 87 % in livestock) and tetracycline (8% in baboons and 48 % in livestock). Among isolates that were resistant to ampicillin or tetracycline, 34 % of isolates were resistant to both antibiotics. Specific resistances to other antibiotics for livestock and wild baboons are summarised in Fig. 4. Out of the 100 randomly selected isolates from

animal faecal samples, 57 were identified as *Escherichia coli*, 24 as *Citrobacter freundii*, 11 as *Kluyvera spp.* 3 as *Klebsiella ornithinolytica*, 3 as *Citrobacter koseri* and 2 as *Citrobacter brakii*.

Antibiotic resistance in livestock and baboons

Resistance to at least one antibiotic was correlated between livestock and baboons across the five localities (Spearman test, $\rho=1$, $P = 0.01$) (Fig. 3). Furthermore, local area identity, but not species identity, substantially increased the likelihood of a model testing the effects of these variables on the resistance to at least one antibiotic in animals (*i.e.* model with area and species identity as fixed effects: AIC= 1379, model with only local area: AIC= 1377, model with only species: AIC= 1395, and model with no fixed effect: AIC= 1397). There was also a strong correlation in the specific resistance to each type of antibiotic between livestock and baboons when all data across localities was analysed together (Spearman rank test, $\rho = 0.95$, $P < 0.001$, Fig. 4). This relationship was further confirmed in each of the five localities analysed separately (Spearman rank test, $\rho > 0.85$ and $P < 0.01$ in all cases).

Antibiotic-resistant bacteria in waterholes

Several species of enterobacteria expressing antibiotic resistance were identified among water samples (Table 1). There was no difference in the prevalence to at least one antibiotic between isolates from natural and artificial waterholes (Proportion test, ratio= 1.27, $P > 0.05$). However, multi-resistance was significantly higher in isolates from natural waterholes (Wilcoxon test, $W = 2127$, $P < 0.001$). The highest levels of resistance in isolates from waterholes were associated with neomycin (85% in natural and 72% in artificial) and ampicillin (77% in natural and 43% in artificial). There was no correlation in the specific resistance to each type of antibiotic between isolates from artificial and natural. (Spearman

rank test, $\rho = 0.55$, $P > 0.16$). A higher number of bacteria species were found in natural waterholes, although sampling is unbalanced and relatively small. The comparison of antibiotic resistant phenotypes within the correspondence analysis suggests that resistant profiles in bacteria from waterholes are more diverse and unique than profiles from baboons or livestock bacterial isolates (Fig. S1, Supplementary Material).

DISCUSSION

Our results show a high level of antibiotic resistance in the enterobacteria gut flora of baboons, livestock and also in bacteria present in artificial and natural waterholes across different localities of the Tsaobis Nature Park, Namibia. This result confirms the role of commensal enterobacteria as a major reservoir for antibiotic resistant bacterial strains, which can subsequently be spread into more pathogenic bacteria (Marshall, Ochieng & Levy, 2009). Strong similarities in both the prevalence and the nature of resistance between livestock and baboons across different local areas of livestock-baboon overlapping territory suggest that antibiotic resistance is transmitted between livestock and wild baboons. Rather unexpectedly, high levels of antibiotic resistant bacteria were also observed among water, highlighting its role as a driver of antibiotic bacteria transmission among wild and domestic animals.

Studies focusing on the prevalence of antibiotic resistance in wildlife have suggested that high (or even low) levels of resistance among wild populations including baboons are associated with transmission from a human or domestic animal source of antibiotic resistance (Cole et al., 2005; Rolland et al., 1985; Thaller et al., 2010). However, the spatial distribution of resistance prevalence within the livestock-wildlife landscape (*i.e.*, across localities) has very rarely been quantified. In this study, the positive correlation in antibiotic resistant prevalence of isolates from livestock and baboons collected among five localities strongly suggests a link in the resistance of livestock and wild baboons. This is also confirmed by the

similarity in resistant phenotypes and the high levels of resistance observed for antibiotics commonly used in either livestock (*e.g.*, tetracycline) or humans (*e.g.*, ampicillin) treatment. Alternative explanations for the correlated high levels of resistance observed among livestock and baboons include a strong independent selection for antibiotic resistance within each host (Mather et al., 2011). However, this hypothesis is less likely to explain our results because wild baboons do not experience any known selective pressure for antibiotic resistance, and environmental natural selection for antibiotic resistance cannot explain the differences in prevalence between different localities in a rather homogeneous semi-arid environment such as Tsaobis. Further longitudinal data over several years should help understanding whether antibiotic resistance is constantly introduced from livestock to baboons or whether it is now persistent and circulating in baboons without constant spillover.

The transmission of antibiotic resistance between bacteria from livestock and baboons can be mediated by either the direct transmission of resistant bacteria or by the transmission of genetic material conferring antibiotic resistance between different bacteria (Normark & Normark, 2002). In fact, horizontal gene transfer allows the transmission of resistant material between phylogenetically diverse bacteria species, including the exchange of gene cassettes, transposons and plasmids (de la Cruz & Davies, 2000; Maiden, 1998; Ochman, Lawrence & Groisman, 2000). Horizontal gene transfer is also a main mechanism for the transmission of resistant material associated with tetracycline and ampicillin (Chopra & Roberts, 2001; Sutcliffe, 1978) and transfer of plasmids is very common between members of the *Enterobacteriaceae* family (Mayer, 1988). The circulation of plasmids or cassettes can also generate the expression of resistance to several antibiotics for the same bacteria strain. However, in our sampling, only 33% of isolates resistant to tetracycline or ampicillin were resistant to both antibiotics. Thus, our findings encourage further work in order to

discriminate whether similarities in antibiotic resistance between livestock and baboons are due to the transfer of bacteria *per se* or by the exchange of resistant-conferring material.

This study highlights the role of home range overlapping in the transmission of resistance from livestock to wild baboons, with animals living in the same area having more similar antibiotic prevalence than animals from the same species group (baboons or livestock) living in different areas. Because baboons and livestock very rarely drink from the same waterholes, waterholes are not expected to be a major transmission pathway for the transmission of resistance between these two hosts. However, foraging overlapping does occur, suggesting that defecation and subsequent transmission of resistance in the soil or pasture may be a key mechanism. In fact, enterobacteria shed by livestock can survive for several days in the soil or pasture, depending on factors such as temperature and moisture (Avery, Moore & Hutchison, 2004; Chee-Sanford et al., 2009; Cools et al., 2001; Topp et al., 2003). Furthermore, there are multiple mechanisms for the transfer of resistant genes between bacteria species in the soil, in fresh water, or from gut bacteria to indigenous soil and water bacteria (Chee-Sanford et al., 2009). Thus, the transmission of antibiotic resistance through indirect territory overlapping may be a widespread phenomenon occurring at the interface between livestock and wild animal populations.

High levels of antibiotic resistance among bacteria collected from water samples suggest that water may facilitate the transmission of resistance between animals. But why are levels of antibiotic resistance so high and antibiotic resistant profiles so diverse in bacteria from water samples? Our results show that antibiotic multi-resistance was higher in isolates from natural waterholes than in isolates from artificial waterholes, and no correlation was found in the specific resistance to each antibiotic between them. The maintenance and selection of high levels of antibiotic resistance in bacteria collected among natural waterholes

could be linked to the high levels of heavy metals in the area such as copper, which has been associated with the resistance to several antibiotics (Baker-Austin et al., 2006; Berg, Tom-Petersen & Nybroe, 2005). Selection of heavy metals might also contribute to the maintenance of resistance within the soil and baboons. In contrast, the resistance observed in artificial waterholes could be more linked to the contamination of bacteria from livestock. However, a larger sample size of enterobacteria will be required to compare the antibiotic resistance between waterholes and animals across different localities. For example, the more diverse and unique patterns of resistance in bacteria from water compared to isolates from animals (Fig. S1) could be explained by the presence of bacteria species that may not be present in high proportions in the gut of animals such as *Serratia spp.*

Overall, this study suggests that high levels of antibiotic resistant bacteria may be transmitted between livestock and baboons by simple territory overlapping. Thus, antibiotic resistance could be used as a measure of potential bacteria transmission risk between wildlife and livestock in different systems. This study also highlights the role of environmental transmission by water consumption as an important factor in the transmission of antibiotic resistant bacteria among wild or domestic animals.

