EFFECT OF LANDSCAPE FRAGMENTATION ON BAT POPULATION DYNAMICS AND DISEASE PERSISTENCE IN URUGUAY

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Immunology and Infectious Diseases

MONTANA STATE UNIVERSITY
Bozeman, Montana

January 2021

DEDICATION

To my students, former current and future, this journey was all about you.

ACKNOWLEDGEMENTS

This work would not have been possible without the help, support, and encouragement I received from many different people. Several institutions provided support for this process: Fulbright Commission in Uruguay together with the National Agency for Research and Innovation granted the funds for the first two years. The Universidad de la República allowed me to keep my position and even advance my career during the PhD, providing a come back to. The IIBCE welcomed me during the last semester of the dissertation and provided ample work flexibility to allow me to finish the PhD. The America Society of Mammalogists and Bat Conservation International provided additional scholarships for this work. My advisors and mentors during my whole career have played a big role in shaping mi work: Enrique, Juan, Eduardo, Álvaro, Carolina, Adriana, and Sandra put me in the way to be able to face this challenge, and during it Raina decided to support a weird project not directly related to the lab's main work. My family has been an incredible support and inspiration for my career. From my parents to my siblings and cousins, to my uncle whose career in tropical diseases has been a beacon. Lots of friends gifted their time and affection and I not always paid them back fairly. Among them, Guillermo, Gonzalo, and Devin stand out. I left many people outside this page, but not away from from my thoughts. Two incredibly special people played a huge role in my transit through the PhD: Majo and Pau were my partners-in-crime at different stages of this adventure and without their encouragement and support this would have been a much lonelier experience. I will be forever grateful for the time and adventures I shared with each of them.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

The transmission of pathogens into novel host species, a process known as spillover, requires a series of conditions to align in space and time. A series of imperfect barriers prevent the jump of pathogens from one species to others. These may include the distribution and abundance of the primary host, the survival of the pathogen in the environment and the susceptibility of the recipient host to the pathogen. Only when permissive conditions align in time and space can the spillover occur. Spillovers may be relatively rare events and the understanding of the dynamics of the barriers is constrained by the ability of detecting and analyzing such events. Systems where spillover does not occur, despite apparent presence of all required conditions, provide an opportunity to understand barriers preventing inter-species transmission. Vampire bat-borne rabies in Uruguay provides such an opportunity. Despite large and stable livestock density, presence of vampire bats, and circulation of the virus in close proximity, the country did not experience livestock rabies outbreaks until 2007. Here we combined historical review, field sampling, and statistical and mathematical modeling to understand the factors driving the emergence of rabies in Uruguay in 2007 and the previous absence of the disease. Our results suggest that rabies outbreaks in the country are spatially and temporally associated with fragmentation of grasslands. We showed that proposed increased connectivity among colonies, in response to fragmentation, is sufficient to explain longer persistence of the virus in the bat colonies, allowing more opportunities for virus transmission to livestock. We showed that connectivity has a strong effect on rabies persistence and that reproductive seasonality and population turnover have only marginal effects compared to connectivity. As connectivity driven by shared feeding areas might not be detectable by genetic analyses of the bats, we proposed the use of a widespread virus persistently infecting bats as a marker to trace connectivity across colonies. Combined, the results presented here provide tools that can be applied to intervene and apply countermeasures to prevent spillover.

CHAPTER ONE

GENERAL INTRODUCTION

Understanding Pathogen Spillover Events

Pathogen transmission across species, known as spillover, requires specific conditions to align in time and space (Plowright et al. 2017). Several imperfect barriers have to be breached in order for cross-species transmission to occur (Plowright et al. 2017). Understanding the spatiotemporal dynamics of such barriers is challenging, as spillover events are comparatively infrequent, and their occurrence is usually revealed by the later development of an outbreak or the manifestation of symptoms in the receiving host (Plowright et al. 2017). In order to predict the occurrence of spillover events and to be able to intervene with countermeasures to prevent or reduce the risk of spillover events, those barriers need to be understood (Reaser et al. 2020). Systems where pathogens were expected to emerge, given the presence of reservoirs, susceptible recipient hosts and interactions among them, present an opportunity to understand factors that prevent the transmission across species. In this sense, Uruguay presents an interesting system to understand vampire-bat-borne rabies and its transmission into livestock.

Rabies is an infectious disease, caused by a Lyssavirus, that still produces tens of thousands of human deaths a year (Velasco-Villa et al. 2017). Reports of disease in livestock, consistent with the symptoms of rabies, following attacks by vampire bats are almost as old as early European presence and livestock introduction in the Neotropics (La Condamine 1745, Botto Nuñez et al. 2019a). However, definitive evidence linking vampire

bat attacks and rabies transmission to cattle was not reported until 1911 in a study of a rabies outbreak in southern Brazil (Carini 1911). Since then, vampire-bat-borne rabies had been detected all across South America, with the exception of Chile and, until 2007, Uruguay (Escobar et al. 2015).

Uruguay had rabies cases in dogs since the English invasions to the Rio de la Plata basin in early 1800, and until 1984 when the last case of a rabid dog was reported, in the eastern region of the country (Botto Nuñez et al. 2019a). This circulation of urban rabies also resulted in sporadic human cases, with the last confirmed cases occurring during an outbreak in 1964-1965 (Botto Nuñez et al. 2019a). During that same epidemic, two cases in livestock were confirmed, but the transmission was linked to infected stray dogs (Botto Nuñez et al. 2019a). After 1984 and until 2007 there were no cases of rabies in the country (Guarino et al. 2013, Botto Nuñez et al. 2019a). Moreover, until 2007 no cases of rabies in wild animals were detected, and no transmission was reported that was independent from the domestic dog cycle (Guarino et al. 2013, Botto Nuñez et al. 2019a). In October 2007, an outbreak of paralytic bovine rabies was declared in the northeastern region of the country bordering Brazil, and vampire bats were confirmed as the source of infection (Guarino et al. 2013). At the same time, local research initiatives were focused on surveilling non-hematophagous bats to assess circulation of rabies in urban colonies (González et al. 2009, Guarino et al. 2013).

Uruguay has one of the highest densities of cattle on the South American continent and this density has been fairly stable for centuries (Botto Nuñez et al. 2019a). Livestock was introduced into the country early, even before large colonial settlements (Botto Nuñez

et al. 2019a). This allowed for breeding of large herds of cattle, making it one of the most notable features of the territory for visitors, even before towns or cities were founded (Toller 1715). The country's territory is dominated by grasslands, and forests are restricted to river margins, so introduction of livestock was not directly linked to deforestation, and livestock density increased rapidly before population declines of large native herbivore (Botto Nuñez et al. 2019a). Current livestock population is strictly monitored, with 100% coverage of herd monitoring and around 90% electronic individual tagging, both for meat traceability and for animal health monitoring. Also, rabies vaccination in livestock changed in response to the outbreak, first as focal vaccination around confirmed cases (2007-2008) and later with vaccination of all areas with viral circulation (2008) and then the authorization of the vaccine for the whole country in 2009.

One major change in land use in the country was the marked increase in exotic forestry (mainly *Eucalpytus spp* and *Pinus spp.*) for wood and cellulose production, that was promoted by the government during the 1990s, by the bestowal of tax benefits for the forestry industry (Botto Nuñez et al. 2019a). This process, happening grassland country, led to the uncommon trend of increased forest coverage, and a special type of landscape fragmentation process (Botto Nuñez et al. 2020). The increase in forestry areas, combined with sustained livestock abundance, altered the spatial distribution of livestock, fragmenting the grazing areas (Botto Nuñez et al. 2020).

Vampire bats were recorded comparatively late in the country, but that was probably due to lack of systematic zoological surveys, rather than a late introduction of the species to the area (Botto Nuñez et al. 2019a). Moreover, the country has records of the

most ancient know vampire bat fossils in South America (Ubilla et al. 2019). Vampire bat transmitted rabies has been present both in Argentina and Brazil for decades, even in areas close to the border.

Under these conditions of high and sustained density of cattle, strict surveillance in a small and accessible country, vampire bat presence and rabies virus circulation in neighboring countries, the lack of detection of rabies cases before 2007, and the sudden emergence of rabies in livestock provides an excellent framework for studying determinants of the spillover process.

Dissertation Outline and Objectives

Given the conditions outlined above, the main goal for the dissertation was to describe the dynamics of vampire bat-borne rabies in Uruguay in order to understand the factors that determined the emergence of the disease in 2007.

As the outbreak occurred in an area with dramatic changes in landscape structure following commercial exotic afforestation, we aimed to understand the effect that land use changes may have on disease emergence. In particular, we were interested in studying the effect of grassland fragmentation on rabies persistence and spillover. We hypothesized that grassland fragmentation results in smaller scattered patches of livestock. This pattern would increase sharing of feeding areas by previously isolated vampire bat colonies, and therefore, increase connectivity of these colonies. Finally, increased connectivity would promote persistence of rabies infection in vampire bats, facilitating spillover to livestock.

Given these hypotheses, we propose four main predictions to be tested:

- Rabies outbreaks should be spatially associated with grassland fragmentation

- Bat colonies in fragmented landscapes should be more connected than those foraging in
  unfragmented grasslands
- The absence of outbreaks in the south should be explained by lack of persistence (fading
  out) of the pathogen after introduction, rather than by absence of pathogen transmission
  because of isolation
- Increased connectivity among vampire bat colonies should allow pathogen persistence

To test each of the four predictions we designed a combination of field, laboratory and modelling approaches that would inform each other. The design of the entire project followed the framework of model guided fieldwork (Restif et al. 2012), to optimize field research.

We first performed a historical review, compiling the available information on livestock abundance and distribution, vampire bat distribution, and rabies occurrence in the country to provide a historical framework to understand the emergence of rabies in the country. This analysis. presented in chapter two, proposed four scenarios that could explain the time and location of the outbreak. Of those scenarios, we believe the available information only supports the occurrence of a landscape change that affected the circulation of the virus in vampire bats, allowing it to spillover into livestock.

Chapter three presents a spatial statistical analysis to test the prediction of spatial and temporal association between rabies outbreaks and grassland fragmentation. Our

analysis discarded a null hypothesis of an invasion wave from Brazil as a single explanation for the outbreak occurrence. We proposed that as response to grassland fragmentation, vampire bats might have increased their home-ranges, increasing the likelihood of contact among colonies. We found a strong effect of winter temperatures on the model, and we proposed that temperature might limit the ability of vampire bats to respond to changes in food distribution, as they are already at their physiological limit in Uruguay.

We could not directly assess the connectivity question, as available methods focus on reproductive contacts (that would leave a genetic footprint on the population) or exchange of individuals across colonies. Also, current methods like telemetry, proximity loggers, and fluorescent dyes have implementation problems and limitations (Hoyt et al. 2018, Ripperger et al. 2020). We therefore capitalized on the ongoing field work in the country and fieldwork in Belize to test the use of a persistent and apparently low pathogenic virus as a prospective population marker to study connectivity among bat colonies. Chapter four presents the result of this preliminary work, showing that gammaherpesviruses could serve as population markers for connectivity and movement tracking in vampire bats.

Finally, we integrated the results from the field work into a dynamic model framework to test in silico the assumption of the effect of increased connectivity on pathogen persistence in a metapopulation. Chapter five presents a scalable and easily implemented model that assesses the dynamic effects of connectivity on rabies persistence in a spatially explicit metapopulation. We also analyzed the effects of reproductive seasonality and population turnover over on pathogen persistence, and presented field estimates for these parameters.

CHAPTER TWO

THE EMERGENCE OF VAMPIRE BAT RABIES IN URUGUAY WITHIN A HISTORICAL CONTEXT

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Germán Botto Nuñez

Contributions: Performed the historical research and wrote the review

Co-Author: Daniel J. Becker

Contributions: Co-wrote and edited the review

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Contributions: Co-wrote and edited the review

Manuscript Information

Germán Botto Nuñez, Daniel J. Becker, Raina K. Plowright

The emergence of vampire bat rabies in Uruguay within a historical context

Status of Manuscript

___ Prepared for submission to a peer-reviewed journal

___ Officially submitted to a peer-reviewed journal

___ Accepted by a peer-reviewed journal

_X_ Published in a peer-reviewed journal

Epidemiology and Infection, 147:e180, DOI 10.1017/S0950268819000682

Epidemiology and Infection

cambridge.org/hyg

The emergence of vampire bat rabies in Uruguay within a historical context

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Review

Cite this article: Botto Nuñez G, Becker DJ, Plowright RK (2019). The emergence of vampire bat rabies in Uruguay within a historical context. *Epidemiology and Infection* **147**, e180, 1–8. https://doi.org/10.1017/S0950268819000682

Received: 17 September 2018
Revised: 21 February 2019
Accepted: 18 March 2019

Key words:
Chiroptera; historical analysis; rabies (animal); spillover; Uruguay

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Abstract

Pathogen spillover from wildlife to humans or domestic animals requires a series of conditions to align with space and time. Comparing these conditions between times and locations where spillover does and does not occur presents opportunities to understand the factors that shape spillover risk. Bovine rabies transmitted by vampire bats was first confirmed in 1911 and has since been detected across the distribution of vampire bats. However, Uruguay is an exception. Uruguay was free of bovine rabies until 2007, despite high-cattle densities, the presence of vampire bats and a strong surveillance system. To explore why Uruguay was free of bovine rabies until recently, we review the historic literature and reconstruct the conditions that would allow rabies invasion into Uruguay. We used available historical records on the abundance of livestock and wildlife, the vampire bat distribution and occurrence of rabies outbreaks, as well as environmental modifications, to propose four alternative hypotheses to explain rabies virus emergence and spillover: bat movement, viral invasion, surveillance failure and environmental changes. While future statistical modelling efforts will be required to disentangle these hypotheses, we here show how a detailed historical analysis can be used to generate testable predictions for the conditions leading to pathogen spillover.

Introduction

For pathogen spillover to occur, several hierarchical conditions have to be present and aligned [1]. First, an infected reservoir population must be present [2]. In structured populations, demography and behaviour of the reservoir hosts are critical components of pathogen persistence [3]. Alongside persistence of the pathogen, shedding of the pathogen and contacts among reservoir and spillover hosts must overlap in space and time [1]. Finally, the detection of these realised spillover events is itself dependent on the frequency and intensity of spillover as well as and the sensitivity of the surveillance system. Comparing these conditions in times and locations where pathogen spillover does and does not occur presents opportunities to understand the factors that shape spillover risk. In Latin America, after advances in the control of canine rabies, bat-borne rabies continues to threaten human and animal health and the number of reported cases has been increasing in recent years [4, 5]. Although bat-borne rabies has been observed throughout Latin America since the 1900s, this disease is a relatively new phenomenon in Uruguay. As a case study, Uruguay therefore presents a novel introduction of a virus into a monitored and large livestock population.

The first bat-borne paralytic rabies outbreak in livestock was detected in Uruguay in 2007, and the common vampire bat, *Desmodus rotundus* (É. Geoffroy Saint-Hiliare, 1810), was confirmed as the source [6]. In 1 week, 193 cows died from rabies, costing the country around $2 million in immediate vaccination alone [7–9]. Rabies virus isolated from vampire bats or livestock from this first year of outbreaks showed high-genetic similarities but divergence from isolates from southern and northern Brazil [6]; however, due to low sample sizes, data were not sufficient to provide a putative origin for the Uruguay outbreak. No rabies sequences from Uruguay have been published or made available from official veterinary laboratories following this initial assessment. Similarly, limited data are available on vampire bat population structure, with a small number of samples suggesting that vampire bats from northern Uruguay are virtually indistinguishable from those in southern Brazil [10].

The absence of bovine rabies in Uruguay until 2007, and its presence only in the northern region of the country thereafter, likely reflects a change in some of the aforementioned conditions (e.g. reservoir distribution, disease surveillance) to allow the occurrence of the 2007 outbreak and subsequent cases. Ideally, a careful statistical analysis of reservoir host distribution, population density, environmental factors and surveillance systems would facilitate differentiating between the various drivers of rabies virus emergence. However, as described above, much of the quantitative data required for such an analysis is absent in Uruguay.

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For example, the country lacks data on vampire bat colony size, connectivity and foraging patterns [11], reflecting broader issues with limited research in field mammalogy in Uruguay [12]. In this paper, we therefore present a historical contextualisation for rabies emergence in Uruguay to identify and develop testable hypotheses to differentiate the drivers of emergence. More broadly, we highlight how historical context should be considered as a key component of studying wildlife disease ecology and pathogen spillover.

Uruguay is a special case compared with other Latin American countries affected by vampire bat rabies. Uruguay's predominant landscape is grassland, and forests are restricted to riparian areas [13, 14]. Livestock were introduced during the early 1600s and grew to high densities well before wildlife prey populations were significantly reduced by overhunting. In contrast to most Latin American countries, Uruguay's forest coverage has since increased, although this change is due to an increase in industrial forestry. This trend makes Uruguay very distinctive from both a South American and a global perspective [15, 16]. Furthermore, because livestock-related goods are the main export in Uruguay [17, 18], the cattle population is strictly monitored: 100% of livestock is under herd traceability and over 80% are under individual electronic traceability systems [19]. Herd traceability began in 1827, was codified in 1973–74, and this law was extended to all livestock in 1996 [19]. Therefore, shifts in bovine surveillance are an unlikely explanation for the recent emergence of bat rabies. The expansion of rabies into Uruguay therefore may instead reflect a change in the distribution of the reservoir host or a change in environmental conditions that promote viral transmission, persistence or detectability.

The common vampire bat, *D. rotundus* (É. Geoffroy Saint-Hiliare, 1810), is responsible for most cases of rabies in Latin America [4, 20]. *D. rotundus* and the two other vampire bats (*Diphylla ecaudata* and *Diaemus youngi*) are the only three obligate sanguivorous mammals. *D. rotundus* depends almost exclusively on mammalian blood [21]. As this resource is extensively available, vampire bats have a widespread distribution that may be constrained by temperature because they have poor homoeothermic capacity [21]. Their sanguivorous diet facilitates the transmission of rabies virus through saliva [21–23]. Rabies virus is likely transmitted through frequency-dependent processes such as grooming, blood sharing and aggressive interactions within vampire bat populations. Metapopulation dynamics (specifically, the immigration of infected individuals) may promote viral persistence [24, 25]. Since the introduction of livestock to Latin America, domestic animals are commonly the predominant prey for vampire bats [26, 27]. The intensification of livestock rearing into forested regions or in areas with otherwise small-scale cattle rearing likely drives dramatic dietary shifts, especially combined with defaunation processes (e.g. as in Uruguay). In Mexico, an extrapolation from passive surveillance confirmed an estimated 90 000 to 100 000 rabies-related cattle deaths per year [28]. In Peru, active surveillance corrected for underreporting estimated >400 deaths per 100 000 cattle in 2014 from vampire bat-borne rabies [29].

In some areas of Latin America, increased deforestation and the corresponding reduction of wildlife populations may trigger an increase in vampire bat predation on cattle and increase risks of rabies outbreaks [20, 30–33]. Intensification of cattle production also increases the availability of prey for vampire bats and allows bat populations to increase and disperse [27, 34]. This phenomenon of population increase driven by changes in livestock production is likely dependent on the landscape and the history of each site. For example, in central-southern Brazil, forest fragmentation for grazing areas and croplands has replaced natural wildlife prey with livestock, facilitating vampire bat predation on cattle [35]. In northern Brazil, mining or logging activities in the forest increased contact between humans and vampire bats leading to increased risk for human rabies [20, 30, 32, 36]. In some cropland areas, livestock were removed from residential yards so that humans became the most accessible prey for vampire bats [20, 30]. The increase in rabies in Uruguay has contrasting mechanisms as forest coverage has increased through commercial afforestation and agricultural expansion has led to substitution of natural grasslands.

We propose that the recent emergence of vampire bat-borne rabies in cattle in Uruguay in 2007 could be explained by one or more of the following hypotheses:

- Vampire bats recently extended their range into Uruguay.
- Rabies recently invaded Uruguay, where vampire bats and cattle have been historically distributed.
- Rabies has been recently detected in Uruguay, despite previous circulation in both vampire bats and cattle.
- Vampire bats and rabies have been present in Uruguay, but recent environmental changes have allowed spillover into livestock. These changes have allowed rabies virus to persist in bat populations and cause epidemics in bats that lead to epidemics in cattle.

Given the cattle surveillance in Uruguay, and the accessibility of the entire country, we assume that if an outbreak had occurred, it would have been detected. To assess historical evidence for these alternative hypotheses for viral emergence, we review historical records on: (i) the distribution of vampire bats and the circulation of virus in both (ii) vampire bats and (iii) cattle.

Recent range expansion of vampire bats into Uruguay

In general, range expansions might be explained by changes in climatic limiting conditions, changes in the distribution of food resources and changes in roost abundance and availability [37, 38]. We collected all historical records of *D. rotundus* in Uruguay, since European colonisation, to examine historical support for the hypothesis that *D. rotundus* has recently expanded its geographical distribution into Uruguay (see supplementary information and Table S1 for a detailed description). We also compiled information available on changes in roost availability and food sources.

Historical records

The first record of *D. rotundus* in Uruguay was in 1933, but there were reports of cows being attacked by vampire bats for several years beforehand [39]. At that point, *D. rotundus* was considered rare in Uruguay. Less than 40 years after this first record, *D. rotundus* was confirmed in several locations around the country, suggesting a widespread distribution [40–42]. However, this pattern of new records and locations does not necessarily suggest expansion of the vampire bat range but rather an incursion of researchers into formerly unexplored roosts (Fig. 1). There is even a previous description of a cave in south-central Uruguay where a description of bats occupying the site is consistent with *D. rotundus* [43]. While this is still an unconfirmed report, it supports the idea that *D. rotundus* has occupied Uruguay for longer than documented reports or captures suggest. Only one roost in

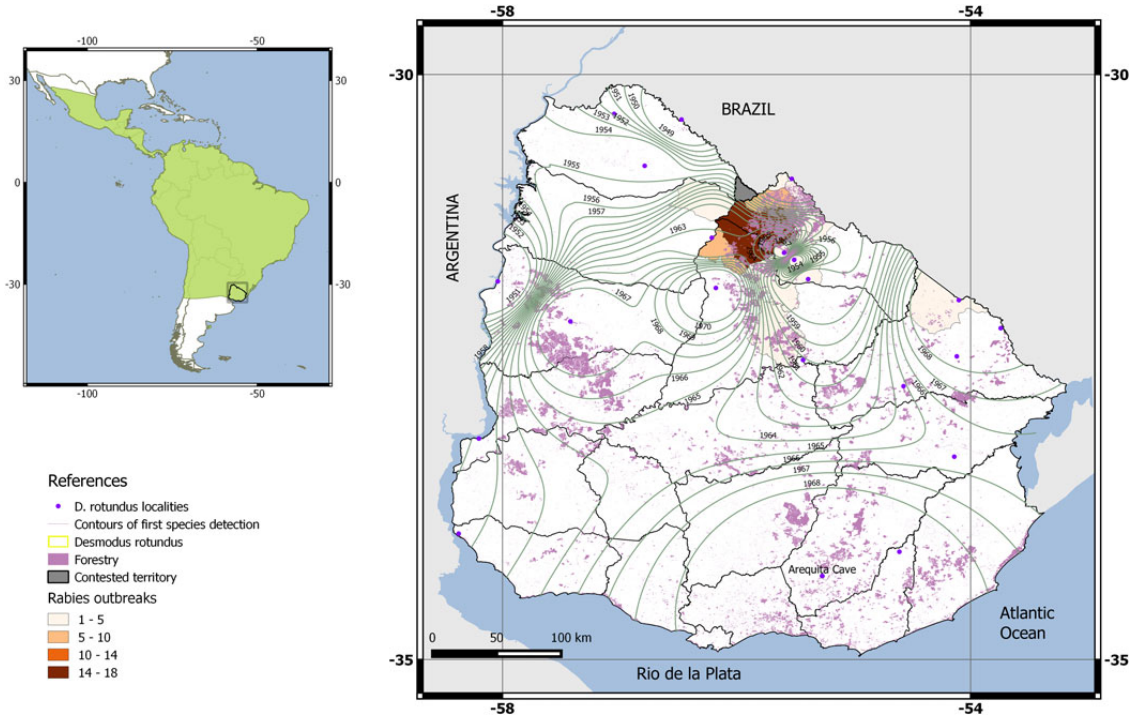

Fig. 1. Map showing the continental location of Uruguay, the accepted current distribution of *D. rotundus* (IUCN, 2012), the localities for *D. rotundus* mentioned for Uruguay by Langguth & Achaval (1972), and the contours generated by interpolation of the date of first record of the species in each locality (see supplementary material and Table S1 for detailed discussion). The Arequita cave in southern Uruguay is shown. The cumulative number of ranches affected by rabies outbreaks in Uruguay is presented according to the official information provided by the Uruguay's Ministry of Livestock Agricultures and Fisheries.

south-central Uruguay may have been recently colonised by *D. rotundus*. The Arequita cave was visited by mammalogists several times between 1890 and 1980, and although a number of bat species were recorded, *D. rotundus* was not found there until 70 years after the first recorded visit [39–42, 44].

Roosts

Roosts used by *D. rotundus* in Uruguay are mostly caves, abandoned buildings and abandoned mining tunnels [45]. Recent changes in roost availability include short-lived mining activities of the early 1900s and changes in the distribution of rural workers in the late 1900s and early 2000s that may have provided other housing structures [13, 46–49]. However, both processes provide a limited number of new roosts, probably insufficient to explain an expansion of vampire bats. Moreover, while some of the first reports of the species in Uruguay were related to these structures [40, 41], as soon as new areas were explored, the species was detected in many long-available natural roosts. *D. rotundus* is now considered abundant and widespread throughout Uruguay, based on the number of roost registered and the detection of vampire bats by acoustic surveys and mist-netting [11, 45, 50–53].

Food sources

Livestock were introduced into Uruguay during European colonisation in the late 1500s and early 1600s, mostly through the missionaries from the Company of Jesus [54, 55]. By the 1630s, cattle were abundant in the Uruguayan territory, with estimated minimum 100 000 animals according to Hernandarias [55]. The northern coast of the Rio de la Plata estuary was not occupied by Europeans during most of the 17th century, and livestock were managed free range and not heavily exploited until 1710 [55]. From this point, there is discrepancy among different documentary sources on the number of cattle in the territory of Uruguay (see supplementary material for a detailed discussion and Table S1). However, considering several hundred thousand pieces of leather were exported from Montevideo annually, the cattle population likely was very large [56, 57]. Other direct reports of exports from Montevideo, published notes from travellers, and historic reports from inhabitants, also support the notion of a large cattle population [56, 58]. By 1908, the livestock population was already 8.2 million cattle and 21.5 million sheep as assessed by censuses [59]. According to the last official estimation, the current population is 12.4 million cattle and 7.3 million sheep [60]. The presence of high concentrations of livestock precedes the large declines of wildlife (Fig. 2, Table S1). There is no evidence that climatic conditions or the availability of food and roosts have limited *D. rotundus* populations in Uruguay for the past 100 years.

Uruguay has historically been described as grasslands with forest in the rivers' banks [61], where several wild mammals could have served as prey for *D. rotundus*, including pampa's deer (*Ozotoceros bezoarticus*), marsh deer (*Blastocerus dichotomus*),

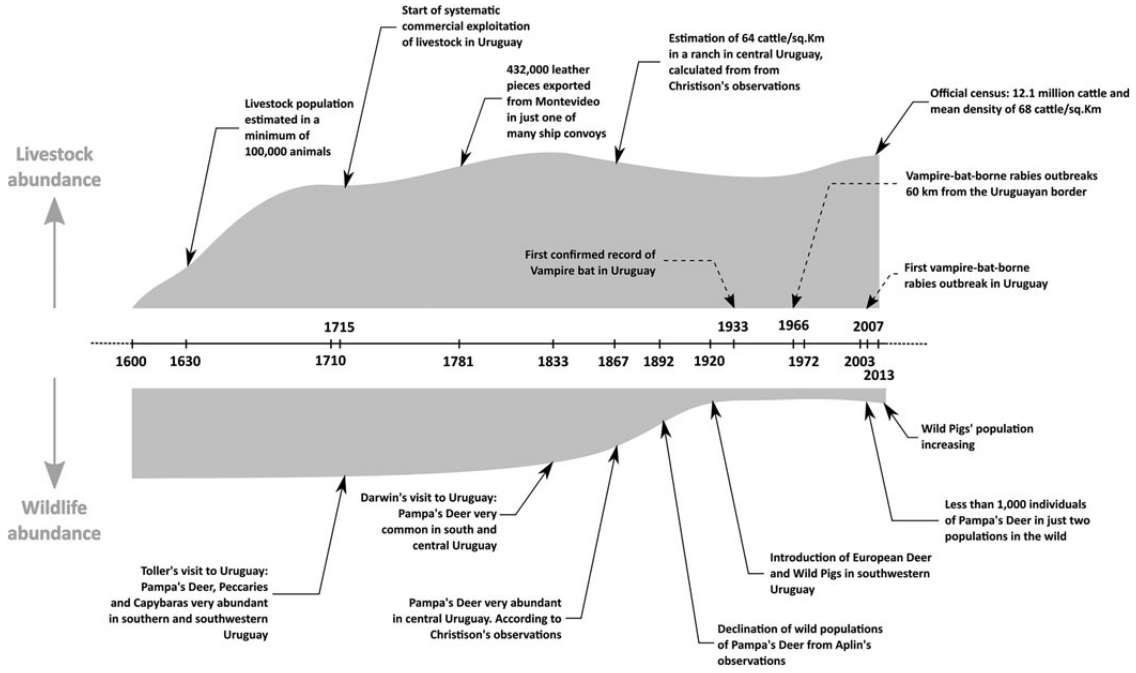

Fig. 2. Timeline of livestock and wildlife abundance, vampire bat records and bovine rabies outbreaks in Uruguay. See the text and supplementary material and Table S1 for details.

brocket deer (*Mazama gouazoubira*), peccaries (*Pecari tajacu*) and capybaras (*Hydrochoerus hydrochaeris*) [58, 62–69]. With the exception of capybaras, these wildlife prey species now have low densities, restricted geographic ranges or are locally extinct [45]. This severe defaunation occurred by the end of the 1800s and the early 1900s, when livestock populations were already established. Moreover, during the early 1900s, wild pigs, goats, Asian buffalo and two species of exotic deer were introduced into Uruguay and formed established wild populations [45, 70]. Wild pigs are now widely distributed in Uruguay and could be a food source for *D. rotundus* [70–72]. Axis deer also exist in relatively high densities in the south of the country [70].

Climate

In relation to climate, more than 40 years ago, McNab proposed that the 10 °C mean minimum isotherm for the coldest month was a key predictor of the geographic range limit of *D. rotundus* [73]. This limit was proposed in accordance with feeding habits of *D. rotundus* and their energetic limitations. Vampire bats are highly sensitive to cold and dehydration owing to their protein-based diet, inadequate lipid stores and high rates of evaporative water loss [73–75]. Cooler climates increase the amount of energy *D. rotundus* must expend to maintain normal temperature, requiring larger bloodmeals. As bloodmeal size is limited by body size and flight capacity, this isotherm restricts the *D. rotundus* distribution [73]. New records of the species after McNab's work have all fallen within his proposed range limit. For instance, records in southern Uruguay, and new records in Mexico and Argentina fall inside the proposed limit [45, 76–78]. Interestingly, the *D. rotundus* distribution does not overlay with cattle distributions in the southern or northern limits (Fig. S1). In Argentina and Mexico, cattle are present on both sides of the 10 °C isotherm, but *D. rotundus* is only present on the side of each isotherm that is closest to the equator. In some areas such as the province of Buenos Aires (Argentina) where *D. rotundus* is absent, cattle densities are higher than in central Argentina where *D. rotundus* is present [79]. Combined, this evidence suggests that the 10 °C isotherm is a good proxy for the *D. rotundus* distribution limit.

While an increase in air temperature has been observed for the region during the 1900s and is expected to continue in the future [80], vampire bats already occupy the entire country. Hence, overall distribution of the species in the country may not be affected. However, behavioural changes (such as feeding habits) might be expected in response to temperature shifts. Increases in minimum temperature and decreases on the frequency of cold nights might impact flight ability of vampire bats, making them able to forage over longer distances [73].

Two recent studies analysed the potential range expansion of vampire bats [81, 82]. One concluded that an extensive expansion into North America is unlikely [81]. Although the other proposed a future range expansion [82], predictions of this species distribution model notably did not include the southernmost area of the known distribution (including Uruguay).

The possibility of recent rabies introduction into Uruguay

As shown above, the historical record provides no support for the hypotheses that *D. rotundus* recently expanded into Uruguay.

Alternative explanations for the recent emergence of bovine rabies in Uruguay are therefore (i) a recent invasion of the virus into Uruguayan bat populations or (ii) a change in conditions leading to increased viral persistence in vampire bat populations and an increased probability of transmission to livestock.

The first report of vampire bat-borne bovine rabies was in 1911, about 700 km from the Uruguayan border in the state of Santa Catarina, southern Brazil [83, 84]. Since this first-reported outbreak, vampire bat-borne rabies in cattle has remained common in the area near the Uruguay–Brazil border [85, 86], suggesting sustained circulation of rabies virus in *D. rotundus*. Even if the late discovery of *D. rotundus* in Uruguay was a reflection of a host expansion process, in 1966 there were already reports of vampire bat-borne rabies in southern Brazil within 60 km of the Uruguayan border [87]. By that time, *D. rotundus* was known to be present in several localities through Uruguay [40, 42]. Rabies in the neighbouring Brazilian southern state of Rio Grande do Sul has been present for at least 60 years. According to one study, between 1964 and 2008, rabies in Rio Grande do Sul has shown a cyclic behaviour with epidemic pulses [88]. Another study, in the same Brazilian state, showed that between 1985 and 2007, only 2 years (1996 and 2001) have had no reported bovine rabies cases in the same state [89]. Accordingly, it is unlikely that rabies virus only recently invaded into Uruguay in 2007. Given the sustained circulation of rabies in southern Brazil, longitudinal seroprevalence in northern Uruguay is needed to understand whether the virus exhibits more sporadic dynamics (perhaps suggesting a more recent invasion) or more endemic dynamics (suggesting longer-established virus) [24, 25].

Recent detection of circulating rabies

One alternative hypothesis is that rabies has been endemic in Uruguay but was only recently detected through surveillance. However, livestock in Uruguay are subject to robust surveillance, and the small country has no inaccessible or remote areas that are not monitored [19]. Moreover, retrospective studies conducted on samples from cattle that died from undiagnosed neurological disease have tested negative for rabies. In 2011, samples from 193 cattle that died from neurological signs between 1999 and 2011 were tested with direct immunofluorescence, immunohistochemistry and histopathology techniques [90, 91]. Immunohistochemical approaches have proven to be reliable to detect rabies virus in formalin-fixed samples from livestock and wildlife in retrospective studies [92]. No samples were positive for rabies before the 2007 outbreak, suggesting that the absence of notified cases does not reflect a failure in surveillance [90, 91]. The introduction of bovine rabies into Uruguay is therefore likely to be a recent phenomenon. While disease surveillance and livestock tracing system in Uruguay are adequate, publicly available systematic reports on livestock and wildlife testing are needed both from the perspective of surveillance and for the data needed to test the proposed hypotheses.

Recent environmental changes leading to persistence and spillover

The historical records reviewed above indicate that recent changes in the distribution and abundance of vampire bats or livestock are unlikely to be the main driver of vampire bat rabies emergence in Uruguay. Rabies virus has been circulating in nearby southern Brazil for at least 100 years. The absence of detected bovine rabies cases before 2007 is unlikely to be explained by a failure in disease surveillance, given the robust monitoring of livestock throughout Uruguay.

An alternative hypothesis for the recent spillover of rabies may be a change in pathogen dynamics (e.g. persistence) within vampire bat populations. In general, factors that contribute to pathogen persistence in bat populations include population size, seasonal reproduction, hibernation and connectivity among roosts [93, 94]. Rabies virus transmission is likely to be frequency-dependent in vampire bats, and thus colony size may have little or no effect on rabies transmission [24]; furthermore, the historical records suggest that colony sizes are unlikely to have dramatically changed in the years prior to the outbreak. While culling practices (e.g. use of vampiricides) are associated with increased rabies transmission in vampire bats [24], culling practices only began in response to the 2007 outbreak and thus cannot explain the emergence event. A shift in vampire bat reproduction (e.g. due to seasonality [95, 96]), stemming from climatic factors is also an unlikely driver of rabies emergence, given that there is no evidence for a change in climate seasonality in recent years [97, 98]. Because colony connectivity is critical for explaining patterns of rabies virus persistence within vampire bat populations [24, 25], shifts in vampire bat movement and connectivity could explain the emergence of rabies virus in Uruguay.

The most dramatic environmental change that has taken place in Uruguay recently – an increase in forest coverage – overlapped in space and time with the initial rabies virus outbreaks. This change in forest coverage was observed following the implementation of the Forests Act (Law 15.939, 1988) and peaked during the early 2000s. The change in forest coverage was abrupt, with forest plantations increasing 60.8% from 764 825 to 1 230 013 ha between 2000 and 2011 [99]. All recorded cases of vampire bat rabies have occurred in the area of most intense forestry activity, except for one outbreak in 2014 in the Department of Cerro Largo. Increases in forest coverage and consequent decreases in grassland surfaces were not followed by decreases in livestock numbers. On the contrary, livestock density increased in Uruguay during this same period. The concentration of cattle in small, scattered, dense patches could therefore affect the dispersal of *D. rotundus*, thereby increasing inter-colony connectivity and metapopulation dynamics that facilitate rabies persistence [25]. Vampire bats roosting in a landscape with homogeneously distributed livestock may forage in small distances around the roosts, reducing contact among distant colonies. When the roosting areas are embedded within habitat matrices with patchy distribution of livestock prey, vampire bats may have to travel further to feeding areas that may be already used by other colonies, thus increasing contact among colonies. This is supported by observations that vampire bats preferentially feed on livestock and that their movement behaviour will often track the distribution of livestock [96]. Culling activities could also modify vampire bat movement dynamics and increase rabies transmission within vampire bat colonies [24, 31].

Critical data needs remain to quantify the structure and connectivity of *D. rotundus* in this newly forested region compared with neighbouring regions with and without rabies. Furthermore, two important aspects of *D. rotundus*' biology in Uruguay are absent: population density across the country and predation pressure on livestock. While there are no data on the former, the distribution of livestock – a good proxy of bat population size [24] – does not suggest a higher density of *D. rotundus* in the outbreak area Fig. S2). However, this assumption should be tested by assessing vampire bat densities, feeding activity and roost distribution.

Standard acoustic surveys could be used to provide information on bat movement patterns, which can be combined with information on predation pressure of vampire bats on cattle. A recent study showed that vampire bats from the north are almost indistinguishable from southern Brazilian populations [10], suggesting either a southern expansion or a recolonisation of empty roosts after culling activities. More work including southern Uruguayan populations and samples collected before culling activities are necessary to differentiate these hypotheses. Population genetics could also provide data on population size and roost connectivity [100]. Culling has been focused on the eastern half of the country, starting in 2007 in the northeast in response to the outbreak and then extending south. No exhaustive reports of culling activities are available. Systematic assessments of culling are necessary, especially in regard to how they may impact bat dispersal and rabies seroprevalence patterns.

Our survey of the historical record suggests that recent environmental changes that may have modified vampire bat behaviour are a likely driver of rabies virus emergence in Uruguay and that recent host population expansion, viral invasion and improved disease detection are unlikely explanations. Additional analyses can help reject these latter alternative hypotheses. For example, the recent expansion of vampire bats into Uruguay could be tested by assessing the genetic structure of vampire bat populations and the potential effects of culling activities started in 2007 [10, 101, 102]. A genetic analysis of the rabies virus isolates from these outbreaks could also shed light on the previous circulation of the virus in the country; for example, a rabies virus phylogeny was used to show independent invasions of rabies virus into Trinidad from the continent [103]. Rabies virus genetics have also been used to infer the rate of spatial spread in Peru [31]. However, limited rabies virus isolates currently constrain these analyses for Uruguay. Accordingly, further work on viral detection and isolation in vampire bats should be conducted in Uruguay. Last, and most important, the primary hypotheses of environmental change must next be tested with available spatial and temporal data on bat population size and distribution, forest cover, livestock density and rabies outbreaks. Because such analyses will be limited by data scarcity, new data collection efforts are needed to assess this hypothesis.

Conclusion

Given this historical context of vampire bat and cattle distribution in Uruguay, a likely explanation for the recent emergence of vampire bat rabies in Uruguay is the substitution of native grasslands with forest plantations that could have altered vampire bat movement and promoted viral persistence, leading to increased transmission from *D. rotundus* to cattle. Spatial analyses of landscape structure between northern Uruguay (where rabies persists) and neighbouring areas where rabies does not persist could help test this hypothesis. Spatial analyses of epidemiological data could be complimented by field surveys of the population structure, connectivity and feeding behaviour of *D. rotundus* in these same areas. More broadly, our case study on bovine rabies emergence in Uruguay provides an example of how a detailed historical analysis on reservoir host distribution, ecology and disease occurrence can help develop and evaluate alternative hypotheses for understanding the determinants of pathogen spillover. Even when basic conditions for spillover appear to be present, analyses of historical contexts and local landscape characteristics can provide testable hypotheses about pathogen emergence and persistence and should be considered more generally when studying wildlife disease ecology.

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Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268819000682

Funding. GB was funded by a Fulbright Graduate Scholarship, Bat Conservation International, and American Society of Mammalogists. DJB and RKP were supported by the U.S. National Science Foundation (DEB-1716698) and the Defense Advanced Research Projects Agency (DARPA D16AP00113 and DARPA PREEMPT administered though DARPA Cooperative Agreement # D18AC00031). The views, opinions and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government. RKP was also supported by the U.S. National Institutes of General Medical Sciences IDeA Program (P20GM103474 and P30GM110732) and the USDA National Institute of Food and Agriculture (Hatch project 1015891).

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CHAPTER THREE

SYNERGISTIC EFFECTS OF GRASSLAND FRAGMENTATION AND TEMPERATURE ON BOVINE RABIES EMERGENCE

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Manuscript Information

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Synergistic effects of grassland fragmentation and temperature on bovine rabies emergence

Status of Manuscript

___ Prepared for submission to a peer-reviewed journal

___ Officially submitted to a peer-reviewed journal

___ Accepted by a peer-reviewed journal

_X_ Published in a peer-reviewed journal

EcoHealth, 17(2): 203-216, DOI 10.1007/s10393-020-01486-9

EcoHealth
https://doi.org/10.1007/s10393-020-01486-9

ECOHEALTH

Original Contribution

Synergistic Effects of Grassland Fragmentation and Temperature on Bovine Rabies Emergence

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Abstract: In 2007, common vampire bats were the source of the first outbreak of paralytic bovine rabies in Uruguay. The outbreak coincided in space and time with the fragmentation of native grasslands for monospecific forestry for wood and cellulose production. Using spatial analyses, we show that the increase in grassland fragmentation, together with the minimum temperature in the winter, accounts for the spatial pattern of outbreaks in the country. We propose that fragmentation may increase the connectivity of vampire bat colonies by promoting the sharing of feeding areas, while temperature modulates their home range plasticity. While a recent introduction of the virus from neighboring Brazil could have had an effect on outbreak occurrence, we show here that the distribution of rabies cases is unlikely to be explained by only an invasion process from Brazil. In accordance with previous modeling efforts, an increase in connectivity may promote spatial persistence of rabies virus within vampire bat populations. Our results suggest that land use planning might help to reduce grassland fragmentation and thus reduce risk of rabies transmission to livestock. This will be especially important in the context of climatic changes and increasing minimum temperatures in the winter.

Keywords: Spatial autoregressive models, Geographically weighted regression, Desmodus rotundus, Minimum mean temperature, Spillover, Uruguay

Introduction and Purpose

The combination of landscape change and increasing anthropogenic food availability for wildlife may influence wildlife demographics, foraging behavior, and immune responses, affecting the spatial and temporal dynamics of infectious diseases (Gottdenker et al. 2014; Becker et al. 2015). Species' behavioral responses to landscape change in particular may be bounded by their ability to adapt phys-

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10393-020-01486-9) contains supplementary material, which is available to authorized users.

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Published online: 22 July 2020

iologically to changing environmental conditions (Weiner 1992; Wong and Candolin 2015). In particular, low temperatures and associated heat loss might limit the energy budget of species and their ability to respond plastically (Weiner 1992). Hence, populations living close to the limit of the species distribution could exhibit lower plasticity to respond to various environmental changes.

In Latin America, vampire bats (*Desmodus rotundus*) have replaced dogs as the main source of rabies infection, following successful control campaigns of canine rabies in many countries (Vigilato et al. 2013; Velasco-Villa et al. 2017). Vampire bats feed on vertebrate blood (primarily mammals), and this foraging ecology facilitates transmission of rabies virus through saliva. In 2007, an explosive outbreak of paralytic bovine rabies (i.e., 200 cattle deaths within two weeks) was detected for the first time in Uruguay (Botto Nuñez et al. 2019). Vampire bats were confirmed as the source of infection through virus isolation and evident feeding on livestock, and the outbreak cost the country ~ USD 2 million in immediate vaccination expenses (Guarino et al. 2013; Botto Nuñez et al. 2019). Bovine rabies outbreaks have continued to occur sporadically in the northeast region of the country (Fig. 1), suggesting that the virus continues to circulate. The last outbreak was recorded in March 2017 (Botto Nuñez et al. 2019). Rabies has not spread to other areas of Uruguay, despite abundant livestock and the presence of vampire bats throughout the country (Botto Nuñez et al. 2019). Uruguay presents a novel case-study as vampire bat-borne rabies spilled over into a livestock population that has robust surveillance. Given the conditions of the country, it is unlikely that a rabies outbreak would go undetected by animal health authorities, suggesting that the outbreak in 2007 was likely the first introduction of the virus (Botto Nuñez et al. 2019).

Uruguay, in contrast with most other Latin American countries, has experienced an increase in forest cover over the past 30 years (Ministerio de Ganadería Agricultura y Pesca 2012; Hansen et al. 2013). However, rather than reforestation efforts, this change has been caused by monospecific exotic forestry for wood and cellulose production replacing highly diverse native grasslands (Silveira and Alonso 2009). This commercial exotic forestry fragments the native grasslands, replacing them with habitat that provides little or no food for vampire bats. Fragmentation of natural ecosystems has been often associated with pathogen emergence, persistence, and spillover (Schneider et al. 2001; Schneider et al. 2009; Gottdenker et al. 2014; de Thoisy et al. 2016; Rulli et al. 2017; Faust et al. 2018; Mastel et al. 2018). However, grassland fragmentation is underrepresented in the fragmentation literature (Fardila et al. 2017). Forest fragmentation has been associated with pathogen spillover through increased contact between reservoir and recipient hosts (e.g., edge effects) or by changes in abundances and diversity of reservoir species (e.g., dilution effects) (Rulli et al. 2017; Faust et al. 2018). However, the effects of fragmentation on reservoir host movement and subsequent disease dynamics have been less studied (Gottdenker et al. 2014). Moreover, habitat fragmentation is predicted to have strong effects on host movement and infection dynamics, particularly in highly mobile hosts such as bats, but quantifying such changes in natural systems is challenging (Hess 1996; Plowright et al. 2011; Becker et al. 2017; Becker et al. 2018c; Kessler et al. 2018). Thus, this grassland system provides an opportunity to test different effects of land-use change on pathogen spillover.

Commercial forestry in the 2007 outbreak area increased more than tenfold in the previous 17 years (from 17,967 ha in 1990 to 256,874 ha in 2007 in Rivera and Tacuarembó Departments), and this is now the most forested territory in the country (Ministerio de Ganadería Agricultura y Pesca 2012). During the same period, livestock production in the area also increased (Dirección de Estadísticas Agropecuarias 2011; Dirección de Estadísticas Agropecuarias 2014a). Owing to this simultaneous increase in forestry area and livestock herds, domestic animals, the primary food source of vampire bats (Voigt and Kelm 2006a; Streicker and Allgeier 2016), are now concentrated in increasingly scattered grassland habitat patches. This increase in livestock density may have driven an increase in size of vampire bat colonies, as demonstrated in previous work in Latin America (Streicker et al. 2012; Delpietro et al. 2017; Becker et al. 2018a), while the concentration of this food source in fewer, separated patches could alter vampire bat foraging patterns and population connectivity. Specifically, this shifted distribution of livestock could increase foraging times of bats roosting within forest patches and facilitate more overlap of bats in these shared feeding patches between colonies (Delpietro et al. 2017; Botto Nuñez et al. 2019).

While rabies virus has typically been considered an epizootic pathogen that travels in spatial waves (Delpietro and Nader 1989; Johnson et al. 2014; Benavides et al. 2016), longitudinal studies of vampire bats in Perú suggest that the pathogen instead persists enzootically within this reservoir host (Streicker et al. 2012). Previous data-driven

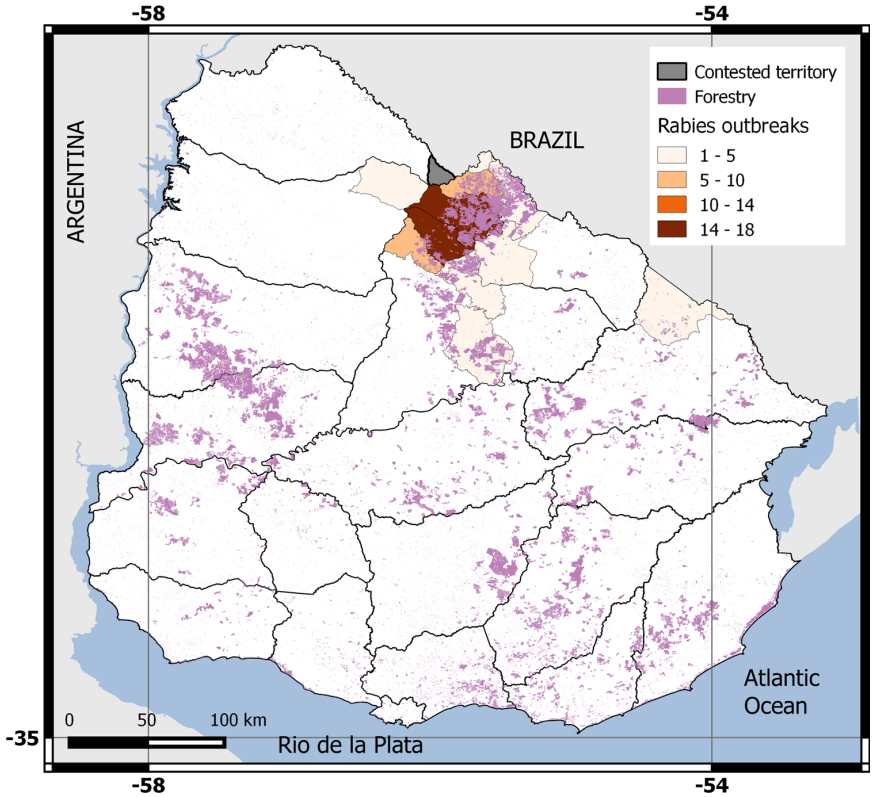

Figure 1. Distribution of ranches with bovine rabies cases in Uruguay from 2007 to 2017, and distribution of exotic forestry areas in 2011. Data from the Uruguayan Ministry of Livestock, Agriculture and Fisheries (MGAP) and the Uruguayan National Environment Office (DINAMA).

mathematical models from Perú also accordingly suggest that rabies cannot persist in isolated populations of vampire bats. Asynchronous connectivity and metapopulation dynamics facilitate persistence of rabies virus alongside frequent (non-lethal) immunizing exposures (Blackwood et al. 2013). Thus, increases in population connectivity and inter-colony interactions at shared feeding sites driven by these shifts in livestock distribution in Uruguay could facilitate rabies persistence.

The plasticity of changing foraging times, in response to changes in livestock distribution, could be constrained by temperature, as heat loss limits bat flight times (McNab 1973; Weiner 1992; Wong and Candolin 2015). Hence, the effects of changes in livestock distribution need to be disentangled from the effects of climate variables. Particularly, minimum temperature of the coldest month has been identified as an important limiting factor for the distribution of vampire bats (McNab 1973; Zarza et al. 2017; Hayes and Piaggio 2018). This finding has two main explanations: lowest temperatures in roost sites determine the energy expenditure necessary to maintain body functions, and air temperature determines heat loss during flight, limiting its duration and foraging distances (McNab 1973).

Here, we used an extensive and spatially explicit dataset from Uruguay to describe the spatial structure of rabies outbreaks across the country and to test the spatial association between rabies outbreaks and landscape transformation. Specifically, we aimed to examine how the distribution of bovine rabies outbreaks was associated with the fragmentation of grazing areas, the density of commercial afforestation, the concentration of livestock, and temperature. We predicted that (a) outbreak densities of rabies would be higher in more fragmented landscapes where livestock is concentrated in separated patches and (b) vampire bats' ability to respond to fragmentation of livestock rearing areas would be limited by minimum winter temperatures.

Methods

Study Area

Uruguay is located in the southern cone of South America (Fig. 1). The climate is temperate with an annual rainfall of 1300 mm and mean temperature of 17°C, with latitudinal variations across the country (20°C and 1600 mm in the north, and 16°C and 985 mm in the coast) (PNUMA 2008). Uruguay is generally flat, with a median elevation of 104 meters above sea level, and with a maximum altitude of 514 m. Uruguay is included in the Uruguayense biogeographic region—a landscape that is dominated by subtropical grasslands, modified by agricultural activities in varying degrees (Evia and Gudynas 2000; PNUMA 2008).

Spatial Data

We obtained the distribution of 2007–2017 rabies outbreaks from the Uruguay Ministry of Livestock, Agriculture and Fisheries. We aggregated the rabies data to the counts of rabies-quarantined ranches (i.e., an individually owned livestock-rearing facility) per police precinct, which are the geostatistical units used in Uruguay (Uruguay Census and Statistics Bureau, INE).

Spatial data on the distribution of livestock were obtained from the National Agricultural Census, which is performed regularly (at least every 10 years) by the Uruguay Ministry of Livestock, Agriculture and Fisheries. The information is publicly available for 2011 at the scale of census tracts (Dirección de Estadísticas Agropecuarias 2011; 2014a; 2014b). The total number of cattle, horses, and sheep were recorded for each agricultural census tract, as these species are common prey of vampire bats (Bohmann et al. 2018) and are raised mostly free roaming in ranches, and so their density can be calculated for large areas. We did not include poultry in this analysis, as these are generally exploited as a secondary food source to domestic herbivores (Bobrowiec et al. 2015). As livestock species vary in size and thus blood available for vampire bats, we used livestock biomass to approximate prey availability (Becker et al. 2018a). Total livestock biomass was obtained by summing species counts multiplied by mean body mass (kg; Jones et al. 2009).

The distribution of forestry and grassland areas was obtained from the National Environment Bureau (Dirección Nacional de Medio Ambiente - DINAMA) for the years 2000 and 2011 (Alvarez et al. 2015; DINAMA 2017), based on interpretation of Landsat TM and ETM+ images following the Land Cover Classification System (Alvarez et al. 2015; Di Gregorio 2016). We used the categories Natural Herbaceous (He), Shrubs (Ar), Palm Groves (Pa), and Naturally Flooded Areas (ANi) as areas compatible with livestock rearing (see Supplementary Table S1 for the Land Cover Classification System legend used in Uruguay and the categories selected). We used the category Forestry Plantations (PF) to indicate non-native forests. Spatial data on average minimum temperature for the coldest month (BIO6) were obtained from WorldClim database version 2 (Fick and Hijmans 2017). All spatial data were projected using the WGS84 / UTM 21S (EPSG 32721) coordinate system and converted into rasters with cell sizes of ~ 100 km^2 and then incorporated into a hexagonal grid of 500 km^2 cells based on weighted averages. Although these data on spatial covariates range from 2000 to 2011 (and rabies data range from 2007 to 2017), the vast majority of rabies cases in Uruguay occurred through 2010 (Figure S1). Following processing spatial data, all analyses were conducted in R (R Core Team 2016).