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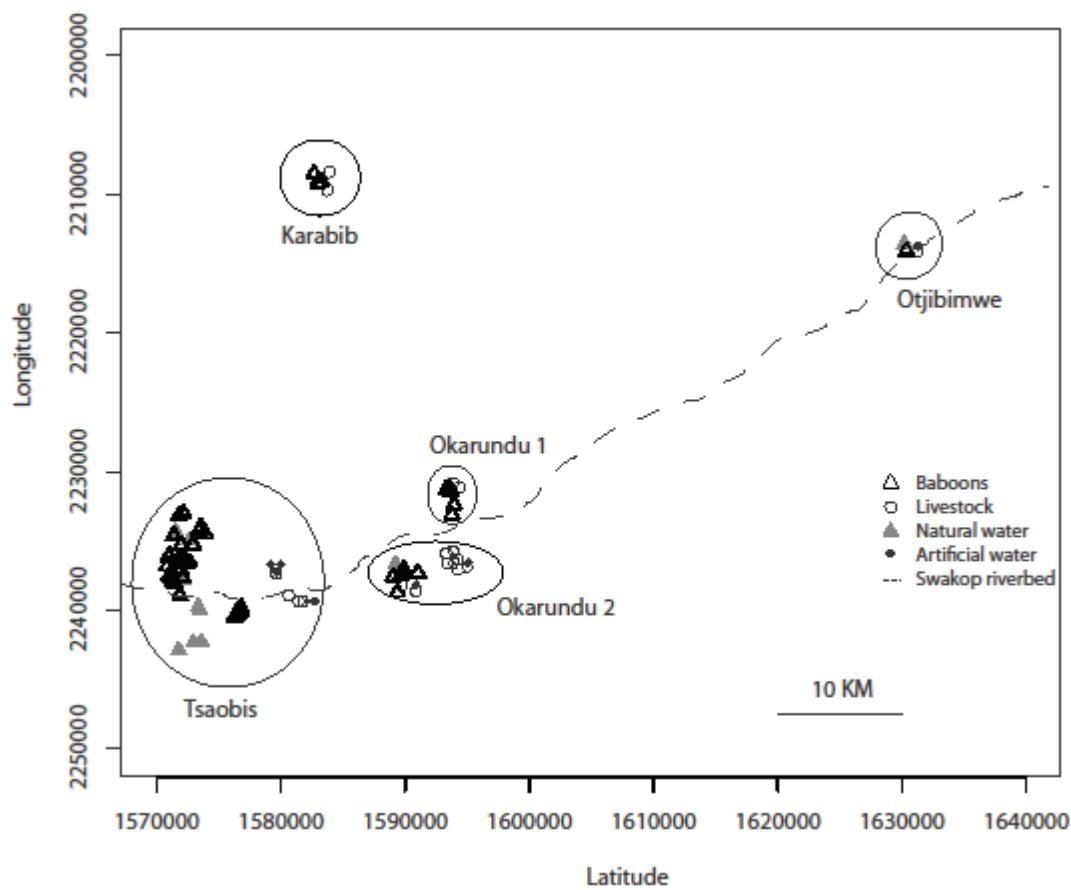
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Table 1. Bacteria species found in both natural and artificial waterholes. The number of isolates identified within each species are shown, along with the prevalence of resistance to at least one antibiotic, and the average number of antibiotics an isolate was resistant to.

Artificial waterholes (N= 41)			
Species	Number	Prevalence	Multiresistance
<i>Escherischia coli</i>	19	63	0.89
<i>Klebsiella oxytosa</i>	12	33	0.3
<i>Enterobacter cloacae</i>	2	100	2
<i>Proteus vulgaris</i>	2	100	2
<i>Citrobacter brakii</i>	1	100	1
<i>Citrobacter freundii</i>	1	100	1
<i>Citrobacter Koseri</i>	1	100	3
<i>Proteus peneri</i>	1	100	1
<i>Serratia marcescens</i>	1	100	2
<i>Serratia odorifera</i>	1	100	1
Natural waterholes (N= 70)			
Species	Number	Prevalence	Multiresistance
<i>Escherischia coli</i>	17	65	0.94
<i>Serratia Marcescens</i>	13	100	3.69
<i>Klebsiella oxytosa</i>	12	100	0.75
<i>Citrobacter Koseri</i>	7	100	3.29
<i>Serratia odorifera</i>	4	100	3.75
<i>Enterobacter cloacae</i>	3	100	1.33
<i>Proteus mirabilis</i>	3	100	4.33
<i>Providencia rettgeri</i>	3	100	4.67
<i>Citrobacter freundii</i>	1	100	3
<i>Edwardsiella hoshinae</i>	1	100	6
<i>Enterobacter sakozakui</i>	1	100	1
<i>Klyuvera spp.</i>	1	100	3
<i>Morganella morgani</i>	1	100	6
<i>Providencia stuartii</i>	1	100	4
<i>Proteus vulgaris</i>	1	100	5
<i>Serratia liquefaciens</i>	1	100	6

441 **FIGURES**

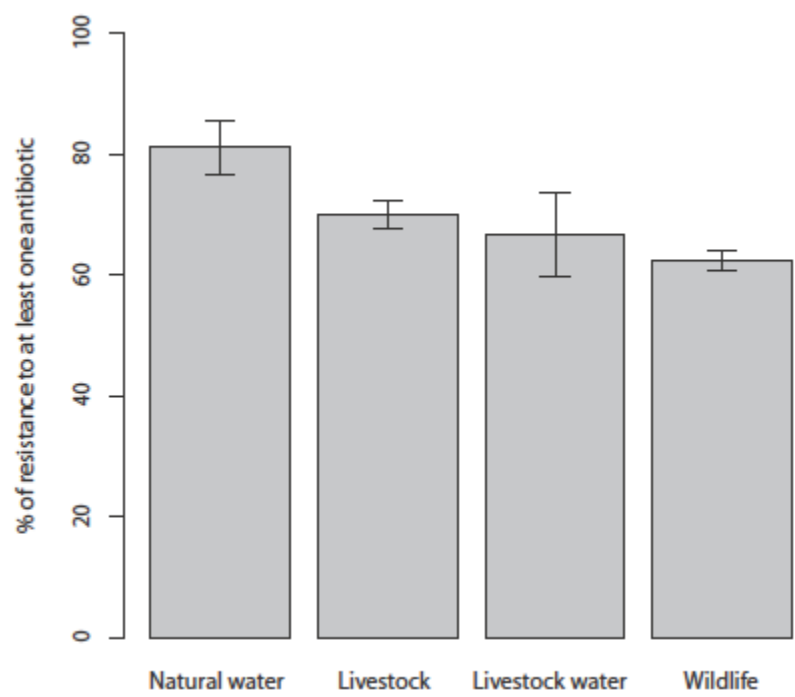
442 Figure 1: Map of collected samples and their location within Tsaobis Nature Park-Namibia