Fragmentation Analysis

For each land-cover layer (years 2000 and 2011), we created one spatial variable for livestock areas and another for non-native forests. Four cell-based metrics were used to assess fragmentation of livestock areas, both for 2000 and 2011: proportion of landscape (*pl*), number of patches (*n*), mean patch size (*mps*), and effective mesh size (*ems*) (Jaeger 2000; Baldi et al. 2006; Baldi and Paruelo 2014). For each cell *c* of the grid, we used the following formulas:

$$pl_c = \frac{1}{A_c}\sum_{i=1}^{n} A_{ic}$$

$$mps_c = \frac{1}{n_c}\sum_{i=1}^{n} A_{ic}$$

$$ems_c = \frac{1}{A_c}\sum_{i=1}^{n} A_{ic}^2$$

where A_c is the area of the grid cell *c*, and A_{ic} is the area of each of the n_c grassland polygon inside the cell *c*. For non-native forests, we calculated the number of patches (*fnp*) and proportion of landscape (*fpl*) both for 2000 and 2011. We also calculated the recent change in the four fragmentation variables (i.e., *n*, *pl*, *mps*, *ems*) and the two forestry variables (i.e., *fnp*, *fpl*) from 2011 to 2000. As fragmentation

variables exhibited collinearity (Figure S2), we performed a principal component analysis (PCA) with the 12 variables of livestock area fragmentation, for 2000, 2011 and the change between 2000 and 2011 (Table S2). The PCA was performed over the correlation matrix, as the scale of the variables included varied significantly, using the function *princomp* from the *stats* package. Using the spectral decomposition (i.e., using *prcomp*) rather than the singular decomposition (i.e., *princomp*) gave similar results, with only inverted loadings on the second component (Figure S4).

Autocorrelation Analysis and Spatial Modeling

To explore the existence of simple spatial trends and to test for spatial stationarity needed to assess spatial autocorrelation, we fit a linear model for rabies outbreak density with the x and y coordinates of the centroids of the grid cells and the xy interaction, as the predictors. As a significant effect was detected, residuals were then used as the response variable for all remaining analyses.

Because rabies data could show spatial autocorrelation (i.e., nearby areas have similar outbreak densities), we calculated a global index on the model residuals via Geary's *C* using the *spdep* package (Bivand et al. 2013; Bivand and Piras 2015; Brunsdon and Comber 2015). This analysis used a Monte Carlo randomization to assess statistical significance and row-standardized spatial weights for the neighbors list.

To next test how landscape changes were associated with the distribution of rabies outbreaks, we fit a spatial autoregressive model (SAR) (Brunsdon and Comber 2015) for the residuals of the linear model, using the *spdep* package, because an ordinary least squares linear model is not appropriate for spatially autocorrelated data (Bivand et al. 2013; Bivand and Piras 2015). We included livestock biomass, the amount of fragmentation in livestock areas, the recent changes in fragmentation, non-native forest land cover, and the mean minimum temperature of the coldest month as predictors. We used a forward selection process, including one variable in each step, based on the improvement of Akaike's information criterion (AIC). We used a difference of two units in AIC as the minimum improvement to include a variable (Burnham and Anderson 2002) and used an intercept-only model as a null model. We assessed the degree of spatial dependence in the model by estimating the value of λ in the fitted model using the spdep package (Bivand et al. 2013; Bivand and Piras 2015; Brunsdon and Comber 2015). To assess whether the model accounted for autocorrelation, we tested the residuals for Geary's *C* (Jetz et al. 2005; Brunsdon and Comber 2015).

To investigate the local variation of the effect of the environmental variables, we next fit a geographically weighted regression (GWR) (Lichstein et al. 2002; Jetz et al. 2005) for the residuals of the linear model, using the *spgwr* package (Bivand and Yu 2017) and the same predictors used for the SAR model (i.e., recent changes in fragmentation, non-native forest land cover, and the mean minimum temperature of the coldest month). This method allows estimating coefficients that vary across space. The varying coefficients can account for local effects not included in the model. Again, we followed a forward selection process (and considered an intercept-only model), but to avoid overfitting, we considered the AIC value for the global model (i.e., with stationary coefficients for each variable) for the selection. We also tested residuals for Geary's *C* to assess whether the top model accounted for autocorrelation.

To obtain the final predictions from the spatial modeling, we added the predictions from the linear regression to the predictions of each of the spatial models (i.e., fitted(LM) + fitted(SAR) and fitted(LM) + fitted(GWR), as both SAR and GWR were fitted using the residuals of the linear regression as the response variable. All data and R scripts to reproduce our analyses are available in a Zenodo repository: https://doi.org/10.5281/zenodo.3735667

Results

Fragmentation Analysis

The first principal component (*pc.frag1*) explained the 46.74% of the total variance (Figure S3) and was related to fragmentation status. *pc.frag1* increased with the increase in number of patches or with the decrease in proportion of landscape covered by livestock areas, the mean patch size, and the effective mesh size. The second principal component (*pc.frag2*) explained the 20.94% of the total variance and was associated with recent change in fragmentation. Negative values in the *pc.frag2* are indicative of increased fragmentation from 2000 to 2011. Both components showed positive and significant spatial autocorrelation, being greater for the second component (*pc.frag1*: $C = 0.25$, p value < 0.01; *pc.frag2*: $C = 0.52$, p va-

lue < 0.01). Fragmentation was more intense in the western and southwestern region (related to intensive crop production, mainly soybean, wheat, corn, and sorghum) and in the northeastern region (related to commercial afforestation) (Fig. 2a, b). Recent changes in forest coverage were related to increased fragmentation in the northeastern region (Fig. 2a–d).

Autocorrelation Analysis and Spatial Modeling

The density of ranches with rabies cases (Fig. 3a) was significantly predicted by longitude, latitude, and their interaction in our initial linear model (adjusted $R^2 = 0.19$, $p < 0.01$, Table S3). Fitted values suggested an underlying northeast-to-southwest gradient in bovine rabies outbreaks, with higher expected values in the northeast and decreasing values in the northeast-to-southwest direction (Fig. 3B, Figure S5). We accordingly found positive and significant autocorrelation ($C = 0.38$, $p < 0.01$) in the detrended residuals (Figure S6, S7). This suggests additional spatial structure in the outbreaks beyond the latitudinal–longitudinal gradient where areas with higher density of cases are clustered. In subsequent spatial models, we used these residuals as our response variable.

We next fitted a SAR model to the detrended density of ranches with rabies cases (linear model residuals). The final model included the change in number of forestry patches (*c.fnp*) and the mean minimum temperature of the coldest month (*temp_jul*; $w_i = 0.98$). This model showed an improvement from the model containing only the change in number of forestry patches (*c.fnp*; $\Delta AIC = 8.22$, $w_i = 0.02$; Table 1) and showed a discrete but significant positive spatial effect ($\lambda = 0.88$, $p < 0.01$). Residuals showed no significant spatial autocorrelation ($C = 0.96$, $p = 0.15$, Figure S8), implying that spatial structure was accounted for by the SAR. Combining the fitted values from the SAR and the linear model for simple trends accounted for a large portion of the variability in the original density of ranches with rabies cases ($R^2 = 0.76$; Fig. 3, A and C, Figure S12 A, Table S5).

The final GWR model included the first component of the fragmentation PCA (*pc.frag1*), the change in number of commercial forestry patches (*c.fnp*), the change in proportion of landscape covered by forestry (*c.fpl*), and mean minimum temperature of the coldest month (*temp_jul*) (Table 2, Figure S9). The differences in AIC and Akaike weights were much larger when evaluating the weighted models than when comparing the global regression models: the Akaike weights were 0.78 and 1.00 for the best model in the global regression and the weighted regression, respectively (Table 2). There was no trend in the values of the fitted coefficients in relation to the values of each variable (Table S4, Figure S10). A trend in the fitted coefficients would have suggested an unaccounted process, linking the explanatory variable with the disease density. While there was some spatial heterogeneity in the distribution of residuals, these were not concentrated in the area of the outbreaks. Residuals again showed no significant spatial autocorrelation ($C = 1.13$, $p = 0.99$, Figure S11). When combined with the predictions from the linear model, the GWR offered a better prediction of the distribution of the density of ranches with rabies cases (Figure 3, A and D; $R^2 = 0.94$, Figure S12 B, Table S5).

Discussion

Rabies virus has been present in the regions neighboring Uruguay (i.e., southern Brazil and northern Argentina) for more than a century, yet vampire bat rabies was not detected in Uruguay until 2007 (Botto Nuñez et al. 2019). Given robust surveillance and historical records of vampire bats, the absence of rabies spillover in Uruguay cannot be explained by failure to detect the disease nor by the absence of the bat reservoir; a recent historical review suggests that contemporary changes in the ecology of rabies may better explain this recent phenomenon of emergence (Botto Nuñez et al. 2019). The largest expansion of commercial afforestation in the northeast region of the country coincides with the emergence of rabies in the area in space and time and could potentially explain the recent outbreaks, which we tested in our spatial analyses.

We hypothesized that concentration of livestock in separated patches might increase contact of previously unconnected vampire bat colonies, increasing pathogen transmission and facilitating pathogen persistence (Botto Nuñez et al. 2019). We in turn quantified the process of patch isolation, as represented by the mesh size and the number of patches, rather than more commonly used measures in epidemiological studies such as edge size, which is important as a proxy for the rate of contact between patches and matrix (Olivero et al. 2017; Rulli et al. 2017; Faust et al. 2018). Fragmentation metrics are diverse, and usually the effects of habitat loss and changes in habitat configuration are not disentangled (Fahrig 2003). In our system, contact rates between the reservoir host (vampire

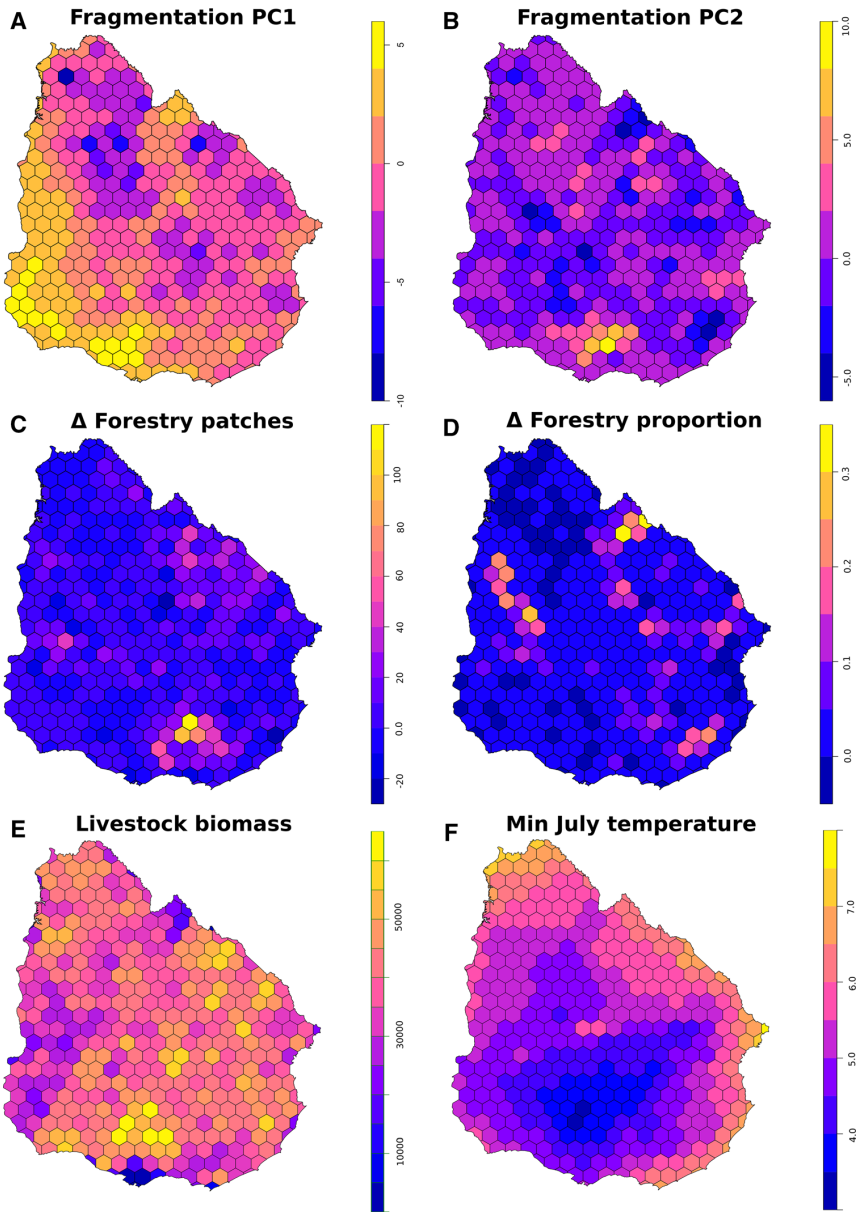

Figure 2. Spatial distribution of predictive variables used for modeling the density of ranches with rabies cases. **A.** Fragmentation PC1 (*pc.frag1*), positive values indicate higher fragmentation of livestock areas. **B.** Fragmentation PC2 (*pc.frag2*), recent changes in fragmentation of livestock areas, negative values indicate increased fragmentation from 2000 to 2011. **C.** Δ forestry patches (*c.fnp*), change in number of exotic forestry patches between 2000 and 2011. **D.** Δ forestry proportion (c.fpl), change in proportion of landscape covered by exotic forestry between 2000 and 2011. **E.** Livestock biomass (*liv.biom*), considering cattle, horses, and sheep. **F.** Minimum Jul temperature (*temp_jul*), average minimum temperature of the coldest month,

bats) and the recipient hosts (livestock) are not driven by edge length, as vampire bats are already using livestock as their main (and perhaps, in many areas, only) food source (Voigt and Kelm 2006b; Bobrowiec et al. 2015; Becker et al. 2018b; Bohmann et al. 2018; Botto Nuñez et al. 2019). In contrast with other studies of the relationship between infectious disease emergence and habitat fragmentation, fragmentation of livestock rearing areas in our system is not

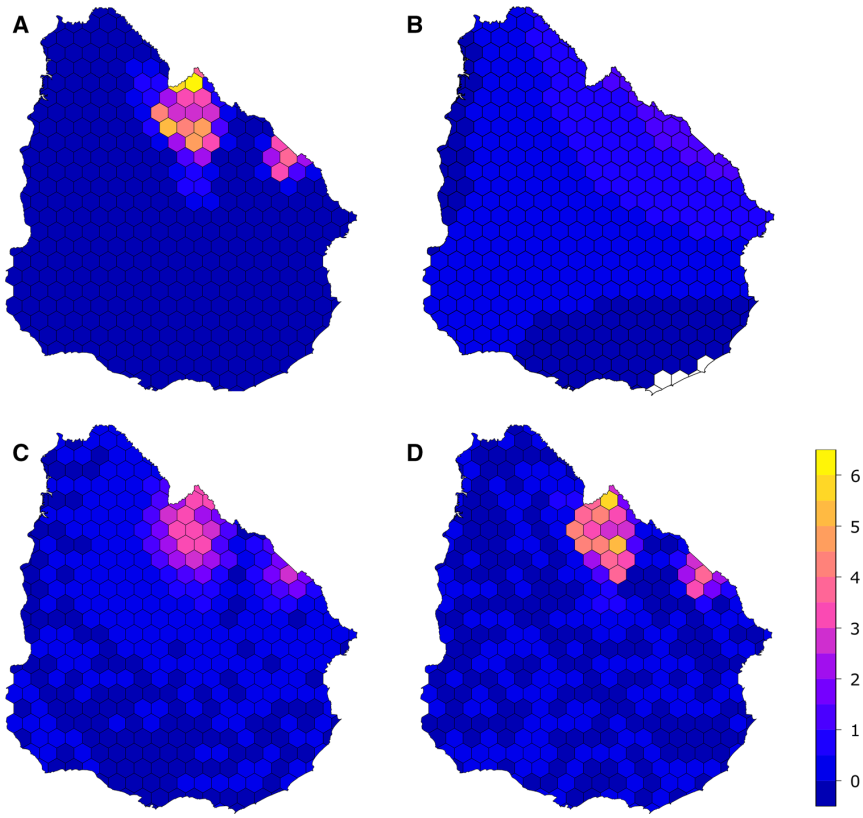

Figure 3. Measured and estimated rabies density: **a** Calculated density of ranches with rabies cases in the regular grid, from the data from official veterinary services. **b** Distribution of fitted values from the linear regression showing a northeast-to-southwest trend on the density of ranches with rabies cases. There are higher expected densities in the northeastern region of the country and decreasing values in the northeast-to-southwest direction (see also Figure S5 for an enhanced color scale for these predicted values). **c** Predicted density of ranches with rabies cases in the regular grid, combining the predictions of the simultaneous autoregressive model and the linear model. **d** Predicted density of ranches with rabies cases in the regular grid, combining the predictions of the Geographically Weighted Regression and the Linear model. Color scale is the same for the four maps.

related to decreased availability or abundance of food or roosts (Olivero et al. 2017; Rulli et al. 2017; Faust et al. 2018). Instead, the direct effect of fragmentation in our system is a change in the spatial distribution of food sources, impacting the foraging strategies of bats and hence the connectivity and contacts between colonies. Thus, a novel approach to fragmentation (in relation to emergence of infectious diseases) was implemented to represent this different proposed mechanism.

We summarized the livestock area fragmentation process in two principal components that described overall fragmentation and recent changes in fragmentation. Both components showed significant spatial structure. Western and southwestern fragmentation areas were mainly related to cropland intensification (particularly soybean production), while northern areas were related to commercial afforestation (Fig. 2a), which has increased recently (more negative values of *pc.frag2*, Fig. 2b) (PNUMA 2008). The commercial afforestation process was highly stimulated by policies promoting and providing economic incentives to forestry ventures (PNUMA 2008).

We detected a spatial trend likely related to invasion of rabies from Brazil, as higher predicted values of rabies outbreaks were observed close to the northeastern border and decreased in the southwestern direction; however, this did not fully explain the spatial distribution of rabies cases (Fig. 3a, b). While an introduction of the virus from Brazil is likely, as outbreaks north of the border have been occurring for more than a century (Teixeira et al. 2008; Barbosa de Lucena 2009), this alone does not explain why

Table 1. Forward selection process for the SAR models. Estimated coefficients and p values (p) are presented for each variable (starred p values are significant at 0.05 level), and differences in Akaike information criteria values in comparison with the best ranking model (ΔAIC), and Akaike weights (w_i) are shown for each model. The first step, considering an intercept-only model, is used as null model.

Variables	Estimate	p	ΔAIC	w_i
Step 1: detrended_residuals ~ 1			14.44	0.00
Intercept	0.00			
Step 2: *detrended_residuals* ~ *c.fnp*			8.22	0.02
Intercept	− 0.04			
c.fnp	0.01	* < 0.01		
Step 3: *detrended_residuals* ~ *c.fnp* * *temp_jul*			0.00	0.98
Intercept	− 0.27			
c.fnp	− 0.04	* < 0.01		
temp_jul	0.04	0.68		
c.fnp:temp_jul	0.01	* < 0.01		

cases in Uruguay only occurred in this area, why they only occurred after 2007, and why pathogen transmission has not expanded since 2007. Rabies cases in Uruguay probably occurred in two independent invasions, both close to the Brazilian border (Figs. 1, 3a), but neither expanded south, despite the presence of bats and livestock throughout Uruguay (Botto Nuñez et al. 2019). This could be further confirmed by phylogeographic analyses if sequences from both foci are made available.

One important spatial measure missing in our study is the local abundance of vampire bats, which is currently unavailable for Uruguay. Although presence and absence data can often be useful for estimating occupancy (which can serve as a surrogate for abundance in some contexts; MacKenzie and Nichols 2004), vampire bats are habitat generalists (Greenhall et al. 1983) and are present throughout Uruguay (González and Martínez-Lanfranco 2010). In lieu of spatial data on vampire bat abundance, livestock abundance might be a useful approximation, as livestock biomass indicates food availability that, along with roosts, limits vampire bat colony size. There is an ongoing survey of vampire bat roosts in Uruguay, but surveillance is higher in or near areas where rabies outbreaks have occurred. Mark–recapture studies would also be useful to estimate vampire bat colony sizes and connectivity.

The SAR model was able to capture the spatial structure of the rabies outbreaks, as the residuals from the model did not show spatial autocorrelation (Figure S8). The positive and significant λ estimate also showed positive spatial dependence in the rabies outbreaks with the two predictive variables included in the final model: the recent change in proportion of landscape occupied by forestry and the average minimum temperature in the coldest month. It is interesting to note that rabies outbreaks did not occur in areas with the largest livestock populations (Botto Nuñez et al. 2019). Livestock populations in Uruguay are large enough to sustain large populations of vampire bats, even in the relatively low-density areas; this could explain the lack of a significant effect of livestock in our analysis (González and Martínez-Lanfranco 2010; Botto Nuñez et al. 2019). In contrast, the minimum temperature in the winter had significative effects (both SAR and GWR models, Tables 1 and 2). While temperature variation is moderate within the country, Uruguay is on the edge of the limit of the tolerance range for the species (McNab 1973; Greenhall et al. 1983). Winter temperatures might influence the length of foraging flights, affecting the ability of vampire bats to modify their home ranges in response to resource's fragmentation.

The GWR model was used mainly as a descriptive analysis to explore the effect of allowing regression coefficients to vary spatially. The results showed significant effects of fragmentation level, the recent changes in forestry, and the coldest temperatures. Allowing spatial variation in the coefficients allowed splitting the regional effects and accounted for unobserved variables, such as the potential influx of the virus from the north. While the fitting process for the GWR model does not guarantee that the model accounted for the global spatial autocorrelation in the response variable, the final model fitted showed no spatial structure in the residuals (Figure S11).

Temperature is a major determinant of the distribution of vampire bats due to their poor thermoregulatory capacity and low tolerance for periods of fasting. Lacking fat storage, vampire bats cannot deal with a series of cold nights where energy invested in foraging flight is not compensated by energy obtained from feeding (McNab 1969; McNab 1973; Greenhall et al. 1983). Uruguay is in the southern limit of the species' distribution, and while *Desmodus rotundus* is present throughout the country, the cold temperatures might limit the distance they can fly to feed in the winter. For instance, if food sources become patchier, vampire bats roosting in higher temperatures could modify

Table 2. Forward variable selection process for the GWR models. The global component of the four fitted GWR is shown. Differences AIC values and Akaike weights (w_i) are shown for each model, in comparison with the best ranking model, both from the global regression (ΔAIC[GR]; w_i [GR]) and from the GWR model (ΔAIC[GWR]; w_i [GWR]). Global estimated coefficients are presented for all included variables, along with the p values (p; starred p values are significant at 0.05 level). Response variable: residuals from the linear model (*detrended_residuals*). The first step, considering an intercept-only model, is used as null model.

Variables	Estimate	p	ΔAIC [GR]	w_i [GR]	ΔAIC [GWR]	w_i [GWR]
Step 1: *detrended_residuals* ~ *1*			35.41	1.60×10^{-8}	162.46	5.28×10^{-36}
Intercept	0.00					
Step 2: *detrended_residuals* ~ *pc.frag1*			15.09	4.13×10^{-4}	282.37	4.83×10^{-62}
Intercept	0.00					
pc.frag1	0.08	*<0.01				
Step 3: *detrended_residuals* ~ *pc.frag1* + *c.fnp*			7.14	0.02	255.74	2.92×10^{-56}
Intercept	− 0.06					
pc.frag1	0.08	*<0.01				
c.fnp	0.01	*<0.01				
Step 4: *detrended_residuals* ~ *pc.frag1* + *c.fnp* + *c.fpl*			2.75	0.20	228.05	3.02×10^{-50}
Intercept	− 0.10					
pc.frag1	0.08	*<0.01				
c.fnp	0.01	*<0.01				
c.fpl	1.92	*0.01				
Step 5: *detrended_residuals* ~ *pc.frag1* + *c.fnp* + *c.fpl* + *temp_jul*			0.00	0.78	0.00	1.00
Intercept	− 0.62					
pc.frag1	0.08	*<0.01				
c.fnp	0.01	*<0.01				
c.fpl	1.95	*0.01				
temp_jul	0.10	*0.03				

(expand) their home ranges. By contrast, those colonies roosting in places with lower temperatures during winter nights might exhibit limited plasticity in home ranges, as the energy needed for foraging is inversely related to temperature. Thus, colder temperatures in the winter might modulate the effect of patchiness of food sources on connectivity. The area where the outbreaks occurred has recently fragmented livestock areas as well as the highest minimum temperatures in the winter (Fig. 2). During the 20th century, there was an overall increase of 0.8°C, where minimum temperatures increased all year round (PNUD 2007). A regional study also concluded that in the period 1960–2000 and based on daily minimal temperatures, there was a consistent trend of increase in temperature of coldest nights and an increase in the number of warm nights (Vincent et al. 2005). This trend is more evident in coastal areas (Vincent et al. 2005). For the next decades, the temperature is expected to keep rising with a total increase of 1.0–2.5°C for 2050 (in relation to the 2000) (Vincent et al. 2005). In this context, and combined with increased land use change and fragmentation of native grasslands (Baldi and Paruelo 2014), understanding how temperature affects plasticity of wildlife to respond to other environmental changes is crucial for predicting potential pathogen spillover risks.

In this work, we showed that the spatial distribution of rabies outbreaks can be explained by a combination of landscape fragmentation and climatic conditions. The recent changes in landscape structure in Uruguay, explained by changes in land use practices, can explain the occurrence of recent outbreaks in the early 2000 s. However, temperature might limit the expansion of rabies into other areas of Uruguay. Our results show that an understudied fragmentation process, such as grassland fragmentation, might have an important effect on the dynamics of infectious diseases. Processes that affect movement of individuals across roosts, such as culling activities, have been proposed to facilitate persistence of rabies virus in vampire bat co-

lonies (Streicker et al. 2012; Blackwood et al. 2013). Similarly, changes in the distribution of food sources can facilitate persistence by increasing contact among colonies. Our results also suggest that physiological limits imposed by temperature affect how species respond to landscape change, modulating how fragmentation may affect the spatial spread of pathogens. More work is needed to disentangle the effects of temperature from the effects of habitat fragmentation. In particular, field studies on how vampire bat home ranges vary across fragmentation and temperature gradients are needed to understand the effects of both variables on vampire bat connectivity and ultimately on rabies virus persistence and spillover risk. Such work would also link habitat and disease data on identical timescales and provide greater inference into associations between environmental change and rabies dynamics in bats and livestock.

Conclusion

The effects of temperature in combination with fragmentation are major factors in determining the spatial pattern of rabies outbreaks in Uruguay. Even when the effects of virus invasion were removed, fragmentation and temperature explained the spatial structure of disease. These results are consistent with our hypotheses that fragmentation could enhance connectivity among vampire bat colonies and promote viral persistence by increasing the rate of immigration of infected individuals. This increased ability of the virus to persist would likely facilitate virus transmission to livestock. The congruence between the conceptual results from both models provided stronger support for the effects of fragmentation on disease emergence. While more experimental support is needed for causal inference and directing policy (such as field-based estimations of vampire bat home ranges across a gradient of fragmented areas), our results suggest that land use planning might help to reduce fragmentation of livestock rearing areas when planning new forestry ventures to help reduce the risk of rabies to livestock. In particular, incorporating metrics of fragmentation of natural grasslands into environmental impact assessment would account for habitat disturbance. Given our results, future work should also analyze landscape fragmentation in the context of climate change, as temperature modulates the responses bat behavior to the fragmentation of food sources.