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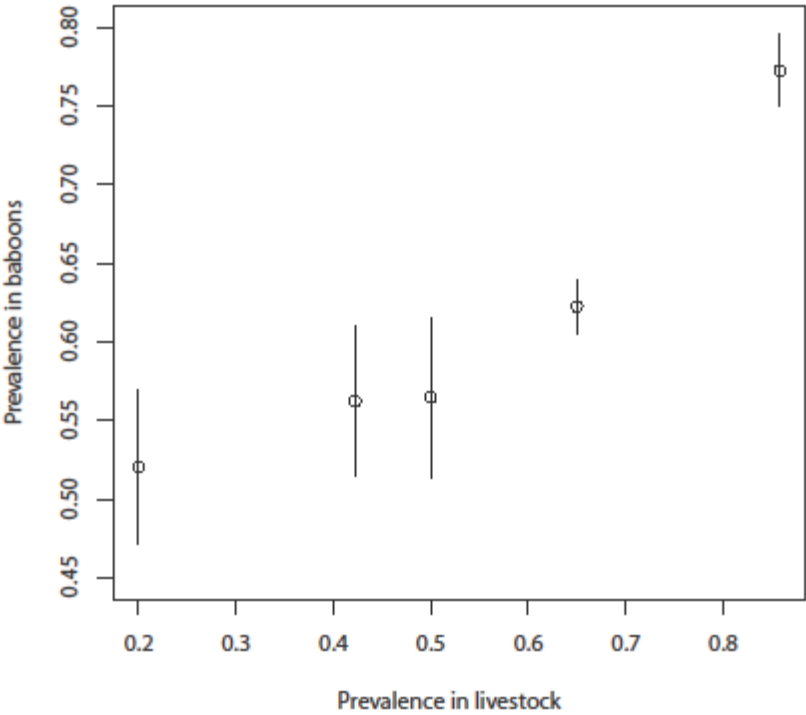
445 Figure 2: Average antibiotic-resistance prevalence by sampling source



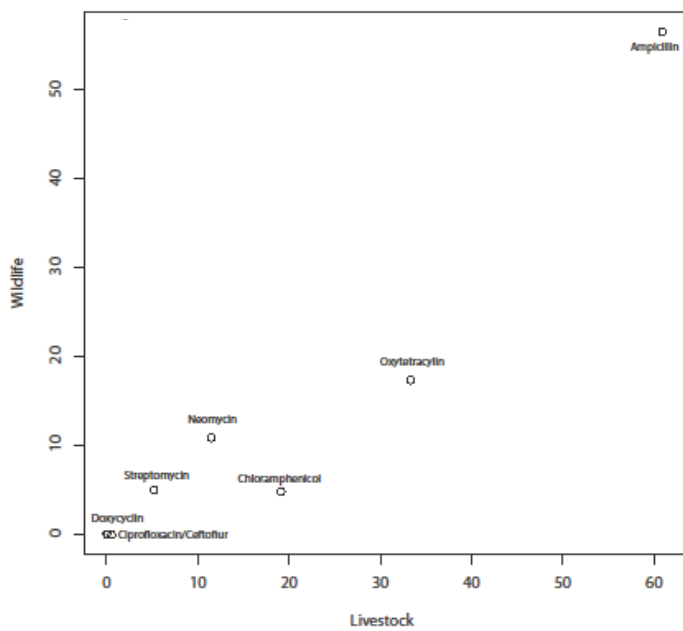
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Figure 3: Relationship of antibiotic-resistance prevalence between livestock and baboons across the five localities. Each point corresponds to a local area of sampling and represents the average antibiotic-resistance to at least one antibiotic. Vertical lines correspond to standard errors of this measure in baboons.



454 Figure 4: Relationship of the prevalence of resistance to each antibiotic between isolates from
455 livestock and baboons. Each point in the graph corresponds to one antibiotic and the
456 coordinates of each point represent the prevalence of resistance to that antibiotic among
457 isolates from livestock (x-axis) and baboons (y-axis).

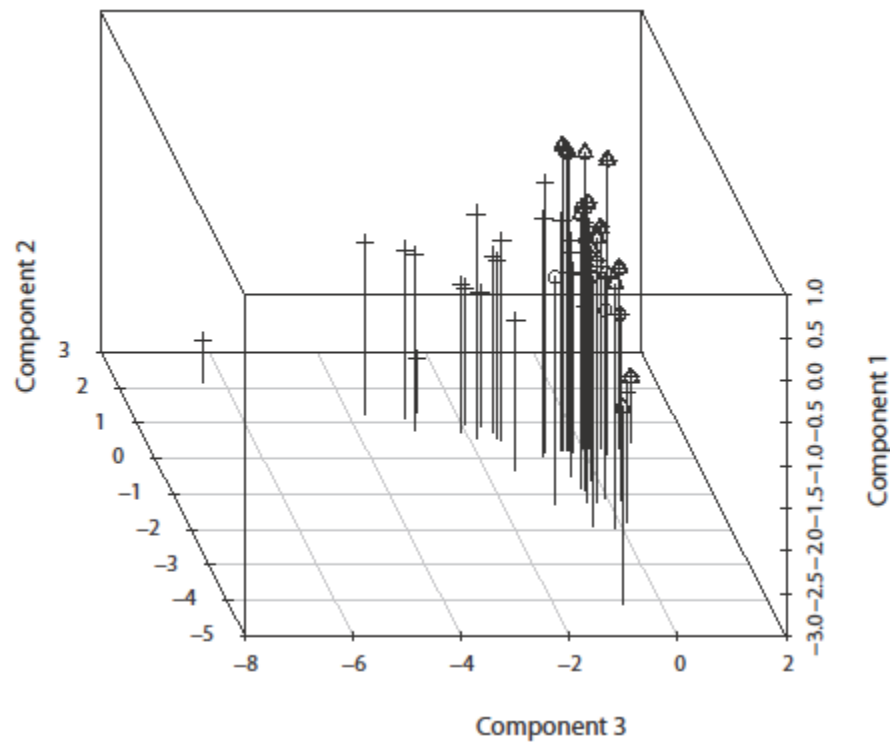


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SUPPLEMENTARY MATERIAL

Figure S1: Correspondence Analysis on resistant patterns. Each bacteria isolate from livestock (rounds), baboons (triangles) and water samples (crosses) is plotted using the value of the three principal components as its coordinates.



Article 5

Bushmeat hunting causes counterintuitive gradients of anti-biotic resistant *E. coli* prevalence in gorillas

Authors: Benavides JA, Escobar-Paramo P, Boyer D, Bodenham R, Godreuil S, Todd A, Raymond M, Walsh PD.

In preparation

Title: Bushmeat hunting causes counterintuitive gradients of anti-biotic resistant *E. coli* prevalence in gorillas

Authors: Benavides JA, Escobar-Paramo P, Boyer D, Bodenham R, Godreuil S, Todd A, Raymond M, Walsh P.

Abstract:

As pathogen spillover from humans and their domestic animals grows as a threat to endangered wildlife such as gorillas, gradients in pathogen prevalence are increasingly used as a “smoking gun” pointing to where spillover has occurred. The almost universal assumption is that pathogen prevalence in wildlife should decrease with increasing distance from the point of spillover. Here, we use a simple Lotka-Volterra predator-prey spatial model to show that, in the common case in which pathogen spillover intensity is correlated with bushmeat hunting intensity, this assumption can be false. If hunting decreases the density of wildlife and, therefore, pathogen transmission rates near the point of spillover, pathogen prevalence in wildlife can actually increase with increasing distance from the spillover point. We also extend the model to show that this conclusion holds even when human-originated antibiotic resistance puts bacterial that spillover from humans at a selective disadvantage in wildlife. Finally, we present data on antibiotic resistance in *E. coli* strains sampled from humans, gorillas and other wildlife in Dzanga-Ndoki Park (Central African Republic), where we show that human disease spillover potential decreases strongly along gradients in wildlife density created by bushmeat hunting. Not only does a strong correlation between humans and gorillas in the relative prevalence of nine antibiotic resistant strains and in the phylogenetic

25 group of antibiotic-resistant strains strongly support spillover from humans, prevalence of
26 resistant strains increases as the intensity of human activity decreases. Thus, these findings
27 support the role of hunting-induced density-dependence in the transmission of bacteria within
28 wild animal populations, along with the use of bacteria genetic data to discriminate between
29 human spillover and endemic circulation of antibiotic-resistant bacteria.
30

Introduction

Spillover of zoonotic pathogens such as the H1N1 and SARS viruses into humans has recently received a great deal of public attention (Garten et al., 2009; Li et al., 2005). What is less widely appreciated is how the encroachment of human populations into once remote areas has amplified the rate at which pathogens of humans and their domestic animals spillover into endangered wildlife (Daszak, 2000; Epstein & Price, 2009). For example, domestic cats are the origin of feline leukemia infections that threaten the survival of the critically endangered Iberian Lynx (Meli et al., 2009). Likewise, domestic dogs are reservoirs for both rabies spillover into critically endangered Ethiopian wolves (Randall et al., 2004) and African wild dogs (Lembo et al., 2007), and canine distemper spillover into a range of endangered felids and canids (Alexander & Appel, 1994; Cleaveland et al., 2000). Human pathogen spillover is a particular threat to the endangered great apes (Wolfe et al., 1998; Woodford, Butynski & Karesh, 2002). Just as their immune similarity to humans has made gorillas and chimpanzees the original reservoirs of leading public health scourges such as malaria and HIV into humans (Keele et al., 2006; Liu et al., 2010; Prugnolle et al., 2010), it also makes them particularly susceptible to human pathogens. In addition, apes live in the equatorial regions where human populations are most rapidly expanding, thereby, amplifying pathogen “rain”, particularly of fecally transmitted pathogens and parasites (Chapman, Gillespie & Goldberg, 2005; Goldberg et al., 2007; Nizeyi et al., 2001; Rwego et al., 2008). Furthermore, human respiratory virus spillover is the leading cause of ape death in the tourism and research programs that represent the best hope for protecting wild apes from other threats (Köndgen et al., 2008; Palacios et al., 2011).