Acknowledgments

GBN was funded by a Fulbright Graduate Scholarship, Bat Conservation International (Student Research Scholarship for Global Bat Conservation Priorities), and American Society of Mammalogists (Latin American Student Field Research Award). RKP was supported by the National Science Foundation (DEB-1716698), the Defense Advanced Research Projects Agency (DARPA D16AP00113), the US National Institutes of General Medical Sciences IDeA Program (P20GM103474 and P30GM110732), and the Strategic Environmental Research and Development Program (RC-2633). This material is based upon work that is supported by the National Institute of Food and Agriculture, US Department of Agriculture, Hatch project under accession number 0185725. The views, opinions, and/or findings expressed are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the US Government. We want to thank the collaboration of Alvaro Soutullo and Marcel Achkar (UDELAR) during the early stages of this research, Angel Segura (UDELAR) for his valuable comments on the data analysis, Nathan Justice (MSU) for his help with the processing of some of the spatial layers and Devin Jones (MSU) for the help with the manuscript editing. The Uruguay´s Ministry of Livestock (MGAP) provided the data on rabies quarantined ranches through a formal request under the Free Access to Public Information Act (Law 18.831) in January 2017 (Exp. 2016/7/1/1/14253).

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CHAPTER FOUR

HERPESVIRUS DIVERSITY IN THE COMMON VAMPIRE BAT (*Desmodus rotundus*) AND ITS POTENTIAL FOR TRACKING CONTACT AMONG BATS

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

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Contributions: Designed the study, collected the samples, performed the lab work, analyzed the data, interpreted the results, wrote, and revised the manuscript

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Contributions: Collected the samples, co- interpreted the results, co-wrote, and revised the manuscript

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Co-Author: Matthew Taylor

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Manuscript Information

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Herpesvirus diversity in the common vampire bat (*Desmodus rotundus*) and its potential for tracking contact among bats

Status of Manuscript

_X_ Prepared for submission to a peer-reviewed journal

___ Officially submitted to a peer-reviewed journal

___ Accepted by a peer-reviewed journal

___ Published in a peer-reviewed journal

To be submitted to: Journal of Animal Ecology

Abstract

Host distribution, movement, and contacts can shape pathogen diversity and evolution. Conversely, within- and between-host population and community pathogen structure can be used to track changes in host movements and population structure over time. For example, species-specific viral infections have been proven to be an effective tool to understand population response of wild felines to human disturbance. Bats are the second most diverse order of mammals and are also cosmopolitan and highly diverse in roosting, movement, and feeding ecology. Chiropterans respond to human disturbance by changes in distribution, community composition, and movement patterns. Some of bats' responses to disturbance are also linked to changes in probabilities of pathogen spillover and disease emergence. Herpesvirus are highly prevalent in bats and are supposed to have low impacts on bat survival, with no known symptoms of infection. Here we used phylogenetic analyses on two viral genes (polymerase and glycoprotein) to assess the relatedness strains infecting vampire bats (*Desmodus rotundus*), and its dependence with geographic structure of host populations. Between 2017 and 2019, we sampled vampire bats from seven colonies in Uruguay and two in Belize, with pairwise distances ranging from eight to 245 km. We show that *D. rotundus* host a cluster of highly related gammaherpesvirus across their geographic range and that likelihood of shared viruses is affected by individual- and colony-level variables. In particular, we show that younger bats and bats using the same roost are more likely to share identical viruses. We propose that herpesviruses are a good system for tracking vampire bat connectivity across colonies,

representing a cost- and time-efficient tool to track movements and response to landscape modifications.

Introduction

A host's movements affect the ecology, diversity, and evolution of its pathogens (Duke-Sylvester et al. 2013, Boulinier et al. 2016). For example, migration can affect the probability of coinfection by and recombination of influenza viruses in wild birds, and contact among colonies of bats facilitates the persistence of lyssaviruses (Olsen et al. 2006, Hill et al. 2012, Streicker et al. 2012b, 2012a, Blackwood et al. 2013, Tian et al. 2015, Horton et al. 2020). Because pathogens usually have short generation times and are more abundant than their hosts, genetic diversity of a microbe can be used to track host movement and contact among hosts (Biek et al. 2006, Duke-Sylvester et al. 2013). For a pathogen to be useful as a population marker, it ideally should cause a persistent infection and have high prevalence, low pathogenicity, large interhost diversity, and high host specificity. Lentiviruses have these attributes, and one lentivirus, feline immunodeficiency virus, was used to infer population dynamics of cougars (*Puma concolor*) in the western United States, including population bottlenecks and movement over decades (Biek et al. 2006, Poss et al. 2008).

While lentiviruses have many of the positive attributes for a community tracking viral pathogen, another class of virus shares many of the same attributes. *Herpesviridae*, the most diverse family within Herpesvirales, an order of DNA viruses that can infect most vertebrates (Davison et al. 2009, Escalera-Zamudio et al. 2016), also may serve as population markers. Viruses in this family infect reptiles, birds, and mammals. Members

of the three subfamilies, *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*, are classified on the basis of viral structure, DNA sequence similarities, life cycles, and pathogenicity (Davison 2002, Davison et al. 2009). Members of each subfamily infect humans, domestic animals, and wild animals. Herpesviruses have been detected in bats worldwide, especially in Africa, Europe, and Asia (James et al. 2020). Initially, cytomegalovirus-like particles were detected in salivary glands of vespertilionid bats by electron microscopy (Tandler 1996), and then betaherpesvirus and gammaherpesvirus DNA was detected in the lungs of European vespertilionid bats (Wibbelt et al. 2007). Herpesvirus diversity in bats appears to be high. Most viruses are species-specific, and there is some evidence of coinfections (James et al. 2020).

To our knowledge, no adequate pathogen model has been used to infer connectivity and movement of bats, despite efforts in this direction (Bergner et al. 2020). Bats are the second most species-rich order of mammals, with 1426 species worldwide (Simmons and Cirranelo 2020). The order Chiroptera, which occurs on all continents except Antarctica, is both species-rich and diverse with respect to ecological traits such as diet, roost use, social structure, and movement patterns (Jones et al. 2009, Kunz et al. 2011, Teeling et al. 2018). Bats serve ecological functions that can are key for ecosystem persistence and can be valued at billions of dollars per year (Boyles et al. 2011). Although bats have been associated with several outbreaks of infectious diseases and spillovers, it is unclear whether they are more likely than other mammals to carry and spread zoonotic viruses (Brook and Dobson 2015, Luis et al. 2015, López-Baucells et al. 2017, Olival et al. 2017, Washburne et al. 2018, Mollentze and Streicker 2020). Spillovers from bats are associated with land-

cover and land-use change (Plowright et al. 2011, Kessler et al. 2018, López-Baucells et al. 2018). Changes in species richness, contact with humans and domestic animals, resource availability, movement patterns, and connectivity among colonies may affect the role of bats in pathogen transmission (Plowright et al. 2011, Becker and Hall 2014, Gottdenker et al. 2014, Rulli et al. 2017, Becker et al. 2018, Faust et al. 2018, Botto Nuñez et al. 2020).

The Neotropics are inhabitated by the most species-rich family of bats, the Phyllostomidae (223 species) (Solari et al. 2019, Simmons and Cirranelo 2020). Despite this high species richness, few bat herpesviruses have been sequenced (Escalera-Zamudio et al. 2016, Wray et al. 2016, James et al. 2020). Most studies used small sample sizes of multiple species of bats. In general, bat herpesviruses are thought to be phylogenetically diverse (Escalera-Zamudio et al. 2016, James et al. 2020). Three studies had large sample sizes. Two were conducted in northern South America (Perú, French Guiana, and Martinique) and one was conducted in Uruguay (Moreira 2019, Griffiths et al. 2020, James et al. 2020). The study in Perú, which focused on betaherpesvirus, encompassed 22 species and 139 samples obtained over four years (Griffiths et al. 2020). Studies in Uruguay and French Guiana and Martinique examined a similar number of species (11 and nine, respectively); work in Uruguay included a greater number of samples (195 versus 77) and was of longer duration (10 versus three years) (Moreira 2019, James et al. 2020). French Guiana and Martinique are dominated by Phyllostomids (60 of 106 species and six of 11 species, respectively) (Simmons and Cirranelo 2020), whereas three of 22 species of bats

in Uruguay are Phyllostomids, and two of those have small ranges (Botto Nuñez et al. 2019b).

In this study, we focused on herpesvirus diversity in a widespread Neotropical bat species that is the main reservoir of rabies virus, the common vampire bat (*Desmodus rotundus*) (Johnson et al. 2014, Delpietro et al. 2017). *Desmodus rotundus* occurs from northern Mexico to Uruguay and central Chile in ecosystems from temperate grasslands to tropical rainforests to coastal cliffs in dry areas of Peru and Chile (Greenhall et al. 1983, Catenazzi and Donnelly 2008, Delpietro et al. 2017, Rodríguez-San Pedro and Allendes 2017).

Understanding movement and connectivity of vampire bats may increase the ability to forecast rabies risks (Streicker et al. 2012b, Blackwood et al. 2013, Benavides et al. 2016, Delpietro et al. 2017). Molecular methods and capture-mark-recapture studies were used to understand the effect of bat movements in rabies transmission and persistence (Streicker et al. 2012b, 2012a, 2016, Blackwood et al. 2013, Benavides et al. 2016, Becker et al. 2020, Bergner et al. 2020). Capture-mark-recapture studies are time-intensive, whereas molecular methods assume that connectivity is reflected in genetic patterns. These methods may overlook non-reproductive interactions and contacts that do not imply immigration. Herpesvirus may be an alternative marker of bat connectivity, especially as it is present in saliva, can be transmitted during social contacts (similar to the transmission route of rabies virus), and infections are assumed to be long-lasting and to have little or no impact on bat health (Wibbelt et al. 2007, Escalera-Zamudio et al. 2016, Wray et al. 2016, James et al. 2020). Moreover, when studying rabies dynamics, having a more prevalent

marker with a similar transmission route (saliva) can be of special interest as a proxy of exposure and contact among bats.

We analyzed herpesvirus diversity in saliva swabs collected in Belize and Uruguay. Both study areas are at marginal locations of the species' distribution but have different species composition of bats and landscape structure, although Uruguay is positioned at the extreme southern distributional limit (Becker et al. 2019, 2020, Botto Nuñez et al. 2019a). We obtained samples for this work by capitalizing on a bat research program established in Belize in 2014 (Herrera et al. 2018, Ingala et al. 2019, Becker et al. 2020) and an ongoing study of *D. rotundus* in Uruguay.

Materials and Methods

Study System

We collected saliva swab samples from vampire bats in Belize and Uruguay (Figure 1). The two colonies of *D. rotundus* that we sampled in Belize have studied since 2014, and connectivity has been estimated with six years of capture-recapture data and microsatellites data (Herrera et al. 2018, Ingala et al. 2019, Becker et al. 2020). We captured bats during two-week sampling periods in 2018 and 2019 in the Lamanai Archeological Reserve and the Ka'Kabish Reserve, both in Orange Walk District. Sites are separated by 8 km in a rainforest fragmented by clearing for agriculture (livestock rearing and crops) (Herrera et al. 2018, Ingala et al. 2019, Becker et al. 2020). Across Uruguay, *D. rotundus* colonies are sampled for rabies surveillance and modeling. We captured individuals in seven colonies in eastern Uruguay from October 2017 through January 2019.

The colonies were in low hills in grasslands that are planted with non-native forests (Botto Nuñez et al. 2020). The distances among colonies ranged from 13 to 245 km. We also included six sequences from a previous sampling (Moreira 2019).

Animal Handling and Sampling

We captured bats with mist nets, hand nets, and harp traps at the entrance or inside of roosts. We held bats in soft-cloth bags until processing (Kunz et al. 2009, Becker et al. 2020). We recorded the sex, age class, and reproductive status of each bat. Field protocols followed the American Society of Mammalogists' guidelines for safe and humane handling of mammals (Sikes 2016) and were approved by the Institutional Animal Care and Use Committees of the American Museum of Natural History (AMNHIACUC-20180123), Montana State University (MSU IACUC 2017-30), and Universidad de la República (CEUA FCIEN ID 481-2017). Bat sampling was authorized by the Belize Forest Department under permits WL/2/1/18(16) AND FD/WL/1/19(09) and by the Uruguay's National Environmental Bureau (DINAMA Res DF137/16 and Res 2/2018).

We obtained a saliva sample from each individual with sterile cotton swabs (Puritan) and preserved samples in 1 mL of DNA/RNA Shield (Zymo Inc.). Samples were maintained in the field at -20˚C in a portable freezer or in liquid nitrogen, and then transferred to -80˚C for permanent storage in the lab.

DNA Extraction and PCR Amplification

We extracted total DNA from samples with the Quick DNA/RNA Pathogen miniprep kit (Zymo Inc.) and eluted in a final volume of 50 μL of RNAase-free water.

DNA extractions were preserved at -80˚C. We confirmed extraction of DNA in samples from Belize with cytochrome B amplification, visualized by 1.5% agarose gel electrophoresis. In the case of negative cytochrome B amplification, DNA again was extracted from the original sample. We used PCR to amplify a 450 bp segment of the CytB gene with two primers designed for *D. rotundus* (Martins et al. 2007), Bat 17A (ACCTCCTAGGAGACCCAGACAATT) and Bat 14A (TATTCCCTTTGCCGGTTTACAAGACC). For the PCR reactions, we used the Fast PCR kit (Qiagen) and a cycling protocol of 95˚C for 5 minutes followed by 35 cycles of 96˚C for 5 seconds, 45˚C for 5 seconds, 68˚C for 12 seconds, and a final elongation phase of 72˚C for 1 minute. All samples from Belize had bands consistent with the expected product size for the host gene cytochrome B amplification, indicating successful DNA extraction. We sequenced five samples to confirm the amplification product and showed homology to published *D. rotundus* CytB sequences (accession numbers: FJ847489 and FJ155477).

For herpesvirus screening, we used a nested PCR protocol with degenerate primers, targeting a 215-315 bp segment of the viral DNA polymerase (DPOL) gene (Vandevanter et al. 1996, Anthony et al. 2015). We performed PCR reactions with the Fast PCR kit (Qiagen). The first round used three primers (5’-3’): DFA (GAYTTYGCNAGYYTNTAYCC), KG1 (GTCTTGCTCACCAGNTCNACNCCYTT), and ILK (TCCTGGACAAGCAGCARNYSGCNMTNAA). The second round used a pair of primers (5’-3’): TGV (TGTAACTCGGTGTAYGGNTTYACNGGNGT) and IYG (CACAGAGTCCGTRTCNCCRTADAT). Both rounds followed the same cycling

protocol: 95ºC for 5 minutes followed by 35 cycles of 96˚C for 5 seconds, 46˚C for 8 seconds, 68˚C for 12 seconds, and a final elongation phase of 72˚C for 1 minute.

For the DOPL positive samples, we amplified a 450 bp segment of the gene encoding glycoprotein B (gB) with a nested protocol designed for *Rhadinovirus* (Ehlers et al. 2007). The first round used two primers (5'-3'), 2759s (CCTCCCAGGTTCARTWYGCMTAYGA) and 2762as (CCGTTGAGGTTCTGAGTGTARTARTTRTAYTC). The second round used the following two primers (5'-3'), 2760s (AAGATCAACCCCACNAGNGTNATG) and 2761as (GTGTAGTAGTTGTACTCCCTRAACATNGTYTC). Both rounds used the same cycling protocol: 95˚C for 5 minutes followed by 35 cycles of 96˚C for 5 seconds, 45˚C for 5 seconds, 68˚C for 15 seconds, and a final elongation phase of 72˚C for 1 minute. PCR amplification products were purified and sequenced by Psomagen. We manually edited and aligned sequence reads in GeneStudio. We built maximum-likelihood phylogenetic trees with PhylML (CNRS, http://www.phylogeny.fr/). We estimated pairwise genetic distances with MEGA 10.1.8. for DOPL sequences for samples from Belize and Uruguay separately and for the gB sequences in samples from Belize. We restricted analyses of genetic distance to sequences clustering in the *D. rotundus*-only clade (Figure 2).

Statistical Analyses

We used logistic regression to separately analyze the predictors (sex, age class, and colony of origin) of herpesvirus prevalence in Uruguay and Belize. We considered virus positive samples those that produced a positive PCR fragment and a sequence with

homology to known herpesvirus genes. Samples from each country were analyzed separately. For each pair of samples we created multinomial variables for sex (i.e. M-M= two males, F-F= two females, and M-F= mixed sex) and age classes (i.e. A-A= two adults, SA-SA= two subadults, and A-SA= mixed age classes). We used a Kruskal-Wallis test to compare pairwise genetic distances of DPOL and gB among pairs of individuals from the same colony and from different colonies. We classified members of pairs from Belize by the identity of the colony (Ka'Kabish or Lamanai Archeological Reserve). We used logistic regression to evaluate the effect of sex, age, year of capture, and colony of origin on the probability of a pair of individuals had identical viral sequences (genetic distance = 0). We compared models by calculating the difference in Akaike's information criterion (**Δ**AIC) between a given model and the model best supported by the data and on the basis of Akaike weights (w_i) (Burnham and Anderson 2002). We assessed whether samples from the seven colonies in Uruguay were consistent with a distance-decay effect on the genetic relatedness of detected herpesvirus by analyzing the correlation between genetic and geographic pairwise distances of the polymerase sequences. We conducted all statistical analyses in R 3.6.2 (R Core Team 2019).

To assess the evolutionary distance of the detected DNA polymerase sequences with other known herpesvirus, we constructed a maximum likelihood phylogenetic tree that included our samples from Belize and Uruguay and relevant alphaherpesvirus, betaherpesvirus, and gammaherpesvirus DNA polymerase sequences retrieved from GenBank. We used Human Herpes Simplex 1 (HSV1) to represent *Alphaherpesvirinae*,

and Kaposi's Sarcoma-related virus (HHV-8) and Epstein-Barr virus (HHV-4) to represent *Gammaherpesvirinae*.

Results

We obtained saliva swabs from 151 vampire bats in Belize and 42 vampire bats in Uruguay. Fifty samples from Belize (33%) and 23 samples from Uruguay (55%) elicited a PCR product that was homologous to a herpesvirus polymerase. Samples from Uruguay had significantly higher prevalence of herpesvirus polymerase than those from Belize (χ^2=5.66, p=0.02). Effects of sex, age, or colony of origin on the prevalence of herpesviruses were not statistically significant (Tables 1 and 2).

To assess the evolutionary distance of the detected DPOL sequences with other known herpesvirus, we performed a maximum likelihood phylogenetic tree, including relevant alpha-, beta-, and gammaherpesvirus DPOL sequences retrieved from GenBank. Alphaherpesvirus formed a monophyletic clade of human, porcine, and bat viruses. *Betaherpesvirinae* also formed a monophyletic clade, with the exception of a virus detected from *Tadarida brasiliensis* from Uruguay (LMM31) that previously was assigned t*o Gammaherpesvirinae*. Samples from Uruguayan vampire bats clustered closely with the *Gammaherpesvirinae* (except one sample, D0552, which clustered with *Alphaherpesvirinae*). In contrast, the Belizean samples clustered closely with both *Gammaherpesvirinae* (n=44) and *Betaherpesvirinae* (n=6) (Figure 2).

The sequences that closely match known gammaherpesvirus DPOL from *D. rotundus* were split between a monophyletic group with relatively good statistical support (89%) and a group that included samples from other bat species in South America

(phyllostomids, molossids and vespertilionids). The monophyletic, *D. rotundus*-only group included samples from French Guiana, Belize, and Uruguay, and one sequence from *Pteropus giganteus* from Bangladesh. On the basis of nucleotide identity, the latter sequence is the closest relative to previously described sequences from *D. rotundus* from Guatemala (Wray et al. 2016) (Figure 2).

To understand the factors that may determine relatedness between viral isolates, we evaluated the effect individual- and colony-level factors on viral genetic distance. We found no difference in genetic distance between pairs of individuals from the same colony or different colonies in Belize (χ^2=0.273, p=0.60) independently of which colony the samples came from (χ^2=1.597, p=0.45). Neither sex (χ^2=0.059, p=0.97) nor age (χ^2=3.403, p=0.18) affected pairwise genetic distances.

Pairwise genetic differences in the polymerase gene (distance=0) were not affected by colony (OR=0.84, p=0.27) or sex (OR=1.23, p=0.29 for F-M vs F-F and OR=1.26, p=0.33 for M-M vs F-F) (Table 3). However, the probability that viruses were identical was greater when both individuals were subadults than adults (OR=2.64, p=0.03). Pairwise genetic distance between one adult and one subadult did not differ from that between two adults (OR=1.09, p=0.66). These differences were not affected by colony of origin (Table 3).

Genetic distances between pairs of individuals from the same colony and different colonies in Uruguay were significantly different (χ^2=5.939, p=0.01). Age (both adults versus one adult and one subadult; χ^2=5.281, p=0.02), and sex (same sex versus one male and one female; χ^2=5.501, p=0.02) significantly affected pairwise genetic distances.

Effects of the colony of origin, sex, and age were statistically significant (Table 4). Pairs of individuals, and pairs of individuals of the same sex, from the same colony were more likely to have identical viruses (OR_{adj}=2.18, p=0.02 and OR_{adj}=3.25, p<0.01, respectively). Pairs of subadults and adults were more likely to have identical viruses than pairs of adults (OR_{adj}=2.41, p=0.05).

In Belize samples, the gB gene was more diverse across samples than the polymerase gene (Figure 3). However, the variability in glycoprotein B among pairs of individuals was not explained by differences in sex, age, year, or colony of origin (p=0.31 to 0.80). Logistic regression models did not explain the likelihood that pairs had identical sequences (Table 5).

Discussion

We conducted a spatially extensive study of herpesvirus infection, virus similarity, and genetic diversity of virus in a widely distributed Neotropical bat species. In Uruguay, where we sampled vampire bats from seven colonies, virus sequences from individuals from the same colony were more likely to be identical than those from individuals from different colonies, and virus sequences from pairs of subadult bats were more likely to be identical viruses than from pairs of adults or one adult and one subadult. Therefore, herpesvirus diversity across space and time potentially could provide inferences on host movement or roosting ecology.

Geographic and ecological diversity may have affected our results. Uruguay is the southernmost extent of the range of vampire bats. We sampled bats in a temperate grassland

that long has been used for livestock rearing (Botto Nuñez et al. 2019a). By contrast, Belize is toward the northern extent of the species' distribution (which extends to northern Mexico; Piaggio et al. 2017). Our sites were in a tropical ecosystem in which forests recently were converted to pasture and crops (Herrera et al. 2018, Ingala et al. 2019, Becker et al. 2020). The composition of the bat community differs between Belize and Uruguay. Belize has higher species richness and a large proportion of phytophagous phyllostomids. Most of the bats in Urugay are insectivorous molossids and vespertilionids (Herrera et al. 2018, Botto Nuñez et al. 2019b, Simmons and Cirranelo 2020). These differences, especially when multiple species occupy the same roost, could affect viral sharing between *D. rotundus* and other species, particularly phylogenetically similar hosts. In Uruguay, we found less evidence of exchanges of viruses between *D. rotundus* and with other species within our herpesvirus phylogeny, and most of the samples clustered in the *D. rotundus*-exclusive clade.

Our results are consistent with previous suggestions that the diversity of herpesviruses in bats has been underestimated (Escalera-Zamudio et al. 2016, James et al. 2020). Moreover, although our results generally are consistent with species-specific herpesvirus infections in bats (Griffiths et al. 2020), the distribution of viral sequences from *D. rotundus* suggest some exchanges with other bat species. Most Gammaherpesvirus sequences from *D. rotundus* in Uruguay cluster in a clade restricted to viral sequences from *D. rotundus*, whereas some samples from Belize clustered outside that *D. rotundus*-only clade (Figure 2). The absence of other phyllostomids in the Uruguayan colonies and the low relative species richness of that family in temperate regions could explain this

observation. By contrast, bat community in Belize is more diverse and especially with higher species richness of phyllostomid bats. The results are consistent with the suggestion that a core group of herpesviruses infect *D. rotundus* throughout its range, and that these viruses sometimes are transmitted to other phyllostomids in more taxonomically diverse areas. If further studies are consistent with this pattern, *D. rotundus*-exclusive herpesvirus sequences might be used to understand intraspecific connectivity, whereas viruses that are shared among genera might provide inferences about interspecific connectivity. The use of herpesvirus sequences as markers of intraspecific and interspecific interactions would be especially welcome given that technologies to record such interactions in the field, such as ultraviolet-fluorescent dust and lightweight proximity loggers, still pose logistical challenges (Hoyt et al. 2018, Ripperger et al. 2020). Additionally, understanding intraspecific and interspecific connectivity may help explain the persistence of pathogens across extensive areas. Measures of relative connectivity could be used for modeling the transmission of rabies virus within and among bat species and for differentiating the roles of intraspecific and interspecific contacts in persistence of the pathogen.

We detected a higher prevalence of herpesviruses in Uruguayan bats than Belizean bats, but we did not identify significant predictors of infection. Betaherpesvirus and gammaherpesvirus sequences from Belizean *D. rotundus* were more variable than those from Uruguay (Figure 2). By contrast, previous analyses in Uruguay, not restricted to *D. rotundus*, indicated that age was a strong predictor of prevalence of infection, with adults more likely to be infected (Moreira 2019). However, in our work, age class was significantly associated with the genetic similarity of viruses.

Bats from the same colony in Uruguay were more likely to have identical viruses than those from different colonies (Table 4). We did not observe this effect in samples from Belize (Table 3), where the two sampled colonies are within 8 km and exchanges of individuals have been observed multiple times across multiple years (Becker et al. 2020). In Uruguay, we have observed only one exchange of bats among colonies during two years with 353 captures, and this exchange was between roosts less than 1 km apart. These observations are consistent with known movements of *D. rotundus*. In Brazil, banding data suggested 2-3 km foraging distances, with low roost fidelity (Trajano 1996). Telemetry studies, also in Brazil, indicated that daily movement distances generally are short (1 and 0.45 km average for males and females, respectively), and that farms from which *D. rotundus* bites on livestock were reported were within 7 km of occupied *D. rotundus* roosts (Rocha et al. 2020). Long-term research in Argentina provided evidence that most exchanges are between shelters less than 1 km apart, and that exchanges was driven by dispersal of juvenile males (Delpietro et al. 2017).

A potential explanation for the higher probability that subadults had genetically identical viruses is a more diverse herpesvirus community in older hosts. Older hosts may have more-diverse viral sequences due to persistent, latent infection combined with enhanced susceptibility to new infections. Age-related increases in prevalence and seroprevalence of herpesvirus have been observed in wild and captive animals, and human populations (Olsen et al. 1998, Juhász et al. 2001, Flach et al. 2002, Craig et al. 2005, Buckles et al. 2007, Cho et al. 2009). In artiodactyls, evidence suggests early gammaherpesvirus infection (either horizontal transmission in newborns and young

animals or vertical transmission during pregnancy), followed by establishment of life-long latent infections (Flach et al. 2002). Data from gammaherpesvirus infections in wild sea lions suggest a similar pattern (Buckles et al. 2007). Latent herpesvirus infections are linked to immunosenescence, compromising the ability of persistently infected individuals to respond to new pathogens by reducing CD8+ lymphocytes subpopulations and triggering immunosuppressing states in the hosts with latent infections (Khan et al. 2004, Cho et al. 2009, Smithey et al. 2012, Dupont and Reeves 2016). The immune aging process can be enhanced by herpesvirus infection and increase the likelihood of new infections in older individuals. These processes increase the prevalence of herpesvirus infection with age and decrease the likelihood of finding identical viruses in older individuals as we observed here.

Other studies have indicated that younger vampire bats have higher viral diversity, probably because their immune systems are immature (Bergner et al. 2020). Even if the rate of infection decreases over time, if bats acquire more infections as they become older, and infections are persistent, more-diverse herpesvirus communities are likely in older individuals. Similarly, in cougars, feline immunodeficiency virus, another life-long infection, was more prevalent in adults, which might be exposed at any time during their life (Biek et al. 2003).