One reason that the magnitude of the disease threat is only now being fully appreciated is that blood and other diagnostic tissues have been notoriously difficult to collect from free ranging wildlife (Leendertz et al., 2006). Many human pathogens also have distinct but closely related forms circulating independently in wild primates (Davies & Pedersen, 2008; Pedersen et al., 2005). Thus, even when diagnostic material is available there is often debate as to whether the origin of infection is truly human spillover or just enzootic circulation. Non-invasive sampling methods and molecular phylogenetic analyses are gradually resolving these diagnostic problems (Archie, Luikart & Ezenwa, 2009; Beja-Pereira et al., 2009; Garten et al., 2009; Johnston et al., 2010; Leendertz et al., 2006). However, they are as yet only available for a handful of pathogens (Archie et al., 2009). They also entail a high cost and level of technical sophistication to deal with issues such as the erroneous phylogenetic inferences that can occur when multiple strains co-infect a single host (Kirsanovs et al., 2010; Razzauti et al., 2009). These costs and technical requirements put them beyond the current reach of many management authorities, particularly in developing countries where financial resources and technical capacity are notoriously limited. Consequently, the most common metric used to infer human pathogen spillover into wildlife is still a positive correlation between the intensity of human activity and pathogen prevalence in wildlife, with prevalence estimated using clinical observations or relatively rudimentary laboratory diagnostics. This is often expressed spatially in terms of a decreasing gradient in wildlife disease prevalence with increasing distance from hotspots of human activity (Acosta-Jamett et al., 2011; Bradley & Altizer, 2007; Goldberg et al., 2007; Gortázar et al., 2008; Otterstatter & Thomson, 2008).

Our purpose here is to suggest that this rule of thumb may often be misleading, particularly in tropical areas where bushmeat hunting consistently creates gradient in wildlife density that directly oppose patterns of disease spillover from humans (Blom et al., 2005;

Kuehl et al., 2009; Walsh et al., 2003a). To illustrate how the affects of such wildlife density gradients on disease transmission rates can produce counterintuitive gradients in disease prevalence, we first present a spatially distributed Lotka-Volterra model in which pathogen particles are the prey and immune antibodies are the predators. The model is intentionally oversimplified to more clearly convey how a balance between the intensity of immune response in individuals and the rate of transmission of pathogens between individuals determines what spatial pattern of pathogen prevalence will emerge. We also extend the model to show how two human pathogen strains can persistently co-circulate in wildlife, even when one strain is competitively inferior, and that this strain can reach highest prevalence in the area of least intense human disease spillover.

To illustrate that these ideas have real value for understanding the dynamics of human disease spillover, we present data on the prevalence of antibiotic-resistant *Escherichia coli* strains isolated in fecal samples from critically endangered western gorillas and other wildlife in and around the Dzanga-Ndoki National Park of Central African Republic. Antibiotic-resistant bacteria in wildlife including apes is typically assumed to be the result of bacterial transmission from humans or domestic animals (Goldberg et al., 2007; Guenther, Ewers & Wieler, 2011; Literak et al., 2010; Rolland et al., 1985; Rwego et al., 2008; Skurnik et al., 2006). Furthermore, antibiotic resistance may also represent an ape conservation problem because in gorilla groups habituated for tourism or research, antibiotics can be used to treat apes against both primary bacterial infections (Hastings & B, 1991; Ryan & Walsh, 2011). Because the genetic material conferring antibiotic resistance can be transmitted between unrelated bacterial strains (Courvalin, 1994; de la Cruz & Davies, 2000; Ochman, Lawrence & Groisman, 2000), the circulation of resistance material can become decoupled from that of the bacterial strains in which they were originally transmitted (e.g. from humans to wildlife). Thus, the study of antibiotic-resistant bacterial transmission represents a particularly complex

challenge in distinguishing whether antibiotic resistant strains originated from human spillover or from endemic circulation. This has led to a lingering debate about whether antibiotic resistance observed in wildlife populations is really the consequence of spillover from humans or the result of local environmental selection pressures that maintain similar genetic material in both humans and wildlife in a particular area (Allen et al., 2010; Goldberg et al., 2007; Guenther et al., 2011). Here we illustrate how the relative distribution of resistance and the genetic background of bacterial strains can be used to disentangle this issue.

MATERIAL AND METHODS

THE MODEL

One strain model

To represent the dynamics of parasite infection within each host individual we use a Lotka-Volterra model in which the immune system is the predator and parasites the prey. In this model the abundance of parasites S within a given host located at a lattice of (i,j) coordinates follows the dynamics:

$$\frac{dS^{(i,j)}}{dt} = g \times S^{(i,j)} \times \left(1 - \frac{S^{(i,j)}}{K}\right) - a \times A^{(i,j)} \times S^{(i,j)} + S^{(i-1,j)} \times \beta^{(i-1,j)} + S^{(i+1,j)} \times \beta^{(i+1,j)} + S^{(i,j-1)} \times \beta^{(i,j-1)} + S^{(i,j+1)} \times \beta^{(i,j+1)}$$

where g is the per-capita growth rate of the parasite, K is the parasite carrying capacity within the host and the parasite mortality rate, a , is proportional to A , the concentration of parasite-specific antibodies in the host. Antibody response to infection follows the dynamics:

130
$$\frac{dA^{(i,j)}}{dt} = r \times A^{(i,j)} \times S^{(i,j)} - \mu \times A^{(i,j)}$$

131 with antibody production increasing at a rate, r , proportional to parasite abundance and
 132 antibody concentration decaying at rate μ . In order to make the model more intuitively
 133 accessible we intentionally ignore innate and cell-mediated immunity. Adding these forms of
 134 immunity changes the model's quantitative dynamics but not its qualitative behavior.

135 To represent parasite transmission we place one host each at node of a 51 x 51
 136 square lattice and assume that parasites excreted by one host are consumed by its four nearest
 137 neighbors at a rate, β , proportional to parasite abundance of the source host. At the lattice
 138 boundary, we impose absorbing boundary conditions to mimic the finite nature of most
 139 habitat blocks containing endangered wildlife.

140

141 **Bushmeat hunting effect**

142

143 We initially assume that the rates of transmission parasite transmission between neighbors do
 144 not vary across the lattice (*i.e.* $\beta^{(i,j)} = \text{constant}$). To represent the effect of bushmeat hunting on
 145 host densities, we make the transmission rate β linearly proportional to distance from the
 146 lattice edge.