Regarding herpesvirus diversity, in our study we observed only a few sequences related to betaherpesvirus in samples from *D. rotundus* from Belize (Fig 2), but betaherpesvirus consistently has been detected in *D. rotundus* samples from Perú, Guatemala, and French Guiana (Wray et al. 2016, Griffiths et al. 2020, James et al. 2020),

and a study in Perú found 80-100% prevalence of betaherpesvirus infections across *D. rotundus* colonies (Griffiths et al. 2020). The lower prevalence in our samples from Belize might be explained by the gammaherpesvirus bias reported in the primer set we used for polymerase screening (Vandevanter et al. 1996, Anthony et al. 2015). Given this bias and the screening approach we used, we cannot rule out beta and gamma coinfections in our sample.

Coinfections might explain both the higher prevalence of gammaherpesvirus in our sample and the higher relatedness of viruses in younger individuals. If adults have higher herpesvirus diversity, the likelihood of finding identical sequences will decrease with age, and use of PCR coupled with Sanger sequencing will provide one sequence per sample. Coinfections with different strains of herpesvirus were detected in French Guiana and Martinique with cloned PCR products (James et al. 2020). Sequence cloning allows for the detection of coinfections, but not for full characterization of the within-host viral community, because the resolution is limited by the number of clones (Niel et al. 2000, Fu et al. 2008, Van Rooyen et al. 2013, Motohashi 2019). By contrast, metabarcoding might allow for the characterization of those communities, especially because the primers already in use for herpesvirus screening allow for detection of variability and assessment of species-specificity in the infections (Wray et al. 2016, James et al. 2020). Moreover, the expected length of the amplicons (215 to 315 bp) (Vandevanter et al. 1996, Ehlers et al. 2007, Anthony et al. 2015) is compatible with metabarcoding methods used to assess diet structure (Galan et al. 2012, Forin-Wiart et al. 2018).

We found greater variability (larger pairwise distances) for the gB sequences than for the DPOL sequences (Figure 3 A, B, D). This is consistent with the expectation of greater variability in a sequence related to cell entry than in a tightly regulated enzyme, such as the polymerase. However, this is inconsistent with the results of a study in which variability in the polymerase sequences was greater (James et al. 2020). The amplification of the gB gene had lower sensitivity than the polymerase, as reported previously (Ehlers et al. 2007, 2008). Greater variability would improve the resolution of connectivity inferred by herpesvirus diversity, but the smaller number of sequences also could constrain the analyses by reducing the number of positive samples. In our study, the differences in the gB gene were not explained by differences in age, sex, colony of origin, or year of capture (Table 5). Hence, the polymerase, if combined with the assessment of intrahost viral communities, may be more relevant for connectivity studies. Even with lower variability than that of a lentivirus such as feline immunodeficiency virus (Biek et al. 2006, Poss et al. 2008), our results indicate that genetic similarity of herpesviruses affected by age class and colony identity, making it useful for understanding host ecology and host-virus interactions. This lower variability constrains the temporal and spatial resolution of the analyses, but it might still provide information on non-reproductive contacts that would be underrepresented when using host genetic markers.

Our analyses support the hypothesis that herpesviruses can serve as population markers in vampire bats. Prevalence is consistently high (Wray et al. 2016, Moreira 2019, Griffiths et al. 2020, James et al. 2020), ensuring large sample sizes of positive individuals. Low or no pathogenicity has been suggested, although this has not been established in wild

bat hosts (Wray et al. 2016, Griffiths et al. 2020, James et al. 2020). Knowledge of herpesvirus biology suggests that infections are long-term. Our results, in combination with those of other recent studies (Escalera-Zamudio et al. 2016, Wray et al. 2016, Griffiths et al. 2020, James et al. 2020), suggest generally strong host specificity, at least for the subset of the herpesviruses strains that infect vampire bats. The use of these viruses as a molecular marker for tracing contacts of vampire bat populations needs to be tested in wild bat populations, and compared with host genetics and host movement studies, including capture-recapture and telemetry studies.

Our ongoing capture-mark-recapture study in both Uruguay and Belize will allow us to directly test whether herpesvirus infection affects survival and how age affects viral prevalence and diversity. Linking survival analyses to infection status, the presence of coinfections, and bat immune phenotypes could shed light on the capacity to use herpesvirus-vectored vaccines for rabies control in *D. rotundus*, as has been recently proposed (Griffiths et al. 2020). In particular, describing the effects of latent beta and gammaherpesvirus infections on immunity in wild bats, along with characterizing age-dependent transmission patterns, might inform selection of a viral vector candidate and identification of demographic subgroups within bat populations as vaccination targets.

Understanding how bat populations move and connect is highly relevant to public health and conservation. Bat responses to landscape modification by humans can trigger pathogen spillover and disease emergence, and affect population viability. In this context, a tool that allows for monitoring of changes in population structure and spatial dynamics is of great value for practical decision making. Long-term monitoring of bat movements

typically is cost, time, and effort intensive. We propose that a molecular approach to track bat movements offers promise for studies of bat ecology and infectious disease.

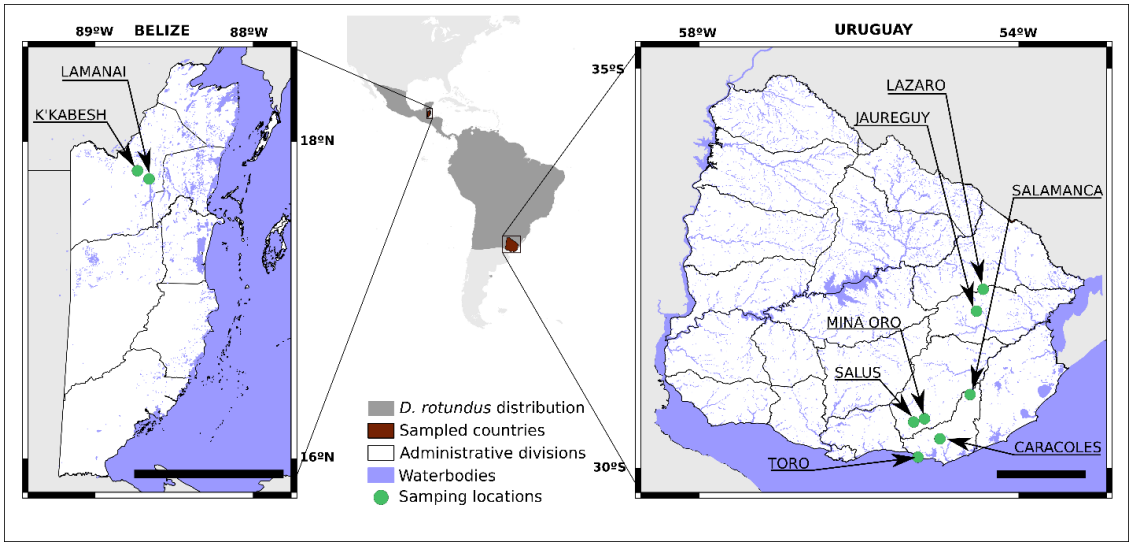

Figure 1. Sampling locations in Belize and Uruguay and distribution of *Desmodus rotundus* according to the International Union for Conservation of Nature. Scale bars represent 100 km.

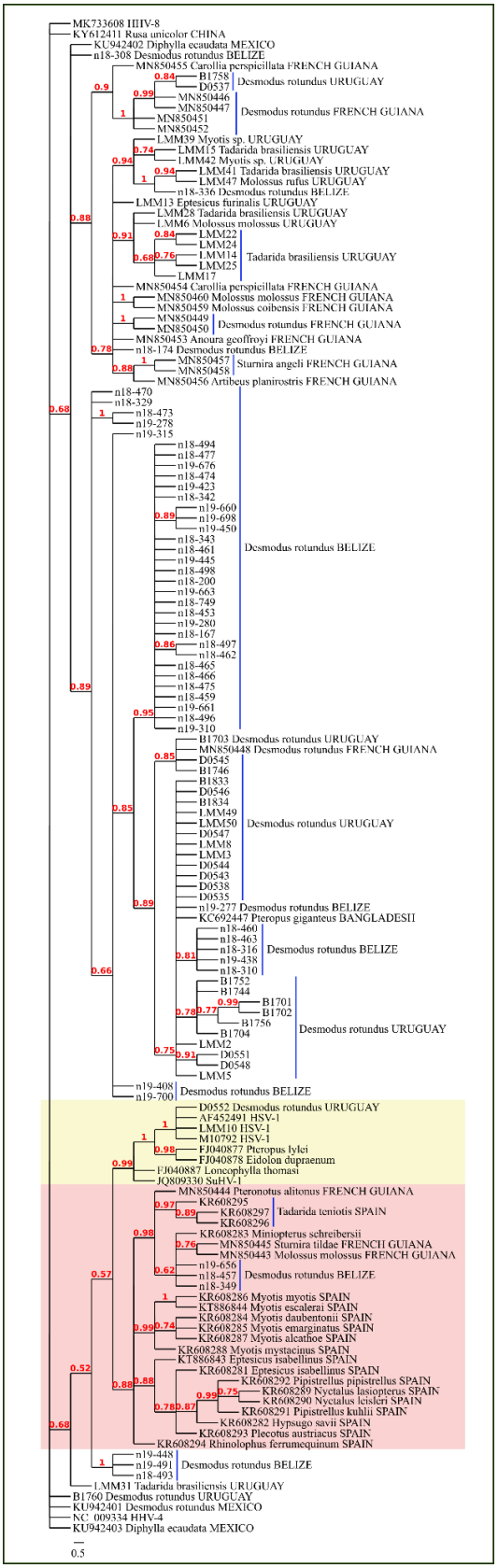
MK733608 HHV-8
KY612411 Rusa unicolor CHINA
KU942402 Diphylla ecaudata MEXICO
n18-308 Desmodus rotundus BELIZE
MN850455 Carollia perspicillata FRENCH GUIANA
B1758
D0537
Desmodus rotundus URUGUAY
MN850446
MN850447
MN850451
MN850452
Desmodus rotundus FRENCH GUIANA
LMM39 Myotis sp. URUGUAY
LMM15 Tadarida brasiliensis URUGUAY
LMM42 Myotis sp. URUGUAY
LMM41 Tadarida brasiliensis URUGUAY
LMM47 Molossus rufus URUGUAY
n18-336 Desmodus rotundus BELIZE
LMM13 Eptesicus furinalis URUGUAY
LMM28 Tadarida brasiliensis URUGUAY
LMM6 Molossus molossus URUGUAY
LMM22
LMM24
LMM14
LMM25
Tadarida brasiliensis URUGUAY
LMM17
MN850454 Carollia perspicillata FRENCH GUIANA
MN850460 Molossus molossus FRENCH GUIANA
MN850459 Molossus coibensis FRENCH GUIANA
MN850449
MN850450
Desmodus rotundus FRENCH GUIANA
MN850453 Anoura geoffroyi FRENCH GUIANA
n18-174 Desmodus rotundus BELIZE
MN850457
MN850458
Sturnira angeli FRENCH GUIANA
MN850456 Artibeus planirostris FRENCH GUIANA
n18-470
n18-329
n18-473
n19-278
n19-315
n18-494
n18-477
n19-676
n18-474
n19-423
n18-342
n19-660
n19-698
n19-450
n18-343
n18-461
n19-445
n18-498
n18-200
n19-663
n18-749
n18-453
n19-280
n18-167
n18-497
n18-462
n18-465
n18-466
n18-475
n18-459
n19-661
n18-496
n19-310
Desmodus rotundus BELIZE
B1703 Desmodus rotundus URUGUAY
MN850448 Desmodus rotundus FRENCH GUIANA
D0545
B1746
B1833
D0546
B1834
LMM49
LMM50
D0547
LMM8
LMM3
D0544
D0543
D0538
D0535
Desmodus rotundus URUGUAY
n19-277 Desmodus rotundus BELIZE
KC692447 Pteropus giganteus BANGLADESH
n18-460
n18-463
n18-316
n19-438
n18-310
Desmodus rotundus BELIZE
B1752
B1744
B1701
B1702
B1756
B1704
LMM2
D0551
D0548
LMM5
Desmodus rotundus URUGUAY
n19-408
n19-700
Desmodus rotundus BELIZE
D0552 Desmodus rotundus URUGUAY
AF452491 HSV-1
LMM10 HSV-1
M10792 HSV-1
FJ040877 Pteropus lylei
FJ040878 Eidolon dupraenum
FJ040887 Loncophylla thomasi
JQ809330 SuHV-1
MN850444 Pteronotus alitonus FRENCH GUIANA
KR608295
KR608297
KR608296
Tadarida teniotis SPAIN
KR608283 Miniopterus schreibersii
MN850445 Sturnira tildae FRENCH GUIANA
MN850443 Molossus molossus FRENCH GUIANA
n19-656
n18-457
n18-349
Desmodus rotundus BELIZE
KR608286 Myotis myotis SPAIN
KT886844 Myotis escalerai SPAIN
KR608284 Myotis daubentonii SPAIN
KR608285 Myotis emarginatus SPAIN
KR608287 Myotis alcathoe SPAIN
KR608288 Myotis mystacinus SPAIN
KT886843 Eptesicus isabellinus SPAIN
KR608281 Eptesicus isabellinus SPAIN
KR608292 Pipistrellus pipistrellus SPAIN
KR608289 Nyctalus lasiopterus SPAIN
KR608290 Nyctalus leisleri SPAIN
KR608291 Pipistrellus kuhlii SPAIN
KR608282 Hypsugo savii SPAIN
KR608293 Plecotus austriacus SPAIN
KR608294 Rhinolophus ferrumequinum SPAIN
n19-448
n19-491
n18-493
Desmodus rotundus BELIZE
LMM31 Tadarida brasiliensis URUGUAY
B1760 Desmodus rotundus URUGUAY
KU942401 Desmodus rotundus MEXICO
NC_009334 HHV-4
KU942403 Diphylla ecaudata MEXICO
0.5

Figure 2. Maximum likelihood tree for the Herpes polymerase gene. Node support are calculated using approximate likelihood ratio test (aLRT). Nodes with support values below 0.5 were collapsed. Tree includes our samples from Belize and Uruguay and sequences of herpesviruses from other bats and terrestrial mammals that are archived in GenBank.

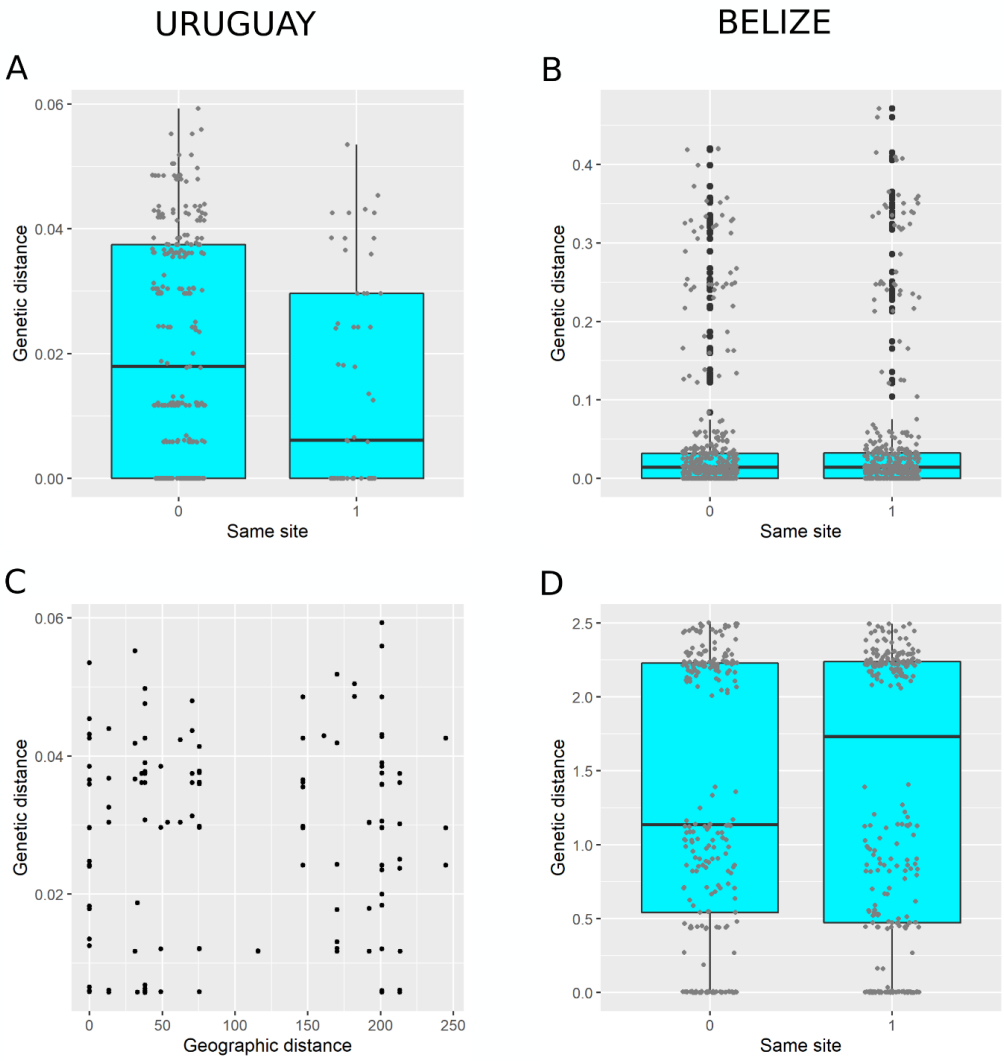

Figure 3. Pairwise genetic distances for polymerase sequences in samples from *Desmodus rotundus* from Uruguay (A and C) and Belize (B) and for glycoprotein B sequences from *D. rotundus* from Belize (D). In A, B, and D, pairs of individuals were from different colonies (0) or the same colony (1).

Table 1. Logistic regression models for herpesvirus infection in samples from Belize. **Δ**AIC, Akaike information criterion; w_i, Akaike weights. For each variable, we present point estimates for regression coefficients and p-values. A null model (intercept-only) is also included.

Variables	**Estimate**	**p-value**	**ΔAIC**	**w_i**
HV+ ~ 1			14.91	0.00
Intercept	-0.70			
HV+ ~ Site			13.46	0.00
Intercept	-0.42			
Site (LAR)	-0.47	0.18		
HV+ ~ Age			0.00	0.76
Intercept	-0.79			
Age (subadult)	0.68	0.52		
HV+ ~ Sex			13.00	0.00
Intercept	-0.75			
Sex (male)	0.10	0.35		
HV+ ~ Site + Age + Sex			2.36	0.23
Intercept	-0.49			
Site (LAR)	-0.48	0.20		
Age (subadult)	0.72	0.19		
Sex (male)	-0.12	0.76		

Table 2. Logistic regression models for herpesvirus infection in samples from Uruguay. **Δ**AIC, Akaike information criterion; w_i, Akaike weights. For each variable, we present point estimates for regression coefficients and p-values. A null model (intercept-only) is also included.

Variables	**Estimate**	**p-value**	**ΔAIC**	**w_i**
HV+ ~ 1			0.02	0.30
Intercept	0.19			
HV+ ~ Site			1.85	0.12
Intercept	-0.41			
Site (JAUREGUY)	-0.44	0.70		
Site (LAZARO)	0.81	0.47		
Site (MINA ORO)	1.50	0.31		
Site (SALUS)	2.35	0.09		
Site (TORO)	0.00	1.00		
HV+ ~ Age			0.41	0.25
Intercept	0.32			
Age (subadult)	-1.42	0.24		
HV+ ~ Sex			0.00	0.30
Intercept	-0.47			
Sex (male)	0.96	0.16		
HV+ ~ Site + Age + Sex			4.71	0.03
Intercept	-0.27			
Site (JAUREGUY)	-0.49	0.70		
Site (LAZARO)	0.81	0.50		

Site (MINA ORO)	1.21	0.44
Site (SALUS)	2.08	0.16
Site (TORO)	-0.29	0.84
Age (subadult)	-1.32	0.32
Sex (male)	0.16	0.89

Table 3. Logistic regression models of the probability that two individuals had identical viral sequences, expressed as genetic pairwise distances = 0 (Distance.0=1), for a subset of the *Desmodus rotundus* samples from Belize (those forming a *D. rotundus*-only monophyletic clade within *Gammaherpesvirinae*). K-K, both individuals from Ka'Kabish; L-L, both individuals from Lamanai Archeological Reserve; L-K, one individual from each site; M-M, both males; F-F, both females; F-M, one male and one female; A-A, both adults, SA-SA, both subadults; A-SA, one adult and one subadult. A null model (intercept-only) is also included.

Variables	Estimate	p-value	ΔAIC	w_i
Distance.0 ~ 1			166.53	0.00
Intercept	-0.91			
Distance.0 ~ Site			167.29	0.00
Intercept	-0.83			
Site (same)	-0.17	0.27		
Distance.0 ~ Site.id			169.07	0.00
Intercept	-1.05			
Site (L-K)	0.22	0.24		
Site (L-L)	0.11	0.64		
Distance.0 ~ Sex			109.04	0.00
Intercept	-1.11			
Sex (F-M)	0.21	0.29		
Sex (M-M)	0.23	0.33		
Distance.0 ~ Age			0.00	0.67
Intercept	-1.07			

Age(A-SA)	0.08	0.66		
Age(SA-SA)	0.97	*0.03		
Distance.0 ~ Year			168.07	0.00
Intercept	-0.86			
Year (same)	-0.10	0.50		
Distance.0 ~ Site + Age			1.40	0.33
Intercept	-1.00			
Site (same)	-0.13	0.43		
Age(A-SA)	0.07	0.69		
Age(SA-SA)	0.97	*0.03		

Table 4. Logistic regression models of the probability that two individuals had identical viral sequences, expressed as genetic pairwise distances = 0 (Distance.0=1), for a subset of the *Desmodus rotundus* samples from Uruguay (those forming a *D. rotundus*-only monophyletic clade within *Gammaherpesvirinae*). Capture site and year of capture could be same or different. M-M, both males; F-F, both females; F-M, one male and one female; A-A, both adults, SA-SA, both subadults; A-SA, one adult and one subadult. A null model (intercept-only) is also included.

Variables	**Estimate**	**p-value**	**ΔAIC**	**w_i**
Distance.0 ~ 1			21.47	0.00
Intercept	-0.90			
Distance.0 ~ Site			15.68	0.00
Intercept	-1.07			
Site (same)	0.88	*<0.01		
Distance.0 ~ Sex			5.67	0.05
Intercept	-1.82			
Sex (same)	1.26	*<0.01		
Distance.0 ~ Sex			3.82	0.12
Intercept	-2.20			
Sex (F-M)	0.38	0.73		
Sex (M-M)	1.70	0.11		
Distance.0 ~ Age			18.36	0.00
Intercept	-0.99			
Age(A-SA)	0.99	*0.02		
Distance.0 ~ Year			23.47	0.00

Intercept	-0.90			
Year (same)	0.01	0.97		
Distance.0 ~ Site + Sex + Age			0.00	0.83
Intercept	-1.99			
Site (same)	0.78	*0.02		
Sex (same)	1.18	*<0.01		
Age(A-SA)	0.88	*0.05		

Table 5. Logistic regression models of the probability that two individuals had identical sequences of the glycoprotein B gene, expressed as genetic pairwise distances = 0 (Distance.0=1), for a subset of the *Desmodus rotundus* samples from Belize (those forming a *D. rotundus*-only monophyletic clade within *Gammaherpesvirinae*). K-K, both individuals from Ka'Kabish; L-L, both individuals from Lamanai Archeological Reserve; L-K, one individual from each site; M-M, both males; F-F, both females; F-M, one male and one female; A-A, both adults, SA-SA, both subadults; A-SA, one adult and one subadult. A null model (intercept-only) is also included.

Variables	**Estimate**	**p-value**	**ΔAIC**	**w_i**
Distance.0 ~ 1			40.92	0.00
Intercept	-2.74			
Distance.0 ~ Site			42.61	0.00
Intercept	-2.64			
Site (same)	-0.21	0.58		
Distance.0 ~ Site.id			43.89	0.00
Intercept	-2.50			
Site (L-K)	-0.14	0.80		
Site (L-L)	-0.50	0.39		
Distance.0 ~ Sex			29.66	0.00
Intercept	-3.00			
Sex (F-M)	0.29	0.58		
Sex (M-M)	0.36	0.54		
Distance.0 ~ Age			0.00	0.99
Intercept	-2.90			

Age(A-SA)	0.82	0.05		
Age(SA-SA)	0.26	0.81		
Distance.0 ~ Year			42.61	0.00
Intercept	-2.64			
Year (same)	-0.21	0.58		

CHAPTER FIVE

VAMPIRE BAT RABIES IN CHANGING LANDSCAPES: HOW LAND USE-INDUCED CHANGES IN POPULATION CONNECTIVITY AFFECT THE RISK OF RABIES VIRUS SPILLOVER

Contribution of Authors and Co-Authors

Manuscript in Chapter 5

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Contributions: Designed the study, analyzed the data, interpreted the results, wrote and revised the manuscript

Co-Author: Richard A. Detomasi Araujo

Contributions: Analyzed the data, interpreted the results, wrote and revised the manuscript

Co-Author: Daniel Streicker

Contributions: Co-designed the study, co- interpreted the results, co-wrote, and revised the manuscript

Co-Author: Raina K. Plowright

Contributions: Co-designed the study, co- interpreted the results, co-wrote, and revised the manuscript

Manuscript Information

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Vampire bat rabies in changing landscapes: how land use-induced changes in population connectivity affect the risk of rabies virus spillover

Status of Manuscript

_X_ Prepared for submission to a peer-reviewed journal

___ Officially submitted to a peer-reviewed journal

___ Accepted by a peer-reviewed journal

___ Published in a peer-reviewed journal

To be submitted to: Ecological Applications

Abstract

Vampire bat borne rabies continues to be a major economic burden for livestock production in Latin America and the Caribbean, and a public health concern in some areas of the region. Previous studies show that immigration is required to maintain rabies virus in colonies, and that population control strategies not only can fail to reduce the transmission to livestock but also can enhance viral persistence in wild populations. More recent studies suggested that changes in movement patterns of the hosts, in response to anthropic landscape fragmentation, may drive livestock rabies emergence. From a disease perspective, connectivity among colonies could reflect infectious contacts in shared feeding areas rather than migration or dispersal among colonies. Here we combine field and modeling strategies to explore how increased connectivity affects the spatial persistence of rabies in vampire bats, and consequently the opportunities for spillover into livestock. We conducted field studies and used empirical data to estimate reproductive success, seasonality of reproduction, and the distribution of bat colonies. We designed a compartmental discrete differences dynamic model to assess the effect of connectivity on pathogen persistence. Our results demonstrated that persistence follows an inverted u-shaped curve in relation to connectivity, where the maximum persistence depends on the colony network spatial structure. Connectivity was the most important factor determining rabies persistence and surprisingly, population turnover only had marginal benefit for persistence and only during conditions of optimal connectivity. If connectivity among colonies responds to land use changes, perhaps managing land use to reduce the risk of

rabies spillover into livestock could be considered as an ecological countermeasure for disease prevention.

Introduction

Vampire bat borne rabies is an important zoonotic disease, that threatens human lives, especially in isolated settings, and causes economic losses, due to livestock disease, in the Neotropics (Johnson et al. 2014, Stoner-Duncan et al. 2014, Freire de Carvalho et al. 2018). There were reports on rabies-like illness in livestock following the attack by vampire bats since the colonial times in the Americas, the first confirmation on the role of this species on bovine rabies was published in 1911 following an outbreak in southern Brazil (Carini 1911).