147

148 **Two-strain model: Antibiotic Resistance**

149

150 The consequences of antibiotic resistance on parasite load can be illustrated by modifying the
 151 equations to represent competition between a wild type strain (S_1) with growth rate g_1 and a
 152 second strain (S_2), for which the production of antibiotic resistance slows growth to some
 153 lower rate g_2 (*i.e.* cost of resistance: (Andersson, 2006). Thus, in this two-strain model, the

abundance of parasites S_1 and S_2 within a given host located at a lattice of (i,j) coordinates

follows the dynamics:

$$\begin{aligned}\frac{dS_1^{(i,j)}}{dt} &= g_1 \times S_1^{(i,j)} \times \left(1 - \left(\frac{S_1^{(i,j)} + \alpha_{21} \times S_2^{(i,j)}}{K} \right) \right) - a \times A_1^{(i,j)} \times S_1^{(i,j)} + S_1^{(i-1,j)} \times \beta^{(i-1,j)} + S_1^{(i+1,j)} \times \beta^{(i+1,j)} + S_1^{(i,j-1)} \times \beta^{(i,j-1)} + S_1^{(i,j+1)} \times \beta^{(i,j+1)} \\ \frac{dS_2^{(i,j)}}{dt} &= g_2 \times S_2^{(i,j)} \times \left(1 - \left(\frac{S_2^{(i,j)} + \alpha_{12} \times S_1^{(i,j)}}{K} \right) \right) - a \times A_2^{(i,j)} \times S_2^{(i,j)} + S_2^{(i-1,j)} \times \beta^{(i-1,j)} + S_2^{(i+1,j)} \times \beta^{(i+1,j)} + S_2^{(i,j-1)} \times \beta^{(i,j-1)} + S_2^{(i,j+1)} \times \beta^{(i,j+1)}\end{aligned}$$

.

The competition coefficients α are taken to be equal to the relative growth rates of the two strains (*e.g.*, $\alpha_{21} = g_2/g_1$).

EMPIRICAL METHODS

Samples collection

Fresh faecal samples were collected from November 2009 to January 2010 inside and outside the boundaries of the Dzanga-Ndoki National Park in south-west Central African Republic (Fig. 3). A total of 112 gorilla fecal samples were collected from gorillas in sixteen wild spcoa; groups, including two groups habituated to the approach of humans. All samples were collected from nests build by gorillas the night before the sampling day (*i.e.* samples collected less than 12 hours after deposition). A total of 68 samples from other wild animals were also collected opportunistically, including one De Brazza's monkey (*Cercopithecus neglectus*) and one unidentified monkey, 18 agile mangabeys (*Cercocebus agilis*) from an habituated group, 14 duikers of several species, 1 bongo (*Tragelaphus euryceros*), 5 river hogs (*Potamochoerus porcus*), 11 forest buffalos (*Syncerus caffer nanus*), and 11 African elephants (*Loxodonta cyclotis*), 4 leopards (*Panthera pardus*), and one unidentified turtle. Fecal Samples were identified to species by BaAka pygmy trackers, using morphological

characteristics of feces and foot prints. Approximately 2 g of fecal material sample was collected from the interior of the fresh fecal stool and placed in a 1.5 ml Eppendorf tube containing 1 ml of sterile and isotonic buffered solution composed of NaCl at 0.9%.

Sixteen samples from human volunteers working in the WWF gorilla habituation program were collected. All volunteers lived in Bayanga and were fully aware of the study goals before giving their oral consent. Collection of WWF staff was approved by the organisation and the park administration, as well as in research permits granted by the Ministry of Sciences and Technology of Central African Republic. Humans were given a sterile plastic bag and were asked to put a small amount of faecal swab on it using sterile gloves and/or a sterile tooth tick provided. Bags containing samples were recovered the next day and transferred to an Eppendorf tube containing the buffer solution. None of the samples presented signs of diarrhoea.

Bacterial isolation

Bacteria from fecal samples were isolated in MacConkey (MC) selective media following the methods described in Quinn (1994) as soon as possible after collection (maximum one week). Previous studies have shown that short-term storage of samples (up to 2 weeks) does not affect the phylogenetic structure, abundance and diversity of microbial communities. Each fecal sample was streaked on a MacConkey (MC) agar plate (VWR) and incubated at 37°C for 24-48 hours. Five isolated pink lactose positive colonies, visually resembling *E. coli*, were transferred into a 200 µl 96 wells microtiter plate containing MC agar. Microtiter plates were then kept at 4°C and shipped to France for further analyses. Once in France, colonies were re-isolated and identified by biochemical testing (API 20E gallery or bioMerieux Vitek). Only one identified *E. coli* isolated per fecal sample was saved in 20% glycerol and stored at -80°C for further analysis.

201 **Analyses**

202 *Antibiotic resistance*

203 Antibiotic susceptibility testing was performed by the agar dilution method (Clinical and
204 Laboratory Standards Institute, 2006). Twofold dilutions of the antibiotics were added in
205 order to obtain the final concentrations superior to critical concentration according to
206 breakpoints provided to National Committee for Clinical Laboratory Standards (NCCLS)
207 guidelines. The antibiotics and dosage levels used were ampicilin ($> 8 \mu\text{g/ml}$), ceftiofur (> 8
208 $\mu\text{g/ml}$), ciprofloxacin ($> 2 \mu\text{g/ml}$), tetracycline ($> 8 \mu\text{g/ml}$), chloramphenicol ($> 8 \mu\text{g/ml}$),
209 doxycycline ($> 8 \mu\text{g/ml}$), neomycin ($> 4 \mu\text{g/ml}$), streptomycin ($> 16 \mu\text{g/ml}$) and
210 sulfamethoxazole ($> 256 \mu\text{g/ml}$). Inocula were prepared in saline to achieve 10^8 cfu/mL (0.5
211 McFarland standard), then diluted 1 in 10 using sterile saline. A multipoint inoculator was
212 used to dispense 1 μL of diluted inoculum to achieve 10^4 cfu/mL per plate with and without
213 antibiotics. Plates were incubated in air at 37°C for 18 h. A fully susceptible *E. coli* (ATCC
214 25922) was used as controls. An isolate was considered resistant if it grew in the presence of
215 the antibiotic and formed a homogenized colony similar to a control colony grown in medium
216 without antibiotic. A replicate was systematically run to confirm each resistance case. To
217 evaluate the reproducibility with the method, independent tests were performed for *E. coli*
218 ATCC 25922, and 5 clinical strains (two tests each).

219 *E. coli* phylogenetic groups

220 Each *E. coli* isolate was assigned to one of the four major phylogenetic groups (A, B1, B2
221 and D) (Lecointre et al., 1998), using a multiplex PCR assay which creates the simultaneous
222 amplification of the *chuA* and *yjaA* genes and a DNA fragment TSPE4.C2 (Clermont,
223 Bonacorsi & Bingen, 2000). This method has been widely used for the genetic description of

different *E. coli* populations given its rapidity and simplicity (Clermont et al., 2000; Escobar-Páramo et al., 2004; Tenaillon et al., 2010), and the equivalence to other more sophisticated methods such as multilocus sequence typing (Gordon et al., 2008). Primers used in this study were those from Clermont et al. (2000).

Spatial analyses

Distance to the closest park boundary for each gorilla group sampled was calculated using ArcGIS X (ESRI), with a map provided by WWF. Only boundaries inside Central Africa were considered, because the park extends on both Cameroon and Congo side. Data on density of gorilla nests per square kilometre was provided by the Convention on International Trade in Endangered Species program for the Monitoring of Illegal Killing of Elephants (CITES, 2007). All statistical analyses were performed in R 2.13.1 (R Development Core Team, 2003). Generalized Linear Mixed Models (GLMMs) were implemented using the lme4 package (Bates & Sarkar, 2007)

RESULTS

Model predictions

What patterns of infection emerge from this system depends on what assumptions we make about the spatial distribution of parasite spillover from humans. Clearly, if we set parasite spillover rate to a level that decreases from high to low as one moves from lattice edge to center, then the intuitive positive correlation between spillover rate and parasite load should emerge. The dynamics change, however, if we concentrate spillover at the edge of the lattice, say at the four corners. In this case three different parasite distributions emerge from the model. First, if parasite growth rate is low relative to the regulatory strength of immunity

(combined effects of the parasite mortality rate, α , and the antibody growth rate, μ), then the effect of spillover on parasite distribution is appreciable only at the point of spillover (Fig 1A). Throughout the rest of the system equilibrium antibodies suppresses parasites to a load close to zero (Fig. 1). Second, if parasite growth rate is high relative to the regulatory strength of immunity, then the parasite will persist at high load within the entire system (Fig 1B). In this case, the additional effect of spillover on parasite distribution will not be appreciable at the point of spillover if the spillover rate, illustrated here as a constant parasite load of fifty, is of a lower order than the parasite growth rate (Fig. 1B). Because the load for each host is a balance between the positive effects of parasite growth and transmission from neighbors and the negative effects of the immune regulation, lowering transmission rate at the edge of the lattice lowers the equilibrium load in the model (Fig. 1C). Thus, parasite load actually increases as one moves into the lattice and away from the point of spillover.

The two-strain model produces qualitatively similar dynamics to the single species model, including the counterintuitive positive correlation between parasite load and distance from the point of spillover (Fig 2). First, if antibody immunity response is low but parasite growth rate is high, the wild strain will exclude the antibiotic resistant-strain from the system because its growth rate is higher and the antibiotic-resistant strain will be suppressed by the host immune system (Fig. 2A). But the important concept illustrated by this model is that strain-specific immune regulation of the wild type strain below its carrying capacity creates “ecological space” for the antibiotic resistant strain. Consequently, when both the antibody response and parasite growth rates are high, the antibiotic resistant strain can circulate persistently in the host population even though it is competitively inferior to the wild type in terms of growth rate (Fig. 2B). In this case, the hunting effect generates the same counterintuitive gradient in load for both strains, although at a lower level in the antibiotic-resistant one (Fig. 2C).

Empirical results

Are humans spreading antibiotic-resistant *E. coli* into wild populations?

The prevalence of bacteria resistant to at least one antibiotic was significantly higher in humans (43.74 %) than in gorillas (15.52%, Proportion test, X-squared= 5.58, $P = 0.01$) and other wildlife (11.76%, Proportion test, X-squared = 6.98, $P < 0.01$). Furthermore, habituated and non-habituated gorillas did not differ in the prevalence of resistance to at least one antibiotic (Proportion test, X-squared=0.03, $P = 0.84$), although sample sizes were small and unbalanced (21 habituated, 91 unhabituated). Among resistant isolates, bacteria were resistant to an average ($\pm$ SD) of 3.15 ± 1.77 antibiotics per isolate in humans, 1.33 ± 0.59 in gorillas and 1.75 ± 0.71 in other wildlife. The highest levels of antibiotic resistant were observed for locally available Amoxicillin (93% of resistant isolates) and Streptomycin (80%). The prevalence of specific resistance to the nine antibiotics tested was correlated between humans and gorillas (Spearman correlation test, $\rho = 0.76$, $P = 0.01$). Prevalence in other wildlife, however, were not correlated with prevalence in either gorillas (Spearman, $\rho = 0.62$, $P > 0.05$) or humans (Spearman, $\rho = 0.39$, $P > 0.05$), although the relatively small sample size for other wildlife provide little statistical power.

The distribution of *E. coli* genetic groups among non-resistant isolates was significantly different in humans than in gorillas (2x4 Fisher exact test, $P = 0.02$) and between gorillas and other wildlife (2x4 Fisher exact test, $P < 0.01$) (Fig. 3). However, among resistant isolates, there was no significant difference in the distribution of *E. coli* genetic groups: either between humans and gorillas (2x4 Fisher exact test, $P = 0.82$) or between gorillas and other wildlife (2x4 Fisher exact test, $P = 0.71$).

299

300 **Distance to park boundaries and multi-resistance**

301

302 The average number of antibiotics to which an isolate was resistant (*i.e.* multiresistance)
303 within each gorilla group was positively correlated with the distance of the sampling location
304 to the boundaries of the park (Spearman correlation test, $\rho = 0.54$, $P = 0.03$, Fig. 4). This
305 result was also obtained at the level of individual gorillas (Poisson GLMM including “group
306 identity” as a random effect and distance to park boundary as a fixed effect, $z\text{-value}=2.18$, $P =$
307 0.02). In fact, no isolate expressing antibiotic-resistance was observed outside the borders of
308 the park (0 out of 19 isolates versus 18 out of 93 inside the park). Gorilla density, expressed
309 as the number of nests per kilometre square, was also positively correlated with distance to
310 park boundary (Spearman correlation te
311 st, $\rho = 0.26$, $P = 0.02$, Fig. 4).

312

313 **DISCUSSION**

314

315 The decrease of parasite prevalence in wildlife hosts with increasing distance to
316 human activity has been often used as evidence for human or domestic animal’s parasite
317 spillover (Acosta-Jamett et al., 2011; Bradley & Altizer, 2007; Otterstatter & Thomson,
318 2008). Here, we explored how bushmeat hunting may influence gradients in the prevalence of
319 human spillover parasites in hunted wildlife species. Our model illustrates that by creating
320 gradients in wildlife host density and transmission rate that directly oppose gradients in
321 human activity, bushmeat hunting has the potential to produce prevalence gradients opposite
322 that of the intuitive expectation, with higher parasite actually *increasing* with distance to the
323 point of spillover. Because hunting is a major driver of wildlife density in Africa (Barnes,

2002; Bowen-Jones & Pendry, 1999; Caro, 1999; Setsaas et al., 2007; Walsh et al., 2003b), our results call for a reassessment of whether human spillover is the source of wildlife infection in populations where wildlife infection prevalence is not positively correlated with levels of human activity. In our model, the shape of prevalence gradients depends on the ratio of inter-individual transmission and the efficiency of the immune response. Thus, we expect that the existence of an unexpected-gradient in parasite prevalence may be particularly relevant to parasites with a long infectious period or parasites that can rapidly increase within hosts before triggering strong immune responses. This is probably the case of most bacteria that proliferate within a host for several days (Grant et al., 2008; Jiang et al., 2000; Levin & Antia, 2001).

Our results also have implications for the growing debate on whether antibiotic-resistant in wild animals is the result of human spillover or the consequence of environmental selection for antibiotic-resistant strains (Allen et al., 2010; Guenther et al., 2011). Our model predicts that antibiotic-resistant bacteria can persist within wildlife populations long after spillover from humans, even if resistance has a cost in terms of parasite population growth rate. It also predicts that the prevalence of even antibiotic-resistant strains in wildlife may, at times, be negatively correlated with levels of human activity. Our data on antibiotic resistance in Central African gorillas show exactly such a negative correlation with prevalence increasing as one moves into remote, high gorilla density areas. This result bears on the human spillover versus environmental selection debate because of our parallel findings on bacterial genotype frequencies. The environmental selection hypothesis assumes that the bacterial population infecting humans is entirely isolated from that of gorillas, with bacterial genotypes in each population experience regimes of selection, drift, and transmission particular to the unique diets, abundances, and other attributes of their respective host species. Thus, even if environmental conditions select independently for the same profile of resistance

material in gorillas and humans, there is no reason to expect that bacterial genotype frequencies should be correlated between humans and gorillas. Such a lack of correlation in genotype frequency is exactly what we observe when we compare human samples to only those gorilla samples that show no resistance. However, bacterial genotype frequencies in gorilla samples that show antibiotic resistance *are* correlated with bacterial genotype frequencies in human samples. This suggests that the resistance patterns observed in gorillas samples are not the result of a long selection process independent of that affecting human parasites. Rather, it strongly implies recent spillover from humans, with rates of antibiotic-resistant bacteria transfer inadequate to homogenize the distribution of antibiotic prevalence within the gorilla population. Also consistent with the conclusion on an anecdotal level, are the high prevalence in gorilla samples of resistance to antibiotics commonly used in the local human population (e.g. ampicillin) and the absence to resistance to antibiotics not used locally (e.g. cefiofur). Although our gorilla dataset is probably the largest sample size ever collected for the study of antibiotic-resistance in African wildlife, a larger sample size would provide more confidence in the robustness of this result.

The deterministic nature and simplicity of our model aimed to show how within- and between-host mechanisms can affect the dynamics and distribution of a parasite at the population level. For example, the two-strain model predicts that a high immune response allows a strain with a lower competitiveness to circulate in the population, because the other strain with a higher growth rate is more suppressed by the immune system. As other levels of complexity are added to this model, other interesting phenomena may emerge. For example, stochasticity in between-host transmission could create areas of low animal density where parasite will go extinct and other areas where transmission rates are high enough to maintain endemic circulation of a parasite. In this case, the effects of hunting gradients could be reflected in a non-linear pattern of parasite distribution. Furthermore, a combination of a high

antibody decay rate and a non-linear immune response to parasite load could generate a re-infection dynamics in the population where the co-existence of strains might be a dynamic process with hosts been recurrently re-infected by one or the other strain.