Pathogen transmission across species, known as spillover, requires specific conditions to align in time and space (Plowright et al. 2017). Several imperfect barriers have to be breached in order for the transmission to occur (Plowright et al. 2017). The presence of the pathogen in the primary host species, pathogen shedding, and contact between reservoir host or pathogen and the recipient host is required for spillover (Plowright et al. 2017). Primary host abundance and distribution, along with pathogen persistence and load are the first determinants of this process (Plowright et al. 2017). As barriers are changing over time and space, the longer a pathogen persists in a community the more opportunities for spillover into recipient hosts. Pathogen persistence might be driven by infection dynamics (e.g., latent infections with recurrent reactivation), host demography (birth pulses), population sizes, waning immunity, migration, and metapopulation dynamics (Lloyd-Smith et al. 2005, Peel et al. 2014)

Different timescales of demographic and transmission processes are critical to explain pathogen persistence in a population (Lloyd-Smith et al. 2005). In particular, from the pathogen side, the duration of the infection, the time from the infection to the onset of symptoms, and the recovery rate are important. Also, the relation of these parameters with the temporal dynamics of the host population: in particular, reproduction timing and seasonality, migratory movements, dispersion, and conformation of social groups. The structure of the population is important as it determines the contact network for each individual. Structured populations are more modular and exhibit different temporal dynamics for the infections. Also, special behaviors within groups can impact the dynamic. In humans, the start of the school year means a great change in the contact network for kids, introducing a highly seasonal driver of infection (Grenfell and Harwood 1997, Grenfell et al. 2001). In wildlife, analogous processes can be represented by juvenile dispersion, seasonal migration, or reproductive swarming behavior. Swarming, for instance, provide an opportunity for increase both in density and also, increase in the proportion of susceptible individuals (Horton et al. 2020). In communities, aside from the proprieties of each population, there are emergent features that derive from the synchronicity (or its absence) across populations. Both in communities or in metapopulations, the asynchrony in infections and influx of susceptibles promotes persistence of infections when populations are connected (at least in intermediate connectivity) (Almberg et al. 2010). On the pathogen side, low pathogenicity and long-term pathogen shedding also promote persistence, by providing a stable source of pathogen. Low basic reproductive number (i.e., the number of secondary infections

expected in a completely naïve population: R_0) also helps persistence by reducing the likelihood of a synchronic massive outbreak that would deplete the susceptible pool. Large outbreaks driven by large R0, especially when combined with short infectious periods and/or slow recovery rates and long-lasting immunity for the recovered individuals, increase the likelihood of epidemic burnouts (Lloyd-Smith et al. 2005)

The common vampire bat (*Desmodus rotundus*) is an obligate hematophagous bat that is distributed from northern Mexico to Uruguay and central Argentina and Chile (Barquez et al. 2015, Piaggio et al. 2017). Feeding habits make the vampire bat a great vector for spillover of rabies virus, as it preys mostly on livestock in human-modified landscapes and rabies is transmitted primarily by bite (Greenhall et al. 1983, Delpietro et al. 2017). Landscape modification has been associated with changes in vampire bat distribution and abundance and it has also been proposed to affect rabies spillover dynamics (Benavides et al. 2016, Becker et al. 2020, Botto Nuñez et al. 2020). Forest clearing for agricultural expansion and livestock rearing has been implicated with changes in rabies distribution in Perú, Brazil, Belize and México (Schneider et al. 2009, Gomes et al. 2010, Fahl et al. 2015, Hutter et al. 2016, Bolívar-Cimé et al. 2019, Santos et al. 2019, Benavides et al. 2020). The increase in resource availability related to livestock rearing might increase contact between vampire bats and domestic animals and also increase vampire bat populations (Benavides et al. 2020). In contrast, in Uruguay, on the southern limit of the species distribution, it has been proposed that grassland fragmentation can also play a role in rabies emergence by changing the vampire bats movement patterns (Botto Nuñez et al. 2020). Grassland fragmentation concentrated livestock in fewer patches,

allowing previously unconnected colonies to share feeding areas (Botto Nuñez et al. 2020). This increased connectivity might alter rabies persistence in vampire bat populations.

In Latin America and the Caribbean, the main strategy to control vampire bat borne rabies in livestock is culling of vampire bat populations to reduce the bite pressure on cattle (Streicker et al. 2012b, Benavides et al. 2020). This strategy has been proposed to be ineffective, at least in some settings; in Perú, as colonies with more intensive culling showed more frequent rabies exposures (Streicker et al. 2012b, Blackwood et al. 2013). Extensive work in Perú has shown that migration between colonies is a key aspect for rabies persistence, and culling is hypothesized to increase migration as culled roosts become available for recolonization (Streicker et al. 2012b, Blackwood et al. 2013, Bakker et al. 2019).

Movement ecology studies in the common vampire bat have been scarce and there is large variation in the proposed home-ranges for the species, ranging from 1 to 20 km (Greenhall et al. 1983, Trajano 1996, Delpietro et al. 2017, Rocha et al. 2020) Many factors, including food availability, landscape disturbance, roost distribution and temperature might affect the movement patterns of the species. Vampire bats are limited in their geographic range by temperature limits, as flying in cold winter nights might require a larger energy expenditure than they might obtain through foraging (McNab 1973). In addition, the species is known for being incapable of tolerating fasting periods longer than 2 nights (McNab 1973, Greenhall et al. 1983).So they might not be able to tolerate environments with a succession of cold nights where they need to fly large distances to get their food.

Reproductive seasonality has been shown to have a large effect on pathogen persistence, with more seasonal reproduction linked to larger outbreaks, but shorter pathogen persistence (because the susceptible pool is replenished over a short period) (Lloyd-Smith et al. 2005, Peel et al. 2014). Vampire bats have been traditionally assumed to be a non-seasonal polyestrous species (Greenhall et al. 1983, Delpietro et al. 2017). In theory, this would allow a trickle of susceptibles into the population, sustaining pathogen persistence, but avoiding large disease outbreaks. However, some seasonal birth pulses have been described, mostly in response to rainfall but not temperature (Nunez and de Viana 1997, Gomes and Uieda 2004, Delpietro et al. 2017). As a widespread species, vampire bats are exposed to a variety of climatic conditions, and some variability in the reproductive dynamic across their distributional range is to be expected. In order to effectively incorporate demographics into disease dynamic modeling, an annual reproductive function should be estimated. Peel et al (2014) proposed a field-based method to estimate wildlife birth pulses in order to be used in population dynamic modeling (Peel et al. 2014). A reproductive pulse was estimated for vampire bats in Perú and incorporated in dynamic disease models (Blackwood et al. 2013, Bakker et al. 2019), but there is no evidence to support that it can be assumed constant across the specie's distribution. As vampire bats have long pregnancy and as capture success might not be equal for adults and newborns, the estimation of birth pulses might be difficult. In some cases, capture and dissection of an entire colony was used to assess the reproductive dynamic of the population (Langguth and Achaval 1972) but those methods might present only a biased approach (depending on the time of year and the type of colony) and might not be

generalizable. Together with reproductive seasonality, global annual reproductive and mortality rates are expected to influence pathogen persistence as faster population turnovers provides faster replenishing of the susceptible pool in the population (Lloyd-Smith et al. 2005).

Here we combine field data and mathematical modeling to describe the effects of connectivity and birth synchrony across colonies on landscape-level pathogen persistence. We considered connectivity across colonies as a process that can involve an indirect contact through an unknown third colony, or more likely contacts at the foraging areas: migration-independent connectivity. We sampled 16 colonies of vampire bats in the southernmost limit of the species distribution to assess the reproductive success and seasonality and used these data to parametrize compartmental discrete-time models, with migration-independent connectivity and rabies transmission.

Materials and Methods

Ethic Statement

All captures were made under two scientific capture permits issued by the environment bureau in Uruguay (Dirección Nacional de Medio Ambiente, Ministerio de Vivienda Ordenamiento Territorial y Medio Ambiente), files 137/16 (approved on January 5th, 2016) and 2/2018 (approved on May 17th, 2018). The animal handling protocol was approved by the Montana State University Institutional Animal Use and Care Committee (Protocol 2017-30 approved on July 10th, 2017) and by the Sciences College - Universidad de la República Committee on Ethical use of Animals (CEUA, protocol number ID481, approved on June 20th, 2017). The biosafety protocol was approved by the Montana State

University Institutional Biosafety Committee (protocol 035-2017, approved on June 14th, 2017).

Study area

Uruguay is situated in the southern cone of South America, in the del Plata basin, between 30º and 35º south latitude, and between 53º and 59º west longitude (Supplementary Figure 1). The main natural landscape of the country are slightly undulated grasslands, with riparian forests along riverbanks (Evia and Gudynas 2000, PNUMA 2008). The climate is temperate with four distinct seasons marked by temperature (Evia and Gudynas 2000, PNUMA 2008). Temperatures show a northeast-southweast pattern with lower temperatures in the south (Evia and Gudynas 2000, PNUMA 2008). The main primary economic activities are livestock production (mostly grassfed), agriculture (where soybean and wheat are main crops) and allochthonous forestry for cellulose and wood production (PNUMA 2008, Botto Nuñez et al. 2020). During the last decades, the country experienced an increase in agricultural activities, especially intensive crop production and afforestation, that led to fragmentation of natural grassland and grazing areas (Evia and Gudynas 2000, PNUMA 2008, Botto Nuñez et al. 2020).

Capture and Handling

We captured vampire bats using mist nets and hand nets (Kunz et al. 2009), at the entrance or inside known shelters across the country (Supplementary Figure 1). Bats were sexed according to external genitalia and pregnancy was detected by abdominal palpation (Racey 2009). Captured bats were wing banded using 3.5mm incoloy bands (Porzana Ltd).

After obtaining samples of blood (blood smear), serum, saliva (oropharyngeal swabs) and skin (wing biopsy), the bats were released at the point of capture.

Reproductive Seasonality Estimation

To describe the reproductive seasonality, we simulated the births from the pregnancy data. We used a sigmoid probability distribution to sample the birth date. The distribution was built assuming that the pregnancy in *D. rotundus* lasts around seven months and that fetuses are usually not detectable by palpation before 2-4 weeks of gestation, when the embryo is about 4 mm long (Delpietro and Russo 2002). We also assumed that detection of pregnancy by palpation is more likely in the last period of gestation. Based on this, we built a decreasing sigmoid probability density distribution to describe the probability of parturition after detection of pregnancy by palpation, following the formula:

$$g(t_d) = c_3 \left[1 - \frac{1}{1 + e^{-c_1(t_d - c_2)}}\right]$$

Where $g(t_d)$ is the probability of giving birth at t_d days after detection of the pregnancy by palpation, c_1 is a parameter controlling the slope of the sigmoid, c_2 fixates the position in time of the change in concavity and c_3 is a constant that can be used to keep the integral of the function equal to 1 in [1, 180]. To simulate the birth date for each pregnancy, we sampled the waiting time (t_d) and added it to the date of capture, using $g(t_d)$ as a probability density. We used c_1=0.1 and c_2=70 as initial values (Supplementary Figure 2), but we then tested the effect of these assumed values on the seasonality. Such an approach has not been using before to our knowledge, and g(t) is just a generic monotonic

decaying function that can be tuned using just two parameters. Higher values of c1 and c2 imply larger uncertainties in the estimation of birth dates from pregnancy (i.e. less ability to predict whether palpated pregnancy is early on its development or terminal).

To account for different capture efforts along the year, for each pregnancy we sampled $10/w_i$ birthdates, where w_i is the proportion of bats from the total sample captured in that week. We then built an empiric distribution of births from the simulated data.

After the simulation of the births distribution, we used a squared cosine exponential function (Peel et al. 2014) to describe the birth pulse:

$$r(t) = k.e^{-s\cos^2(\pi t - \omega)}$$

This function is always positive, with a period of 1 year and has 3 parameters to be estimated, namely the offset (ω), the synchrony (s) and a scale parameter (k). The scale parameter allows us to fit the global annual reproductive rate of the population. To fit the function to the data, we obtained an expected number of births for each month of the year (x_i), by multiplying the total number of observed pregnancies times the proportion of births in that month according to the simulated distribution. In this way, we were accounting for the global reproduction rate and the capture effort. We then used a likelihood function to select the best combination of values for the synchrony and the offset parameter (Peel et al. 2014).

$$\mathcal{L}(data|\Theta) = \max_{\{s,\omega\}} \prod_{i=1}^{i=12} \binom{n_i}{x_i} {p_i}^{x_i}(1-p_i)^{n_i - x_i}, \quad p_i = \frac{\int_0^{t_i} r(u)du}{\int_0^1 r(u)du}$$

We explored the parameter space with 100 values of s in the interval [0, 10], according to a visual exploration of the data and the relation of the synchrony with the

width of the birth peak (Peel et al. 2014), and 100 values of ω in the interval [0.01π, π]. We fitted the scale parameter (k) so the integral of r(t) was equal to the global reproductive rate in the interval [0,1]. As the calculation of x_i already included the correction by sampling effort, we used a constant number of individuals for each month of the year (n_i=30).

To test the effect of our assumptions on the detectability of pregnancies, we carried out a sensitivity analysis to explore the effect of changing c_1 and c_2, on the synchrony of births (s). We tested 20 values of c_1 ranging from 0.05 to 0.50 and 21 values of c_2 ranging from 50 to 90 days. Supplemental Figure 2 shows a graphic representation of the effect the values tested for the sensitivity analysis had on the shape of the curve.

Model Building

For the disease dynamics model we proposed a compartmental model based on two previously published models used to describe rabies dynamics in Perú (Blackwood et al. 2013, Bakker et al. 2019). In particular, we simplified the assumption of the disease stages used by Blackwood (2013) and used instead a single infectious state and one exposed state as included in Bakker (Bakker et al. 2019). We kept the assumptions of the density-independent births (Bakker et al. 2019), the frequency dependent transmission (Bakker et al. 2019) , and infected individuals from neighboring colonies contributing to the force of infection (Bakker et al. 2019) through indirect contacts or sharing of feeding areas The model then includes four classes, namely: Susceptible (S), Exposed (E), Resistant (R), and Infected (I) (Figure 1). To simplify the structure, we did not include age or sex structure in the model. We included the possibility of newborns having maternal immunity, so the

births are split among the susceptible and transient immune classes (according to the proportion of susceptible and non-susceptible individuals, respectively). To better incorporate the effect of neighboring colonies on the force of infection we used a discrete-time model with a time step of 1 day. Contacts are distance-dependent, with probability of contact (w_{ij}) described by an inverse logistic function dependent on the distance between the two colonies (d_{ij}):

$$w_{ij} = 1 - \frac{1}{1 + e^{-v(d_{ij}-u)}}$$

This function allows to control of distance-dependent connectivity with just one parameter (u) that can be interpreted as a distance threshold for connectivity, and it is the distance for which the function takes a value of 0.5. This function has the same shape as the used to model birth dates from pregnancies (see methods: reproductive seasonality estimation), and so v controls the slope of the sigmoid. For steeper slopes, individuals from colonies separated by distances below u, would have daily contact probabilities above 0.5. If the contact happens trough sharing feeding areas, this can be interpreted as frequent co-use of the same livestock patch.

The equations for the transmission model are as follows:

$$S_{i_{t+1}} = S_{i_t} k \int_t^{t+1} e^{-s\cos^2(\pi t-\omega)} - S_{i_t}\beta \sum_{i=1}^{i=25} I_{j_t} w_{ij} + \epsilon R_{i_t} - \mu S_{i_t}$$

$$E_{i_{t+1}} = S_{i_t}\beta \sum_{i=1}^{i=25} I_{j_t} w_{ij} - E_{i_{t+1}}(\tau + \mu)$$

$$I_{i_{t+1}} = \delta\tau E_{i_t} - I_{i_t}(\alpha + \mu)$$

$$R_{i_{t+1}} = \left(N_{i_t} - S_{i_t}\right)k \int_{t}^{t+1} e^{-s\cos^2(\pi t-\omega)} - (1-\delta)\tau E_{i_t} - \epsilon R_{i_t}$$

Susceptible (S) individuals move to the Exposed class (E) by contact with either infected individuals of the same colony (I_i) or infected individuals from neighboring colonies (I_j). The weight of the interaction is defined by the distance to the neighboring colony thorough the threshold function (w_{ij}). Weights for individuals of the same colony (main diagonal of the weights matrix) is set to one. Exposed individuals exit the class at a rate τ and can either develop a transient immune state with no signs of infection (R) with probability 1- δ or develop the disease and become infectious (I) with probability δ. Infectious individuals will eventually die from the disease at a rate α. Immunity in the resistant individuals will wane at rate ε and they will become susceptible again. All classes are exposed to natural (non-disease-induced) mortality (μ). Newborns will distribute among the Susceptible and Resistant classes, in proportions S/N and 1-(S/N) respectively, according to the seasonal birth function estimated before, with parameters c_1=0.1 and c_2=70 (see: reproductive seasonality estimation). We fitted the function so the integral over one year equals the field estimate of global reproductive rate. To make the population stable in time, we fitted the mortality rate (μ) to equal the global reproductive rate, so the expected natural mortality equals the reproductive success. Other parameters from the model (β,ε,δ,τ, and α) were taken from the literature (Table 1).

To obtain an independent estimation of natural mortality rate we reanalyzed the data presented on age distribution for colonies from Argentina and Brazil (Lord et al. 1976). To estimate the expected lifespan (the inverse of natural mortality rate) we assumed

that each observed animal contributes with the estimated age in years, plus 0.5 years (assuming that deaths would occur at the middle of the period) and applied the following formula:

$$\frac{1}{\mu} = \frac{1}{N}\sum_{j=1}^{J} n_j \, (A_j + 0.5)$$

Where N is the total number of observed individuals, n_j is the number of individuals in the age-class j and A_j is the age in years of class j. For this calculation we included all individuals reported as the authors stated that they did not find significant differences among colonies (Lord et al. 1976).

To keep the model stochastic, births, deaths, and transitions among model states were sampled from probability distributions in each step. Births were sampled from a binomial distribution with probability equal to the integral of the birth pulse function over the time step and number of trials equal to the number of individuals (S or N-S depending on the class receiving the births). Deaths, and transitions were sampled from an exponential distribution with the mean equal to the inverse of the appropriate parameter (i.e.: μ, ε, α, τ). Infections were sampled from a binomial distribution with mean equal to $S_{i_t}\beta \sum_{i=1}^{25} I_{j_t} w_{ij}$ for each subpopulation in each time step.

To model the metapopulation structure we used a field sample of vampire bat roosts in Uruguay, containing 25 roosts (Supplementary Figure 7). Pairwise distances were calculated using sp package (Pebesma and Bivand 2005, Bivand et al. 2013).

To assess the effect of our assumptions on the dynamic model, we did a sensitivity analysis of the mortality rate (μ), the birth synchrony (s) and the connectivity threshold (u)

on the long-term landscape-level pathogen persistence, measured as the latest time for which there is at least one infected individual in the metapopulation, running the model three independent times over 50 years each. For the birth synchrony we tested 20 values ranging from s=0 (no seasonality) to s=10. For the mortality rate we tested ten values ranging from $\mu=1.0 \times 10^{-4}$ days^{-1} to $\mu=9.0 \times 10^{-4}$ days^{-1}. All parameters included in the models are summarized in Table 1. All analyses and simulations were run in R 3.6.2 (R Core Team 2019).

Results

Captures

We recorded 353 captures of vampire bats between September 2017 and July 2019 in 16 colonies around Uruguay (Supplementary Figure 1), representing 323 individuals and 30 recapture events. We found 36 pregnant females by abdominal palpation. We estimated the global reproductive rate of the population as 0.102 year^{-1} (95% exact binomial confidence interval: [0.072; 0.138]). The distribution of pregnancy did not show a clear seasonal pattern, however only during warm months the confidence interval is clearly distinct from zero (Supplementary Figure 3).

We estimated the expected lifespan from published data from Argentina and Brazil (Lord et al. 1976) as 8.63 years, which corresponds to a natural mortality rate of $\mu= 0.116$ years^{-1} (or 3.2×10^{-4} days^{-1}). This value is within the confidence interval of the field-estimated global reproductive rate.

Reproductive seasonality

To estimate the reproductive seasonality, we simulated the births using a probability density function ($g(t_d)$) with parameters c_1=0.1 and c_2=0.7. The empiric distribution showed a seasonal behavior of births, with a peak centered around the end of November. Using the simulated empirical distribution of births, the maximum likelihood estimators for the synchrony and offset parameters were $\hat{s} = 4.55$ and $\hat{\omega} = 0.4\pi$. The structure of the likelihood surface suggests that combination as a global maximum (Supplementary Figure 4). The births predicted by the fitted curve show a seasonal distribution with the peak occurring on Nov 23rd and with 95% of the birth occurring within an interval of 203 days (Supplementary Figure 5)

Sensitivity Analysis

The synchrony showed a bimodal behavior in the sensitivity analysis, with respect to c_1 and c_2: for values of c_1 (slope of the sigmoid) above 0.1, independently of the value of c_2 the reproduction seems non seasonal ($s \leq 0.5$). Only for values of c1 below 0.1, c2 show higher importance in determining the width of the birth peak (Supplementary Figure 6). Figure 2 shows the trajectories of the 25 subpopulations over a 50-year period for three distance thresholds: 10, 100 and 300 km. Following identical initial conditions for each subpopulation (S_0=90, E_0=0, I_0=5, R_0=0), the 25 subpopulations show diverse trajectories, owning to the stochastic nature of the model. For the intermediate distance threshold there is longer persistence of the pathogen (time with at least one infected individual in the metapopulation), whereas for 10 and 300 km persistence is shorter. For the most connected network (higher distance threshold), there are more extinction events on the

subpopulations, with just a few of them being able to recover after the clearance of infected individuals from the system (Figure 2).

Pathogen persistence showed an inverted U-shape curve, as a function of distance threshold, with maximum (and roughly stable) values from around 80 to 220 km thresholds (Figure 3A). Persistence crashed at about 250 km thresholds. We evaluated the effect of reproductive seasonality on persistence for three connectivity distance thresholds (10, 100, and 300 km), assessing linear correlation between rabies persistence and synchrony (s). There was no observed effect for either of the threshold distances analyzed, tested by Pearson's correlation coefficient ($r= -0.11$, p-value = 0.42; $r= 0.03$, p-value = 0.83; $r= -0.12$, p-value = 0.37 for 10, 100 and 300 km thresholds respectively) (Figure 3C). The mortality rate, as measure of population turnover, only showed an effect on the persistence for the intermediate (100 km) distance threshold ($r= 0.55$, p-value = 0.01), with higher turnover linked to longer pathogen persistence (Figure 3B). For extreme thresholds (10 and 300 km), there was no detectable effect of mortality on persistence ($r= -0.20$, p-value = 0.40; $r= 0.23$, p-value = 0.33 respectively). The connectivity threshold also shows an effect on bat subpopulations persistence, with higher probability of local extinctions with larger distance connectivity thresholds (Figure 3D, Supplementary Figure 9).

Discussion

We present here a modeling strategy for vampire bat rabies that allows for explicit incorporation of metapopulation dynamics and can easily accommodate field data for both colony distribution, population sizes, and distance threshold for connectivity. Our results suggest that in complex metapopulations, connectivity and population turnover play

important roles in pathogen persistence, but reproductive seasonality has marginal effect on long-term rabies virus persistence in a metapopulation of vampire bats that resembles a real-word distribution of colonies. In our model, connectivity had such a strong effect that only when connectivity allowed for persistence, there was some positive effect of population turnover, extending the duration of pathogen circulation (Figure 3B). For extreme connectivity values, the persistence was so low, that the effect could not be overcome by reproductive synchrony or population turnover (Figures 3B and 3C).

The values for connectivity thresholds depend on the spatial structure of the colonies considered. In our example, the distribution of the 25 roosts harboring the subpopulations shows an aggregated pattern, with three distinct clusters of roosts distributed in the territory (Supplementary Figures 7 and 8). This aggregated pattern implies that at low thresholds those clusters will perform as independent populations, where a rapid outbreak across the cluster followed by a fade out of the infection is expected to occur, leading to low persistence. By contrast, with higher thresholds, all colonies are connected, and might behave as one single big population, where on top of the low persistence of the pathogen, several of the host subpopulations go extinct (Figure 2). The intermediate threshold (100 km) is just between two peaks in the distribution of pairwise distances (Supplementary Figure 8), and it will allow some interaction among clusters of shelters, allowing in turn for asynchrony in the infections and a more spatially complex behavior (Almberg et al. 2010, Horton et al. 2020). Fitting more realistic values of connectivity threshold, as interpreting the biological meaning of parameters v (slope) and u (threshold) from the connectivity function will be impacted by the spatial structure of the

colony network. Unsampled colonies might play a significant role when structuring the connectivity analysis. In the present work we used a sample of colonies that showed the pattern assumed for colonies in the country: an aggregated distribution, with colonies clustering according to orography and types of rocks available.

It is accepted that higher population turnover rates allow for faster replenishing of the susceptible pool, allowing for a faster reentrance of the disease within the subpopulation upon contact with an infected migrant (Lloyd-Smith et al. 2005). However, we only found effect of the mortality on persistence in the intermediate connectivity threshold (Figure 3B). This is probably due to a rapid extinction of the pathogen in the other connectivity settings, following the initial outbreak, that influx of newborns cannot recover. Also, as there is one annual birth peak in the model, is to be expected that if prevalence due to connectivity is too low (around one year), the influx of susceptible through reproduction is not going to revert the epidemic fade-out. Moreover, in our model, not all newborns are susceptible, and thus in the first birth peak, a proportion of the newborns will increase the resistant compartment of the model, contributing to the fade-out (by effectively decreasing the proportion of susceptible individuals).

Population turnover rate is determined in our model by the global natural mortality and the birth rates. To make the population stable over time (in absence of infection), we set these two values to be equal between them and to equal the observed birth rate in field populations. Another strategy to make the population stable in average is to set density dependent birth or deaths, by setting a carrying capacity on the system and making the rates to level off when the population approaches that asymptotic value. The practical advantage

of achieving expected stable populations by equaling the birth and mortality rate is that we can use this model with different populations sizes and without the need of establishing a carrying capacity for each shelter. Conversely it does not accommodate expected rapid population responses to disease induced changes in population. Our model showed that it is both capable of allowing for recovery of populations, and that even after strong disturbances (disease induced deaths), populations can recover (Figure 2, Supplementary Figure 9).

Estimating birth rates and especially mortality rates in the field is challenging. In the case of the vampire bat, as it is a long-lived mammal (Lord et al. 1976, Greenhall et al. 1983, Delpietro et al. 2017), survival estimations in the field are resource and time intensive and captivity studies do not necessarily represent well the longevity in the wild. We estimated the birth rate in the field and used that estimation both for the reproductive rate function and for the mortality rate (Table 1). Our choice of setting equal values for birth and mortality rates is supported by the lack of statistical differences between the birth rate estimated from our field data in Uruguay and the mortality rate derived from life tables built for colonies in Argentina and Brazil. The mortality rate we used in this work is almost three times lower that the estimate used in Perú: $2.8x10^{-4}$ $days^{-1}$ and $8.1x10^{-4}$ $days^{-1}$ respectively (Bakker et al. 2019). The difference is explained by a difference in how lifespan was estimated from age tables.

Given these results, special consideration should be given to population turnover when assessing persistence and especially when modeling a disease in a wild species with very few field-estimations of lifespan. In this context, long term banding initiatives are of

great value for feeding field data into models. Initiatives such as those are underway at least in Uruguay, Argentina, Perú and Belize.

The lack of effect of reproductive synchrony on pathogen persistence was a surprising result, given previous knowledge in the field (Lloyd-Smith et al. 2005, Peel et al. 2014). We tested this relationship for three different points of the threshold spectrum, to account for potential interactions between connectivity and reproductive synchrony (Figure 3C). The values of synchrony tested also cover even beyond the values tested under the sensitivity analysis for the reproductive seasonality estimation (Supplementary Figures 4 and 6). Moreover, our reproductive seasonality function shows a similar behavior to other observations in the region (Delpietro et al. 2017), and if different it has an even stronger pulse in the late spring. The apparent discrepancy between pregnancy and birth seasonality might be explained by small sampling sizes, combined with long pregnancies. Our simulation of birth allows to control for both effects. While we tested indirectly the assumption of detectability of earlier pregnancies, by the sensitivity analysis on c_1 and c_2, this assumption might also be tested in the field (e.g., by using longitudinal data on recapture and pregnancy detection and births in easily accessible colonies).

We showed here, and has been shown before that connectivity is important for bat rabies persistence (Blackwood et al. 2013, Horton et al. 2020). Our innovation here was the use of connectivity without immigration, as means for disease transmission across colonies, and we analyzed that into a spatially explicit scalable metapopulation dynamic model.

Changes in connectivity among vampire bats can be due to changes in individual exchange of bats, for example as in response to culling campaigns that clear roost that can be colonized by vagrant individuals (Blackwood et al. 2013, Benavides et al. 2020). It has been recently proposed that changes in connectivity among colonies as result of increasing home ranges due to landscape fragmentations, could be implied in rabies spillover into cattle in Uruguay (Botto Nuñez et al. 2019a, 2020). That hypothesis, derived from an historical analysis and spatial statistical modeling, together with the results from this work can lay out a framework to understand effects of fragmentation on disease spillover. In addition, we present here a tool that can be escalated to be used as a preemptive tool, by modeling different scenarios of land use change and their impacts on disease transmission and spillover risk.

One important and still missing point is a measure to effectively assess connectivity in the field. Ongoing field work combining wing banding, telemetry and proximity sensors might improve this approach (Becker et al. 2020), and those connectivity measures could be directly fed into our modeling framework. Moreover, undergoing research proposes to use biological markers of connectivity, by analyzing bats pathogens that can be shared among contacting individuals without immigration or reproductive events (Unpub. data). If an effective connectivity measure is found to track the response of vampire bats to land-use changes, the results of this work, in combination with previous published models (Botto Nuñez et al. 2020) could be used to model rabies transmission and spillover risk under different scenarios of land-use change. In such a context, proper land use planning can be used as an ecological countermeasure for spillover prevention (Reaser et al. 2020).

Incorporating field data into reliable models, to then use those models to evaluate intervention scenarios that can reduce spillover risk, might allow to incorporate the disease dimension into effective land planning and also serve as hypothesis generator and tester for intervention studies aiming to reduce spillover risks, both in this and other wild disease systems.

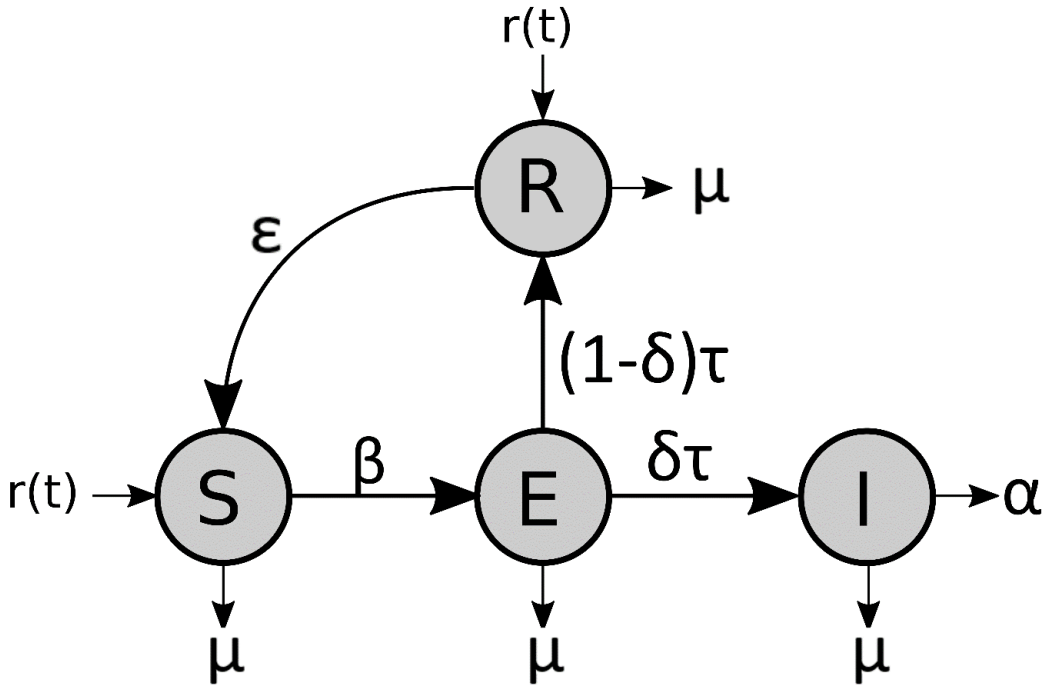

Figure 1. Structure of the compartmental model for rabies dynamics in each subpopulation i. S: susceptible, E: exposed, I: infected, R: resistant (transient immune), r(t): birth function, β: probability of infection given contact, ε: immunity waning rate, μ: natural mortality rate, α: disease-induced mortality rate, τ: inverse of mean time in exposed category, δ: Probability of an exposed individual to develop symptoms and became infectious. See text and Table 1 for estimated values of the parameters and equations of the system.

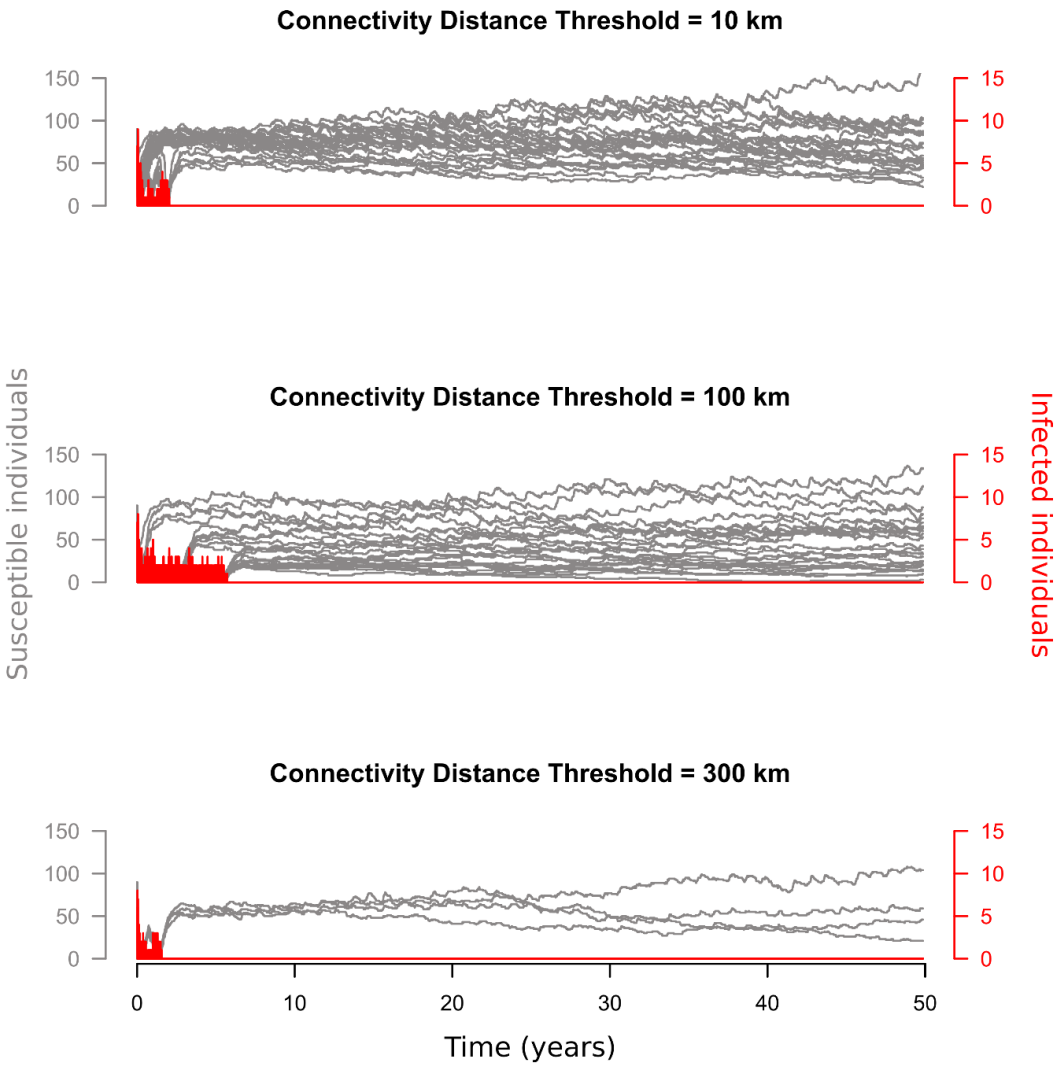

Figure 2. Dynamic of one metapopulation consisting of 25 subpopulations, over 50 years simulations with three different distance thresholds for connectivity. Number of susceptible individuals in each subpopulation are shown in gray (left y-axis), and infected individuals are shown in red (right y-axis). Each line represents the dynamic of one subpopulation. The intermediate distance threshold shows the larger landscape-

level persistence (maximum time with at least one infected individual in the entire metapopulation). With the largest threshold distance, not only the persistence is reduced, but also several populations go extinct within the first 5 years. All subpopulations start at time=0 with 90 susceptible and 5 infected individuals. The model runs with a 1-day time step.

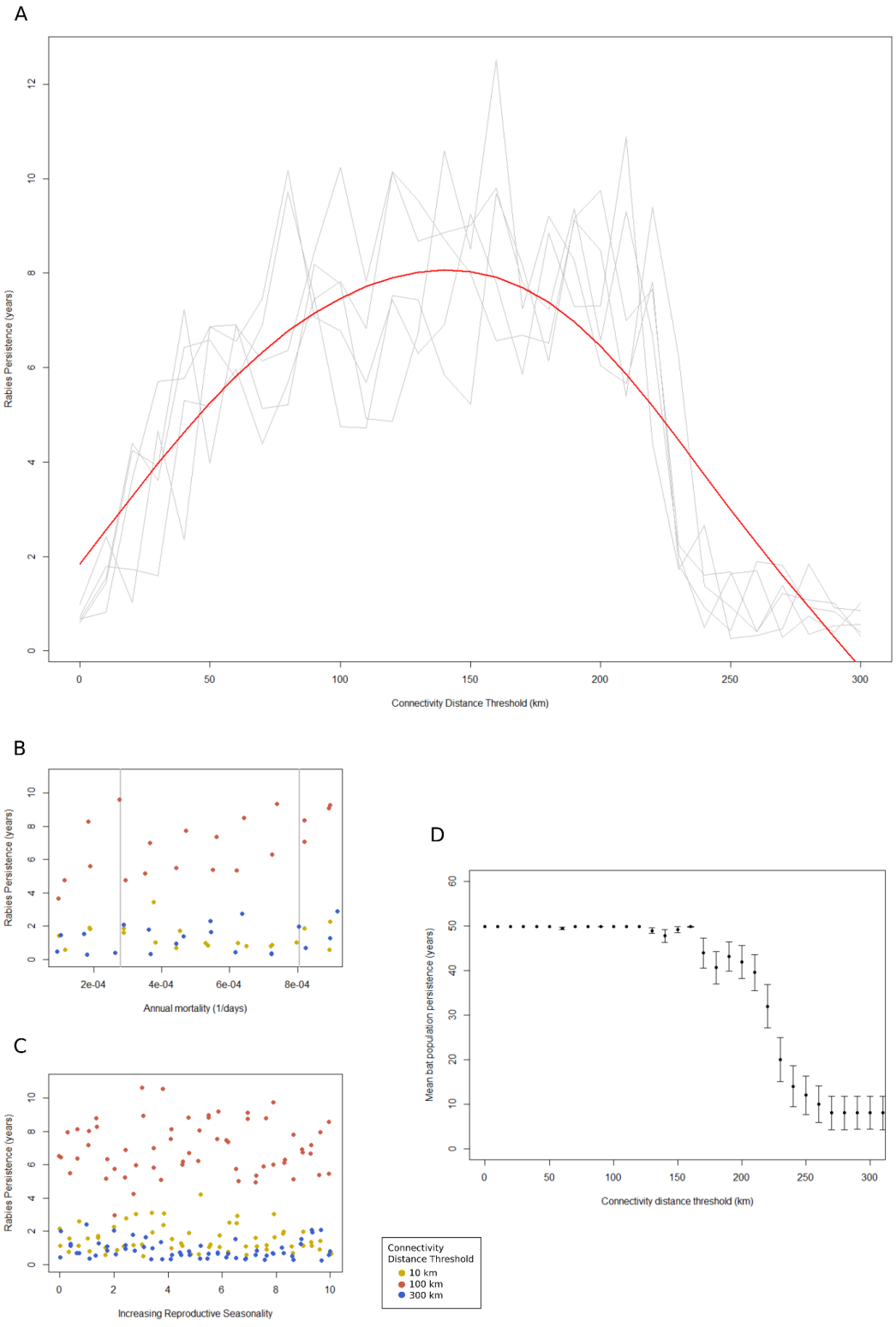
A
Rabies Persistence (years)
Connectivity Distance Threshold (km)
B
Rabies Persistence (years)
Annual mortality (1/days)
C
Rabies Persistence (years)
Increasing Reproductive Seasonality
D
Mean bat population persistence (years)
Connectivity distance threshold (km)
Connectivity Distance Threshold
10 km
100 km
300 km

Figure 3. Effect of connectivity distance thresholds, reproductive seasonality and population turnover on disease and population persistence. **A**: Metapopulation-level persistence, defined as the maximum time with at least one infected individual in the metapopulation over 50-year simulation, as function of connectivity threshold (distance between colonies that renders a 0.5 probability of an infected individual in one colony getting in contact with individuals from the neighboring colony). The red line show an adjusted spline with 4 degrees of freedom. For lower thresholds, each subpopulation function as an isolated colony, where the outbreak fades out. For higher threshold values, the whole metapopulation behaves as one single population, where the infected individuals affect their colony and neighboring colonies with equal probability. For intermediate values, the persistence is maximum as some spatial structure in the metapopulation allows for an asynchronic dynamic of the infection within subpopulations. **B**: Effect of reproductive seasonality on metapopulation-level pathogen persistence, for three different distance thresholds: 10 km (yellow), 100km (red) and 300km (blue). Reproductive seasonality is measured here as the s parameter of the squared cosine function of reproduction (Peel et al. 2014). Whereas the threshold affects the persistence, there is no effect of the seasonality on the persistence in either of the three groups (r= -0.11, p-value = 0.42; r= 0.03, p-value = 0.83; r= -0.12, p-value = 0.37 respectively). **C**: Effect of population turnover, measured through annual global mortality, on metapopulation-level pathogen persistence, for three different distance thresholds: 10 km (yellow), 100km (red) and 300km (blue). The two gray vertical lines show the global annual mortality values used in this study (2.8×10^{-4} $days^{-1}$) and in

Bakker et al. 2019 ($8.1x10^{-4}$ $days^{-1}$) as reference. For lower (10 km) and higher (300 km) connectivity thresholds there is no effect of population turnover on pathogen persistence (r= -0.20, p-value = 0.40; r= 0.23, p-value = 0.33 respectively). For the intermediate connectivity threshold there is a positive linear association between population turnover and pathogen persistence (r= 0.55, p-value = 0.01). **D**: Effect of connectivity distance threshold on survival of the subpopulations. There is a monotonic decrease in subpopulation survival with the increase in connectivity (see Supplementary Figure S9).

Table 1. Parameters used in the dynamic model and sources

Parameter	Description	Value	Source
Reproductive Cycle Estimation			
c1	Slope parameter for the probability density function of birth given detectable pregnancy	0.1	This study
c2	Time after pregnancy detection for which probability of parturition is 0.5 of the maximum probability	70 days	This study
c3	Scale parameter for the probability density function of birth given detectable pregnancy	Set so the integral of the function over 180 days is equal to one	
k	Scale parameter for the birth pulse function	Set so the integral of the reproductive function over one year equals the global field-estimated birth rate ($2.8x10^{-4}$ $days^{-1}$)	
s	Synchrony for the birth pulse function	4.55	This study
ω	Offset for the birth pulse function	0.40	This study
Connectivity among subpopulations			
v	Slope parameter for the connectivity as function of pairwise distance	0.0005	This study
u	Distance threshold for connectivity: distance between colonies where infected individuals have a 0.5 weight on infection in neighboring colonies	Variable	This study
Disease modeling			
β	Probability of transmission given contact with an infected individual	0.9322064	Bakker et al. 2019

δ	Probability of an exposed individual to develop symptoms and became infectious	0.10	Bakker et al. 2019
τ	inverse of mean time in exposed category	1/21 $days^{-1}$	Bakker et al. 2019
α	Disease-driven death rate.	1/11 $days^{-1}$	Bakker et al. 2019
ε	Immunity waning rate	1/135 $days^{-1}$	Bakker et al. 2019
μ	Natural mortality rate	$2.8x10^{-4}$ $days^{-1}$	This study. Set to be equal to the annual birth rate for population stability

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

Here we combined historical research, field surveys, lab work and mathematical and statistical modeling to understand the factors determining the occurrence of rabies spillovers from vampire bats into livestock.

The historical review is a powerful tool to identify key factors affecting the process. In the case of Uruguay, the analysis showed that the most likely explanation for the prior absence of rabies outbreaks and the emergence in 2007 is related to a change in landscape structure that affected the population dynamics of bats. This is supported by the absence of evidence of the vampire bats or the virus entering the system recently. The records on livestock abundances and trade, together with historical records of wildlife, allow us to infer that there was no recent change in ecological factors limiting the abundance or distribution of vampire bats in the country. Historical records of rabies in Brazil and Argentina support the notion that rabies virus was circulating in areas neighboring Uruguay, and there was no clear invasion pattern that could explain recent introduction of the virus in the country. Lastly, strict surveillance on cattle movements and health make unlikely the scenario of a rabies outbreak going undetected in the country (Botto Nuñez et al. 2019a).

The most drastic change in landscape structure in the country is the substitution of native, diverse grasslands for exotic forestry for wood and cellulose production (Baldi and Paruelo 2014, Botto Nuñez et al. 2020). This process intensified during the 1990s through government's policies, including tax exceptions, to promote afforestation. The process was

so extreme that Uruguay stands out in the global context for its increase in forest coverage in the last decades (Hijmans et al. 2005). The side result of the increased forest coverage is the fragmentation of grasslands, an understudied process that has been overlooked in its potential effects on disease transmission (Faust et al. 2018, Botto Nuñez et al. 2020). Here, we applied spatial statistical modeling to describe the grassland fragmentation process in the country and to assess the effect of this process on rabies emergence. We showed that rabies outbreaks are spatially correlated with grassland fragmentation. We also showed that winter temperatures play an important role in determining outbreak distributions, whereas livestock density has no detectable effect. We proposed that grassland fragmentation impacts the distribution of feeding areas for vampire bats, forcing them to increase their home ranges. Increased home ranges combined with scattered feeding areas where livestock is more concentrated might increase the connectivity among colonies. Moreover, as Uruguay is at the southernmost limit of the vampire bat distribution, only those colonies exposed to high enough winter temperatures might increase their home ranges in response to fragmentation. Vampire bats are known to be limited by winter temperatures as exposure to cold nights during foraging could entail larger energy expenditure, by means of heat loss, than energy obtained from foraging (McNab 1973, Hayes and Piaggio 2018). Our results then, despite being descriptive, match the known biology of the species and provide a plausible mechanistic for the observed correlation.

Measuring connectivity in wild populations is challenging, and more so in highly mobile small mammals such as bats. Telemetry studies, fluorescent dyes, proximity loggers, wing-banding and molecular markers can be used to assess connectivity. However,

each of those methods have their own limitations. Our proposed mechanism for increased connectivity does not imply exchange of individuals among colonies (but rather sharing of feeding areas) nor reproductive events. In addition, the landscape process under study is recent, especially compared with the lifespan of the species. Hence, molecular methods focused on the bats might be useless for assessing connectivity in this context. Marking methods are time and effort intensive, while proximity loggers might provide useful information but for reduced time frames and sample sizes. Here we proposed the use of a highly prevalent, and apparently not pathogenic virus naturally infecting bats as a new molecular marker for contact tracing in bats. We showed that vampire bats are commonly infected by a clade of gammaherpesvirus that show high species-specificity. Additionally, these viruses are present in the saliva, so they might be transmitted among bats in the same events that are relevant for rabies transmission. Our results showed that the colony of origin is a good predictor for viral sequence homology among pairs of bats. We also showed that younger bats are more likely to share identical viral sequences among them, than with adults. This is consistent with the knowledge of herpesvirus producing life-long infections and subsequent infections increasing within-individual viral diversity (Olsen et al. 1998, Juhász et al. 2001, Flach et al. 2002, Craig et al. 2005, Buckles et al. 2007, Cho et al. 2009). Our observation not only presents herpesviruses as good candidates for contact tracing in vampire bats, but may also provide inferences about potential viral vectors for wildlife spreadable vaccines (Griffiths et al. 2020). The increasing within-individual viral diversity with age, the apparent low pathogenicity, and the usefulness of these viruses as connectivity marker need further testing. Our field sampling system provides a unique

opportunity to improve these methods, capitalizing data derived from our lab work and from the modeling presented here.

We showed that spatiotemporal distribution of rabies outbreaks is consistent with an effect of grassland fragmentation over transmission of the pathogen. We also proposed a putative mechanism to link both processes, and the rudiments of a molecular tool to evaluate the connectivity among colonies. The last step was to test if increased connectivity might alone, and under realistic field conditions in Uruguay, be responsible for increased persistence in bat populations. We implemented a spatially explicit dynamic metapopulation model to test this assumption *in silico*. We incorporated field data derived from two years of sampling in the country giving reliable estimations of birth rates and reproductive seasonality. We used data from previous models and lab experiments to complete the parameters of the model that we could not estimate from the field. Finally, we assessed the effect of connectivity across a virtual metapopulation on the persistence of the virus in bat colonies. We showed that the viral persistence follows an inverted u-shaped curve in response to connectivity, with maximum values depending on the spatial structure of the metapopulation. We also showed that the connectivity effect was so strong that population turnover and reproductive seasonality only had marginal effects and these effects could only be tested at connectivity values that allowed some persistence.

The tools presented here might be used to design, implement and monitor interventions that serve as ecological countermeasures to manage spillover risk in the system (Reaser et al. 2020). Moreover, the framework developed here, combining

historical review with model-guided field work (Restif et al. 2012, Wood et al. 2012) can be adapted to fit the needs of different systems.

Our statistical models can be used to perform risk assessments on proposed interventions, such as new forestry ventures, incorporating the disease risk into environmental impact assessments. If validated in the field, molecular tools based on viral community characterization can be used to parametrize the effect of landscape disturbance into colony connectivity and this, in turn, can be incorporated into spatially explicit models to predict the long-term effect of interventions, by analyzing competing development scenarios.

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APPENDICES

APPENDIX A

SUPPLEMENTARY MATERIAL TO CHAPTER 2: THE EMERGENCE OF VAMPIRE BAT RABIES IN URUGUAY WITHIN A HISTORICAL CONTEXT

HISTORY OF THE DISCOVERY OF *D. rotundus* IN URUGUAY AND THE SOUTHERN CONE OF SOUTH AMERICA AND URUGUAY

Desmodus rotundus was originally described by E. Geoffroy Saint-Hiliare in 1810 [1]. However, this species was mentioned earlier in several reports of naturalists visiting South America. In his reports of travel along the Amazonas river in 1743, Charles Marie La Condamine described bats that suck the blood from cows, horses, and even humans as common in warm American countries [2]. According to La Condamine's report, these attacks had been described since the early missions in the Amazonas basin during the 1600s. La Condamine even attributed the massive mortality of livestock introduced in Borja (currently in Loreto Department, Perú [4.44° S, 77.59° W]) and in other missions to bites from *D. rotundus* [2]. Those reports were also mentioned by Buffon, but he described *D. rotundus* without direct contact with this species, based only on other's descriptions [3]. In his description of *D. rotundus* ("El Mordedor" [The Bitter] in his non-linnean nomenclature), Felix de Azara reject the report of La Condamine as overstated, arguing that the loss of blood is minimum and that there is no other significant damage produced by *D. rotundus*' bite [4]. Azara himself was bitten by a *D. rotundus,* but he described the bite as a minor wound, claiming that it would be impossible for a livestock herd to be killed by bites from *D. rotundus* [4]. When Azara described *D. rotundus*, he didn't provide the putative distribution for the species [4]. In 1959, Angel Cabrera in restricted the type locality to Asunción (25.30° S, 53.64° W) *sensu* [5], based on the diaries and notes from Azara.

Charles Darwin was the first naturalist after Azara to actually see a *D. rotundus* feeding. He described the feeding behavior in his diary, and that passage is included by Waterhouse in the description of the species [6]. Darwin recorded the species in the central-northern region of Chile (Coquimbo, IV Region; 29.99° S, 71.35° W). He proposed that *D. rotundus* should be absent or unknown in central Chile, citing a report from Molina, who dismissed the presence of "blood-sucking species" in the central region [6], suggesting that the species was restricted to northern Chile only.

Another early record of *D. rotundus* comes from Alcide D'Orbigny, who collected some individuals in Bolivia (San José de Chiquitos, north of Department of Santa Cruz, 17.84° S, 60.74° W) during his travel around South America [7]. Interestingly, even though he travelled throughout the continent, the only mention of the species was from Bolivia [7]. Hermann Burmeister included *D. rotundus* in his "Description Physique de la Republiqué Argentine", noting that the species was probably present in Argentina but restricted to the northern border, given records of the species in southern Paraguay [8]. However, his notes are restricted to individuals collected in Lagoa Santa in Brazil [8] (Minas Gerais, 19.63° S, 43.90° W).

In 1894 J. H. Figueira published a list of the 55 known species of mammals from Uruguay [9,10]. Figueira mentioned five bat species comprising the three families currently recognized in Uruguay (Phyllostomidae, Molossidae, and Vespertilionidae). He considered all five species widely distributed along the country, but he mentioned *Plecotus velatus* (*Histiotus velatus*) as the most common, and especially mentioned the Arequita Cave (Department of Lavalleja, 34.28° S, 56.26° W) as one of the known roosts [9]. These data

are especially interesting as *H. velatus* is an easily distinguishable species due the size and form of the ears. Currently, *H. velatus* do not inhabit that cave; the cave now has colonies of *D. rotundus* and *Myotis* species [11].

In 1929, Collin Sanborn published "The Land Mammals of Uruguay", reporting the results of the Captain Marshall Field Brazilian Expedition during the four-month stay in Uruguay [12]. Sanborn visited the Arequita Cave by the end of December 1926 and collected 43 *Myotis chiloensis ater*. These were later determined to be *M. levis* by Alfredo Ximenez following the identification by Richard La Val on specimens from the National Museum of Natural History in Montevideo [13]. No *Histiotus* were recorded here, so Sanborn argued that it was impossible to know if Figueira misidentified the bats or if the colony of *Histiotus* was expelled and replaced by *Myotis* [12]. The currently accepted idea is that the first identification by Figueira was mistaken. In either case, neither Sanborn nor Figueira made any mention of *D. rotundus* in the cave.

In 1935, the first reference to *D. rotundus* in Uruguay appeared in Garibaldi Devincenzi's work on the mammals of Uruguay [14]. The author referenced ongoing reports of cattle attacked in the north of the country. He instructed several collectors to look for bats in Rivera Department, and specifically for *D. rotundus*, which could be easily differentiated from other bat species from Uruguay. In 1933, one of the collectors submitted a male *D. rotundus* from Centurión in the Department of Cerro Largo (32.14° S, 53.78° W) in the North-eastern region of the country [14,15]. Since this first record, and until the publication of the 1935 paper, no other individual was obtained, so Devinzenci proposed the species to be rare for Uruguay [14]. For some years, the species was supposed

to be low in abundance and restricted to the area where it was first recorded. However, between the first record in 1933 and 1960, *D. rotundus* was confirmed in several localities of the country, suggesting a widespread distribution [16]. It is interesting to note that both Devincenzi and Eduardo Acosta y Lara also visited the Arequita Cave before 1950 and again recorded the presence of *Myotis levis*, but did not mention *D. rotundus* [14,15]. The first record of *D. rotundus* inhabiting Arequita Cave is from 1969, mentioning a small number of individuals [17]. These authors also provide several new localities confirming the widespread distribution of *D. rotundus* in Uruguay [17]. In their 1959 paper, Acosta y Lara described *D. rotundus* in a cave in the south of Uruguay (Salamanca Cave, Department of Maldonado, 34.08° S, 54.61° W), noting that there were also *M. levis* present; however, these were restricted to small fissures in the rock where a knife blade could be barely introduced [16]. The authors speculated that *Myotis* may have been forced into these fissures by the introduction of *D. rotundus* to the cave [16]. In 1972 Alfredo Langguth and Federico Achaval published the only paper on *D. rotundus* ecology in the country, based on a colony from Rivera department [17]. In that paper the authors also provide a list of 21 localities from where *D. rotundus* had been collected [17].