The pertinence of our model to explain the dynamics of *E. coli* transmission and our empirical results depend in at least two major major assumptions of our model. First, we assumed that after introduction, bacteria will growth within the host before its suppression by the immune system. We can call this the “novelty advantage”, delaying the clearance of bacteria in the system if those bacteria are transmitted into a new host before being entirely cleared by the immune system. This assumption is supported by models describing the dynamics of bacteria after introduction within the host (Levin & Antia, 2001). Second, we assumed that different strains of *E. coli* will activate different immune response and that antibody response is proportional to bacterial load. Recent empirical experiment supports the existence of adaptive immunity for commensal bacteria such as *E. coli* and the proportional activation of the immune system depending on the bacterial load (Hapfelmeier et al., 2010; Slack et al., 2009).

The model reveals the importance of density-dependent transmission in the dynamics of a pathogen introduced into wild populations. The effect of host density on disease transmission is one of the fundamental principles of epidemiology in humans, but remains an ongoing debate on wildlife epidemiology (Lloyd-Smith et al., 2005). In particular, empirical data confirming the effects of disease density-dependence in wildlife populations remains scarce (Cross et al., 2010; Lloyd-Smith et al., 2005). Temporal increases of host density have been proposed as a driver of disease increase in systems such as brucellosis in elk (Cross et al., 2010). In this study, we suggest that the spatial distribution of animal density within the landscape can also greatly affect the dynamics and spatial distribution of a pathogen. Other mechanisms shaping the density of wild animals, such as the distribution of food patches in

399 the landscape, may also greatly affect the dynamics of a disease in wild populations. Finally,
400 although we assumed in our model that gorilla density was directly correlated to bacteria
401 transmission rates between gorillas groups, the mechanism underlying this link remains to be
402 determined.

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FIGURES

Figure 1. One-strain model predictions for the distribution of parasite loads. Each host is located within a 51x51 lattice with coordinates (i,j) . The parasite load within each host is plotted under three different scenarios of the model: **A)** High immunity response ($r=0.01$; $a=0.1$) and no gradient in transmission rates ($\beta =0.09$), **B)** Low immunity response($r=0.01$; $a=0.006$) and no gradient in transmission rates and **C)** Low immunity ($r=0.01$; $a=0.006$) and a gradient in transmission rate. The transmission rates was assumed increase linearly from the edge to the centre of the lattice going from $\beta =0.001$ to 0.09. Hosts at each corner of the lattice had a constant parasite load of $S=50$, representing a constant introduction from humans. Other parameter values for all scenarios were $K=100$, $g_1=0.9$ and $\mu=0.01$. Simulations were run until equilibrium was reached.

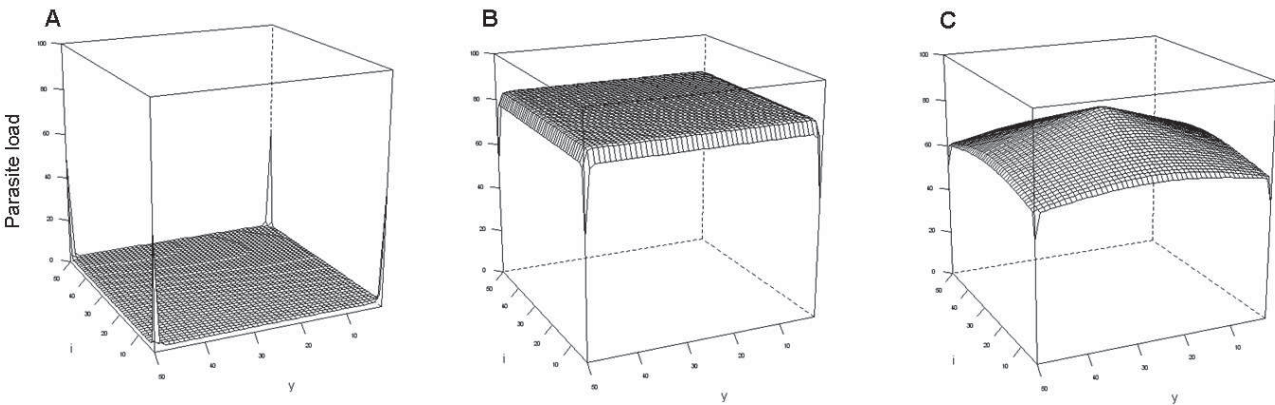
Figure 2. Two-strain model predictions for the distribution of parasite loads. The parasite load of strain S1 (light grey) and S2 (dark grey) within each host are plotted under three different scenarios of the model: **A)** Low immunity response ($r=0.01$; $a=0.001$) and no gradient in transmission rates ($\beta =0.09$), **B)** High immunity response ($r=0.01$; $a=0.006$) and no gradient in transmission rates and **C)** High immunity ($r=0.01$; $a=0.006$) and a gradient in transmission rate. Transmission rates increased linearly from to the edge of the lattice to the centre going from $\beta =0.001$ to 0.09. Hosts at each corner of the lattice had a constant parasite load of $S=50$, simulating a constant introduction from humans. Other parameter values for all scenarios were $K=100$, $g_1=0.8$, $g_2=0.6$ and $\mu=0.01$.

Figure 3. Prevalence of *E. coli* phylogenetic groups in resistant and non-resistant isolates for each host group. The percentage of *E. coli* isolates belonging to each of the four

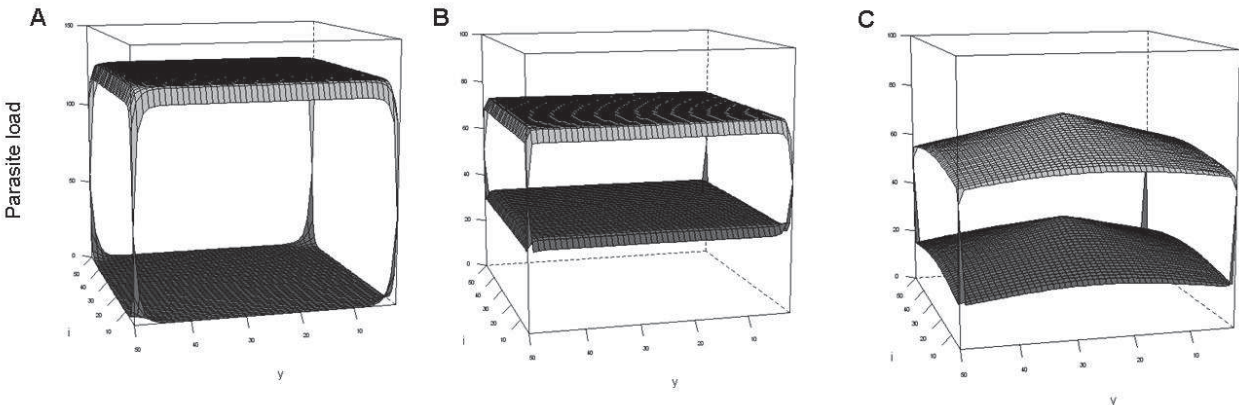
phylogenetic groups (A, B1, B2, D) is shown for resistant (Top) and non-resistant (Bottom) isolates among gorillas, wildlife and humans.

Figure 4. Relationship between distance to park boundary, gorilla density and multiresistance. Left plot: Estimates of gorilla nests/km² from MIKE data are plotted as a function of the distance of each sample to the closest park boundary. The plotted line shows the predicted values of gorilla nests nests/km² from a linear model including distance to park boundary as the explanatory variable. Right plot: The location of each gorilla group sampled in this study is illustrated on the top-left hand corner. The grey surface in the map corresponds to the park area. The graph shows the relationship between the mean number of multiresistance (i.e. number of antibiotics for which an isolate is resistant to) per gorilla group and the distance of that group to the closest park boundary. The plotted line shows the predicted values of mean multiresistance per group from a linear model including distance to park boundary as the explanatory variable.

681 Figure 1
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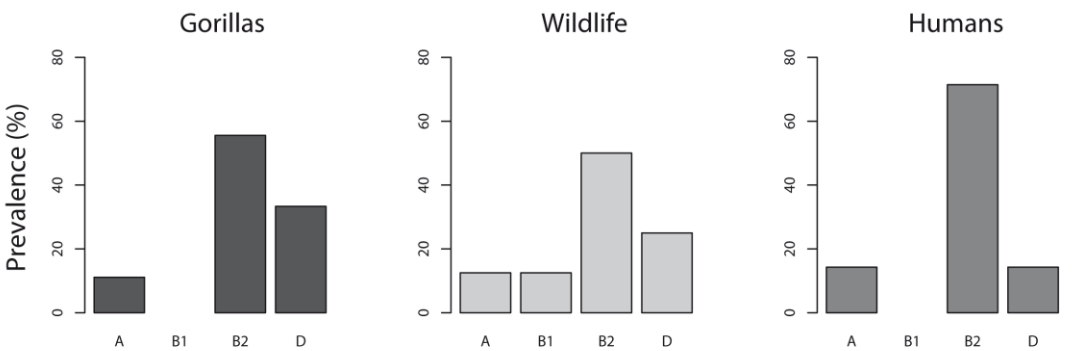
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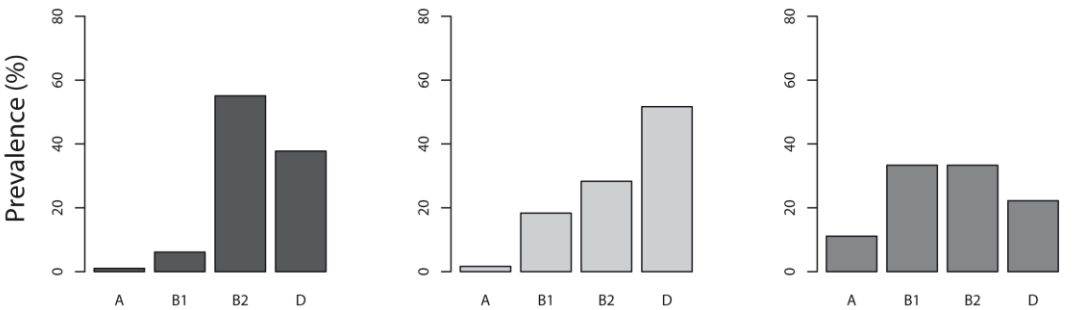
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689 Figure 3
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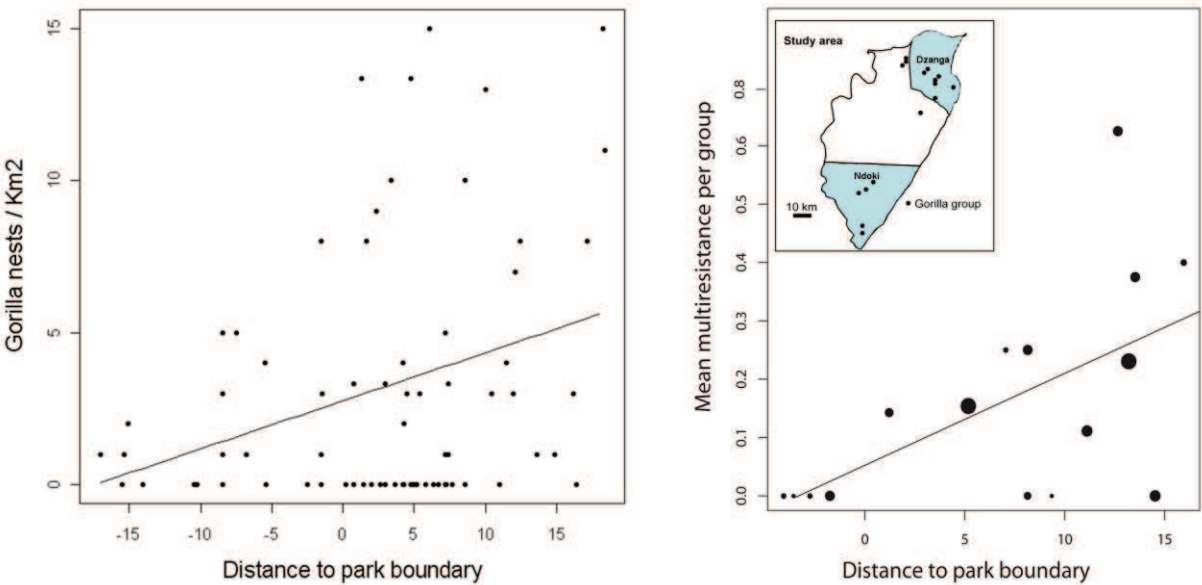
Resistant



Non-resistant



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694 Figure 4
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Résumé

Comprendre l'émergence et la propagation des maladies infectieuses chez les animaux sauvages est devenue une priorité en santé publique et en conservation. En combinant la collecte de données et le développement de modèles épidémiologiques, cette thèse s'est focalisée sur la compréhension de deux phénomènes clés: (i) étudier comment l'hétérogénéité au niveau des individus, des groupes, de la population et de l'environnement influence la propagation de parasites et (ii) quantifier la transmission de bactéries résistantes aux antibiotiques depuis l'homme vers les animaux sauvages dans trois aires protégées d'Afrique (Tsaobis NP- Namibie, Lopé NP-Gabon et Dzanga-Ndoki NP- République Centrafricaine). Les principaux résultats de ce travail montrent que : (1) De multiples facteurs incluant la température, la pluie, l'utilisation du territoire, le genre, l'âge et la condition physique influencent la richesse spécifique de parasites gastro-intestinaux chez le babouin chacma, (2) Les contacts entre animaux autour des points d'attractions de l'habitat peuvent influencer de manière importante la propagation spatio-temporelle d'une maladie, (3) La transmission d'entérobactéries résistantes semble avoir lieu depuis les humains ou le bétail vers les animaux sauvages dans des zones où le contact entre ces populations est élevé, (4) Le gradient de densité de gorilles produit par la chasse peut générer une augmentation de prévalence d'un parasite avec la distance au point d'introduction. Les conclusions de ce travail ouvrent de nouvelles possibilités pour l'étude des maladies émergentes chez les animaux sauvages.

Mots clés: Ecologie de la transmission, résistance aux antibiotiques, modèles épidémiologiques, gorilles de plaines de l'ouest, babouins chacma, *Escherichia coli*

Title : Disease dynamics in complex social systems: the emergence of infectious diseases in primates

Abstract

Understanding the emergence and spread of infectious disease in wild animal populations has become an important priority for both public health and animal conservation. Combining the collection of empirical data with the development of epidemiological models, this thesis focuses on understanding two key issues of wildlife epidemiology: (i) how heterogeneity at the individual, group, population and landscape level affects parasite spread (ii) investigating whether transmission of antibiotic resistant bacteria from humans to wildlife is occurring within three protected areas of Africa (Tsaobis NP-Namibia, Lope NP-Gabon and Dzanga-Ndoki NP-Central African Republic). The main findings of this work indicated that: (1) multiple-scale factors including temperature, rainfall, home range use, sex, age and body condition influence gastro-intestinal parasite richness among wild baboons; (2) animal contacts around 'habitat hotspots' can substantially influence the spatio-temporal dynamics of a disease; (3) antibiotic resistant enterobacteria seem to be spreading from humans/livestock to wildlife when the territory overlap between these two populations is expected to be high; (4) gradients in gorilla density created by bushmeat hunting can reverse the expected pattern of decreasing parasite prevalence with distance to human-spillover. The conclusions of this work open new possibilities for studying the mechanisms explaining the spread of emerging infectious diseases among wild animals.

Key words: wildlife epidemiology; disease ecology; antibiotic resistance; epidemiological-models; western lowland gorillas; chacma baboons; *Escherichia coli*