Currently, the colony of *D. rotundus* in Salamanca Cave is still present, as are *Myotis* (Pers. Obs. GBN). However, in Arequita Cave, a colony of *D. rotundus* and a colony of *Myotis* co-inhabit the same cave but at intervals. The *Myotis* are present in the cave during the spring and the summer; when the *Myotis* colony increases its numbers in mid spring due to immigration the colony of *D. rotundus* is displaced from the main cave to the atrium of the cave (Pers. Obs. GBN). This behavioral pattern was observed by one

of the authors (GBN) and confirmed by the owner of the property, who has systematically observed the cave over many years.

After the work of Acosta y Lara, and Langguth and Achaval [15–17], *D. rotundus* has been registered in many other localities, including localities in the Atlantic cost of the country (Pers. Obs. GBN) and is presumed to be present in the whole country including the rural outskirts of Montevideo city [18]**.** Roosts used by this species in Uruguay are mainly caves, mines, and abandoned buildings (ranging from houses to industrial settlements); the size of the colonies ranges from a few individuals to several hundred or even thousands [18].

Since the first paralytic rabies outbreak in Uruguay in 2007, several campaigns have been conducted by the animal health authorities to reduce *D. rotundus* populations [19–21]. The main procedure for colony reduction is poisoning using Vaseline-based Warfarin pomade (i.e., vampiricide) [19–21]. While the numbers of *D. rotundus* killed in these campaigns remains unclear, several hundred individuals have been collected for diagnostic and surveillance purposes during the first years after the rabies outbreak. The reduction of colonies continues to be one of the main strategies to control the spread of rabies without considering the potential effect of this practice on the circulation of the virus in the bat population [22].

LIVESTOCK INTRODUCTION AND CURRENT ABUNDANCE

Common knowledge in Uruguay states that livestock was introduced in this territory by Hernando Arias de Saavedra (a.k.a. Hernandarias) in the early 1600s [23,24].

However, the introduction by Hernandarias was probably of little impact, because of the relatively small number of livestock; however, the introduction of livestock from the Missionaires from the Company of Jesus was much more important, in number of individuals [23]. The missionary livestock seems to have had several introduction events. First, there was a report of around ten cows bought from the Portuguese ca. 1552 and herded to Paraguay [23,25]. Second, another group of livestock ("several hundred") was brought from Santa Cruz de la Sierra (Bolivia, 17.78° S, 63.18° W) in 1568 [25]. There were two other introductions from Coquimbo (Chile, 29.99° S, 71.35° W) and Santa Cruz de la Sierra around 1557, to Cordoba (Argentina, 31.41° S, 64.19° W) and Santiago del Estero (Argentina, 27.78° S, 64.26° W). These two stocks were used in the foundation of Santa Fe (Argentina, 31.64° S, 60.71° W) in 1573 [25]. The two accepted origins for livestock in Uruguay are the introduction from the Jessuitic Missionares in the north, forming the "Vaquería del Mar" [23] and the two introductions by Hernandarias in southwestern Uruguay [23,25]. The first introduction by Hernandarias was based in the confluence of the Negro and Uruguay rivers in 1611 (33.28° S, 58.40° W). The second introduction was based in the coast of what is now Colonia del Sacramento (Department of Colonia, 34.47° S, 57.84° W) in 1617 [24]. Both the introductions of Hernandarias' consisted of a small number of livestock.

By 1627, Hernandarias estimated the population of livestock in the Banda Oriental (current Uruguay) to be about 100,000 animals [25]. This vision of abundant livestock in the Uruguayan territory by the 1630s is shared by many researchers [23]. The Uruguayan territory was not occupied by the Europeans during most of the 17th century, so livestock

were not heavily exploited until 1710 [25]. The cattle were first raised as free ranging in both banks of the Rio de la Plata [25].

There are few references about the expansion of livestock after this introduction. However, from the reports of early visitors of Uruguay and from scattered references about leather commerce, one can get an idea of the abundance of livestock. In 1715, William Toller arrived Uruguay in his voyage to Buenos Aires from England travelling aboard the Warwick [26,27]. The Warwick's first stop in Uruguay was in Castillos Bay (34.34° S, 53.78° W), in what today is the Department of Rocha, during June 1715 [26,27]. Toller and Dover went ashore several times hunting 10 bulls. Toller noted in his diary that "the plains were full of cattle, but most of them Bulls" [27]. He also described many small deer. During the next stops in the north shore of the Rio de la Plata, Toller mentioned the abundance of cattle. He also mentioned deer and 400 peccaries near the St. Thomas river (today the Rosario river, Department of Colonia, 34.43° S; 57.34° W) [27]. The first observation was about 400 kilometers from where Hernandarias introduced livestock some 100 years earlier. Toller repeatedly refers to the poor soil in the places where he recorded high numbers of livestock. Leather commerce was one of the biggest economic activities during the first years of the Spanish colonies in the Rio de la Plata. According to the "Asiento de Negros", the official Spanish Crown's record of the slave commerce, between 1702 and 1714, approximately 174,000 leather pieces were exported from Buenos Aires (Montevideo was not founded yet) to England [26]. The British South Sea Co., which had an office at Las Vacas stream (Department of Colonia, 34.00° S, 58.29° W), exported around 480,000 leather pieces between 1723 and 1729 [26]. In 1777, 364,534 leather pieces

were exported to Spain from Montevideo and these do not account for smuggling or the slave trade [28]. According to a letter from Perez Castellanos dated in 1787, 321,450 leather pieces where exported from Montevideo in the same year; several came from Buenos Aires, but most were from Uruguay [29]. According to the same author, on March 5th, 1781, a convoy departed from Montevideo carrying 432,000 leather pieces; at the same time, six mail frigates and other ships also carrying leather departed to Europe [29]. Around the same date, in 1780, a livestock census showed 408,000 cattle in just Montevideo's jurisdiction alone [28]. Between 1792 and 1793, 1.6 million leather pieces were exported from Montevideo to Spain [28]. The livestock were raised free-range and with little or no care and were usually killed to sell the leather. During the end of 1800s, ranchers began to castrate bulls in response to the decreasing prices of leather overseas [29]. By 1800, leather exports were stopped due to wars within the viceroyalty. This almost completely stopped cattle slaughtering in the country [28]. In 1806, John Mawe, an English geologist visited Uruguay and was confined to imprisonment in a ranch in central-south Uruguay, in a locality called Barriga Negra (33.97° S, 55.07° W), about 145 km northeast from Montevideo [30]. After his observations of the area, Mawe mentioned the presence of several livestock ranches with herds ranging from 60,000 to 200,000 cattle each [30]. According to census data, there were 2.6 million cattle in 1821, just in possession of Brazilian subjects and in the southern half of the country. By 1887, Brazilians were estimated to possess about 5.2 million cattle (half of that in the south and half in the north) and this would represent less than a half of the estimated 12 million total cattle population in the country [28].

In 1880, David Christison, an English naturalist published the diary of an excursion to central Uruguay, which took place in 1867 [31]. Christison described the country as a continuous grassland with very soft slopes that were rarely interrupted by linear forests associated with some of the rivers. He travelled from Montevideo to San Jorge (Department of Durazno, 32.84° S, 55.89° W) during the autumn. In this district, limited by the Negro river, and the Chileno and Carpintería streams; 60,000 cattle, 100,000 sheep, and 6,000 horses were kept at that time (1880) [31]. Christison estimated the area of the district as 943 km^2 [31] (which is an accurate estimation when compared to the area calculated from modern maps) producing an average density of almost 64 cattle per km^2. While it could be supposed that this density is higher than the national average, Christison's mentioned the abundance of cattle throughout the entire trip, suggesting the density observed in San Jorge was similar to elsewhere in Uruguay. Christison also referenced the recent implementation of wire fencing, not only in San Jorge but also in other areas of the country [31]. Wire fencing was an important advance towards livestock production in Uruguay, improving the management of the herds. During the 1900s, Uruguay began a livestock census that covered the whole country and standardized data collection. In 1908, the agricultural census recorded 8.2 million cattle and 21.5 million sheep [32]. The estimated cattle population was around eight million until the second world war, when a decrease in cattle abundance was observed: 6.3 million in 1943 and 6.8 million in 1946 [32]. While the cattle population in the 1900s was lower than estimates from the previous century, there is an important contribution of sheep that, despite having more fluctuating numbers, averaged more than 17 million during the period [32] (Table S1).

While *D. rotundus* was first recorded in Uruguay during the first half of the 20th century, ample prey was available in great numbers for at least 200 years prior. In 2016, the total estimated livestock population was 12.1 million cattle [33], while in 2011 (the last agricultural census) the population was 10.7 million cattle, 7.3 million sheep, 354 thousands horses, and almost 184 thousands pigs [34]. The density of cattle for 2016 is estimated to be approximately 68 cattle/km^2, slightly above the estimated density for San Jorge in 1867 [31]. In 2016, 2.23 million cattle were slaughtered for domestic consumption or export and 225,000 were exported alive, totalizing almost 2.5 million animals removed annually from Uruguay [33].

NATIVE AND INTRODUCED WILDLIFE IN URUGUAY IN RELATION TO *Desmodus rotundus* POPULATIONS

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In 1715, William Toller arrived to the Rio de la Plata on an English cargo ship from an English trade company on route to Buenos Aires [26]. The diaries of both Teller and the president of company responsible for the cargo ship (Thomas Dover) provide data about the area 100 years after the introduction of livestock [26]. Their first stop in Uruguayan territory was at Castillos' Bay, in what now is the Department of Rocha (34.34° S, 53.78° W) in June, 1715 [26,27]. At this stop, Toller reported the presence of many small deer, probably referring to pampa's deer (*Ozotocerus bezoarticus*) [27]. He mentioned that some members of the expedition reported to have seen bears; this is likely impossible because of X. Raúl Vaz Ferreira, noted that capybaras (*Hydrochaerus hydrochaeris*) may have looked

like bears to the English observers [35]. During that expedition and the subsequent stops in Uruguayan territory, Toller continued to describe wildlife, describing a herd of 400 peccaries after passing Montevideo bay, which he named the St. Thomas river [27]. According to Vaz Ferreira, the St. Thomas river might be the Rosario river (in the Department of Colonia) or the Cufré stream (in the border between Departments of Colonia and San José) [35]. This site is likely the Rosario river (34.43° S; 57.34° W) in the Department of Colonia, judging from the maps on the original manuscript from Toller. There are three recognized species of peccaries in South America: *Catagonus wagneri* (chacoan peccary), *Pecari tajacu* (collared peccary) and *Tyassu pecari* (white-lipped peccary) [36]. The chacoan peccary is restricted to the Gran Chaco in Paraguay and northern Argentina and moves in solitary or small herds of up to ten animals [37]. The other two species are known to inhabit forests; however, according to Felix de Azara's observations, in the absence of forests, *T. pecari* forms large groups and inhabits "pajonales" (grasslands dominated by *Paspalum*, *Panicum* or *Cortadeira spp.*) [38]. *T. pecari* is known to have larger herds than *P. tajacu*, with groups up t hundreds and with anecdotal reports of up to 2000 individuals [39–41]. Vaz Ferreira assigned the observations from Toller to *P. tajacu* [35], which was supposed to inhabit northern Uruguay, even though it is now considered extirpated in Uruguay [18]. Toller also reported deer (probably the same species than in Castillos: *O. bezoarticus*) in the mouth of the Santa Lucia river and in the Department of San Jose [27]. Cattle were referenced constantly during all the trip, and Mr. Dover hunted cattle several times [26,27]. From the wild species observed by Toller, both deer and peccary could have been feasible food sources for *D. rotundus*. In

1806, John Mawe also provided a description of peccaries in central-south Uruguay also describing “considerable herds of small deer” [30]. Given the mention of large herds, Mawe was probably referring to pampa’s deer.

For other potential prey of *D. rotundus*, Azara [38] suggested that tapir (*Tapirus terrestris*) could have occupied the Rio de la Plata, but likely in low abundances. There are no other mentions to this species for the Uruguayan territory, and from Azara’s notes, it does not seem to have been observed that far south [38]. For deer, Azara confirmed the presence of just one species for the Rio de la Plata basin: the Güazú-ti or pampa’s deer (*O. bezoarticus*). Other species that could have been present in the territory alongside those included in Azara’s list are the marsh deer (güazu-pucú; *Blastozerus dichotomus*) and the grey brocket deer (guazú-tí; *Mazama gouazubira*); for the latter, Azara was not aware of this species occurring in Uruguay [38]. The marsh deer is thought to have been present in Uruguay territory, although is now exitirpated, and the grey brocket deer is still present [18]. The grey brocket deer is a small deer, most commonly observed in forests, which could make it less conspicuous than the pampa’s deer. Because Azara only used local names (mostly in the Guaraní language), we relied on the interpretation of Mones & Klappenbach to assign Latin binomial names[42].

Charles Darwin visited the Rio de la Plata during the Beagle’s trip in 1833. From his observations [43][44], we can extract a good description of the Uruguayan territory in the 19th century. Darwin described Uruguay as an “ondulating surface, clothed with turf” [44]. During his stay in Maldonado (southern Uruguay, 34.90° S, 54.95° W), Darwin described the pampa’s as the only highly abundant mammal species [43], present in all the

Rio de la Plata's coast. Another highly abundant species was the capybara. Darwin attributed its high abundance to infrequent hunting due to its low-value fur and unattractive meat [43].

Several species of native mammals were also mentioned in Christison's travel report,. Christison mentioned pampa's deer as "not uncommon" all around the country. He also mentioned a larger deer, perhaps referring to *B. dichotomus*, as previously abundant in the area but then absent for several years. The same comment was made about the jaguar (*Panthera onca*) and the great ant eater (no species mentioned, but could refer to *Mymercophaga tridactyla*), while mountain lions (*Puma concolor*) were seem to be rare but still present [31]. During 1892 and 1893, another English naturalist, Oliver Aplin, visited Uruguay and published on the mammals of the country, mainly based on his observations in the Departments of Rio Negro and Soriano [45]. From the list of mammals presented in this work, deer would have been the most important food source for *D. rotundus*. Aplin mentioned that the pampa's deer was almost extirpated from the Departments of Rio Negro, with only one herd of approximately 20 animals remaining in the area of Merinos (Department of Rio Negro, 32.38° S, 56.90° W) but still abundant in the Department of Florida [45]. For marsh deer, Alpin described this species as rare but still present in the forests along the Uruguay river's banks [45]. It is interesting to note that the same year of Aplin's publication, Figueira also published a paper on the mammals from Uruguay. Figueira considered the pampa's deer to be common throughout the country but especially abundant in Department of Rocha, while he described the marsh deer as present only the north (maybe coincident with Aplin's observations) and southeast of Uruguay [9].

Figueira also considered the grey brocket deer to be rare and only present in Departments of the north and southeast [9]. Figueira also mentioned the collared peccary as almost extinct in Uruguay and only present in the northern region in the Departmetns of Artigas and Cerro Largo [9]. Sanborn visited Uruguay 33 years later and described the pampa's deer as very abundant only in the Department of Rocha, while in low abundances elsewhere [12]. About the marsh deer, Sanborn was told that could still be present in the same area, but in very low abundances [12]. Sanborn did not provide new information on the grey brocket deer, as his comments about this species were only based in Figueira's and Aplin's publications. Today, the pampa's deer is currently limited to two very restricted populations representing two distinct subspecies: *O. b. uruguayensis* in Los Ajos (Department of Rocha, 33.58° S, 54.04° W) and *O. b. arerunguaensis* in El Tapado (Department of Salto, 31.81° S, 56.66° W). These two populations have restricted areas and small population abundance, about 300 individuals in Los Ajos and around 500 in El Tapado [46]. The grey brocket deer is probably present throughout Uruguay, except for the southwest, but not in high densities, and it has been effectively recorded in 10 out of the 19 departments [18,46–48]. As this species uses native and mixed forests as its main habitat, the increase of forestry may have helped it. The marsh deer is considered extinct in Uruguay [18,46], as the last reported record for the species is from 1958 [46].

From these historical reports, we can deduce that the deer populations had declined by the end of the 19th century and the early 20th century. Other native wildlife that could be a food source for *D. rotundus*, such as other deer species, are either extinct (marsh deer) or exist in low abundances (grey brocket deer). Peccary, once possibly abundant, according

to the Toller's and Azara's reports, were absent from historical records or were considered to exist in very low numbers by the end of 19th century; peccaries are currently considered extirpated [18]. The lesser anteater (*Tamandua tetradactyla*) was mentioned by Figueira as rare and restricted to the north and northeastern regions [9]; similarly, *T. tetradactyla* is now considered rare and restricted to the low mountain ranges in northwestern Uruguay [18]. The giant anteater (*M. trydactyla*) is also considered extirpateds [18]. The jaguar (*P. onca*) is now considered extirpated, and the mountain lion (*P. concolor*) is rare, with confirmed records only in the northern half of the country since 1970 [18].

Other native mammals known to inhabit Uruguay are probably not a good food source *D. rotundus*, either for their small size and/or because of their behavior. There is one report of *D. rotundus* preying on capybaras (*H. hydrochaeris*) in Argentina [49], and the capybara has been common throughout Uruguay since colonial times [18,27,35,43].

Other than livestock, there are some introduced naturalized exotic mammals in Uruguay that could represent a food source for *D. rotundus*: two exotic deer (*Axis axis* and *Dama dama*), wild pigs (*Sus scrofa*), goats (*Capra hircus*), and Asian buffalos (*Bubalus bubalis*) [18,50]. The Axis deer (*A. axis*) was introduced in the early 20th century (ca. 1920) in south-western Uruguay (Anchorena's Ranch, Department of Colonia, 34.28° S, 57.97° W), and later one population was translocated to the south-eastern region (Department of Rocha) [50]. Today, this species is distributed almost throughout the whole country but more densely in the Uruguay river basin and in the southern region (Departments of Florida, Canelones, Rocha and Treinta y Tres) [50]. The European deer (Gamo, *D. dama*) was introduced into southern Uruguay in the Department of Florida and has a small

population that has shown little or no dispersion [50]. There are some populations of naturalized goats (*C. hircus*) in the Haedo's and Cuchilla Grande low mountain ranges in the departments of Lavalleja and Maldonado [50]. By the 1980s, two populations of Asian water buffalo were introduced in northern Uruguay: one in Artigas and the other in Rivera departments [18,50]. In Rivera, the population has been restricted to forestry production areas and in Artigas is located in grasslands in the Uruguay River's basin. The animals in Artigas have been individually identified and monitored using the same system as livestock, as part of the system to control foot and mouth disease's control in the early 2000s. Finally, wild boar (*S. scrofa*) are now distributed across the country, occupying mainly forests and wetlands [18,50]. This species was introduced in 1920 in the Department of Colonia (Anchorena's ranch, 34.28° S, 57.97° W) for hunting. Today, wild boar are considered to be agricultural pests, and hunting is permitted and encouraged by the government as a population control measure [50,51]. Studies from southern Brazil demonstrate that *D. rotundus* will feed on *S. scrofa*, especially in forested areas with low livestock density [52,53]. The population abundance estimate of wild boars is not known, but is considered to be currently increasing and widespread in Uruguay [50]. The population size of other wild prey is negligible at a national level, while some species could have some importance for *D. rotundus* at a very local scale..

ON THE GEOGRAPHIC EXPANSION OF *Desmodus rotundus*

Recently, two studies have focused on the potential expansion of distribution range of vampire bats, in response to climate change, and its relationship with increased bovine

rabies risk [54,55]. The first study proposed that the distribution of *D. rotundus* would expand under three of the evaluated climate change scenarios [55]. The second study focused in the potential future expansion into the US, and found that while there are some suitable areas in the Mexico-US border, an extensive expansion is unlikely [54]. The analysis performed in the first paper, however, presents some limitations. In reference to the present work, the distribution model used by these authors fails to predict the current southern limit of the species' distribution [5,55–57]. This limitation constrains the interpretability of the paper's result for the southern area of vampire bat's distribution.

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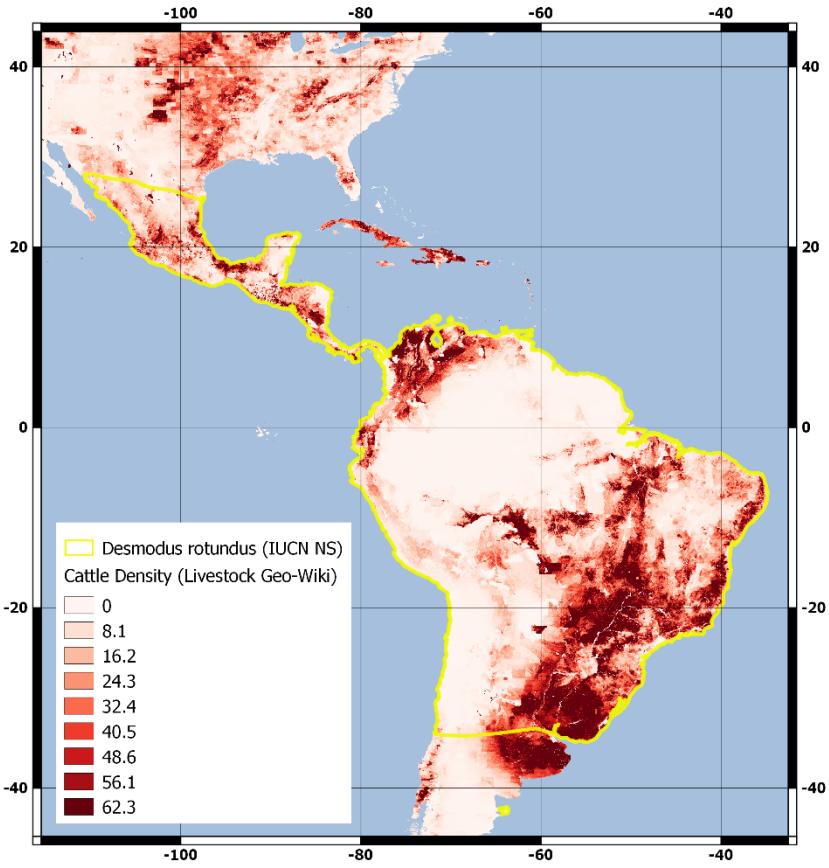
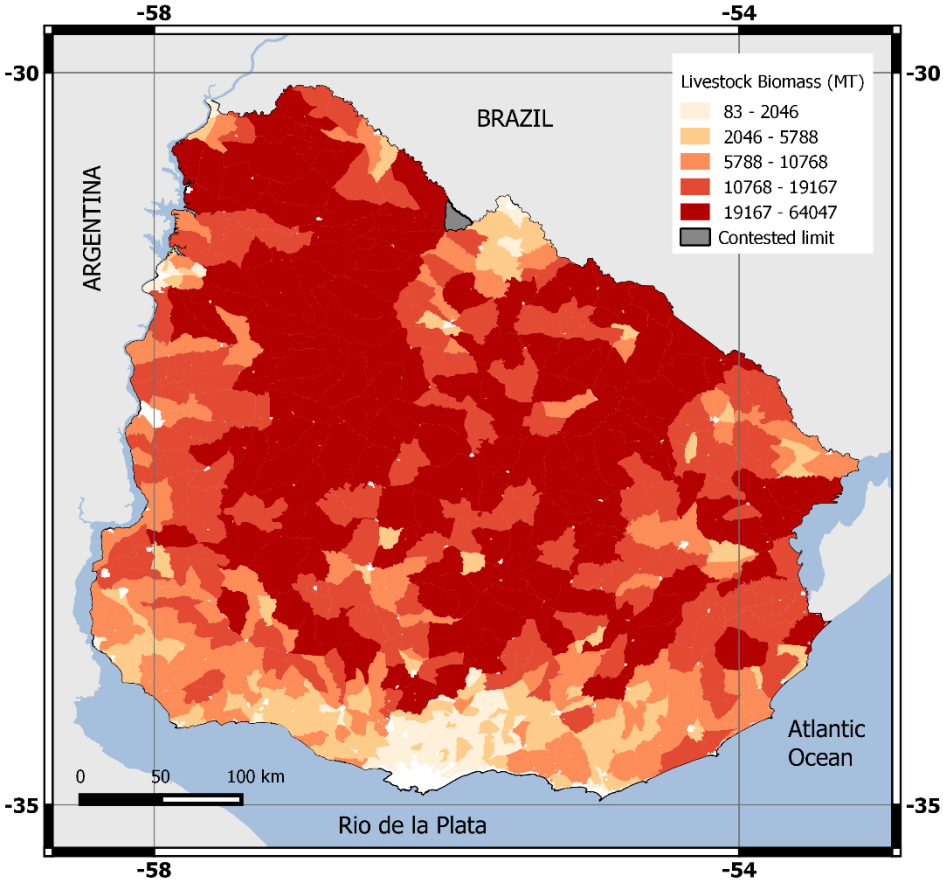


Supplementary Figure S1. Distribution of the common vampire bat (*Desmodus rotundus*) in relation to cattle density. The distribution of *D. rotundus* is not limited by the absence of food sources. Both in the southern and northern limits, there are areas of high density of cattle outside the area of presence of *D. rotundus*. The distribution of *D. rotundus* was obtained from IUCN – Nature Server (Barquez et al. 2015) and the estimated densities of cattle for 2005 (heads of cattle per km^2) were obtained from Livestock Geo-Wiki (Robinson et al. 2014). The southernmost record for *D. rotundus*, in Peninsula Valdés (Chubut Province, Argentina: 42°30’ S, 63°56’ W), corresponds to only one unconfirmed observation of the species (Barquez et al. 1999).



Supplementary Figure S2. Livestock biomass (in metric tons) according to the Uruguay 2011 agricultural census. To obtain the total livestock biomass we multiplied the main total abundance of three livestock prey for vampire bats (cattle, sheep and horses) obtained from the census data, by the average weight for each species, obtained from PanTHERIA database (Jones et al. 2009). Mean weight for the three species is 618, 39 and 403 kg for cattle, sheep and horses respectively. Cattle biomass is currently the best proxy for vampire bat abundance, as there is no direct estimation of population sizes nor a complete inventory of shelters.

Table S1. Historical records on cattle abundance, and wildlife and vampire bat distribution in Uruguay since the 1500s

Year	Cattle	Wildlife	Vampire bats	References
1552 / 1568	Introduction of cattle from Paraguay and Bolivia for the foundation of Santa Fe. Later that cattle were introduced into Uruguayan territory			[1,2]
1611 / 1617	First and second introduction of cattle by Hernandarias			[3]
1627	Estimation of a minimum of 100.000 cattle in Uruguayan territory			[2]
1702 – 1714	174.000 leather pieces exported from Buenos Aires (including Uruguayan ones)			[4]
1710	Start of systematic exploitation of cattle in Uruguay			[2]
1715	Reports of very abundant cattle in the coast by Europeans visitors	Deer (probably pampa's deer) in large herds. Capibaras and peccaries also in large groups near the coast.		[4,5]
1723 – 1729	480.000 leather pieces exported from Colonia			[4]
1777	364.000 leather pieces exported from Montevideo (plus and unknown number of smuggled pieces)			[6]
1780	480.000 cattle in Montevideo's jurisdiction, by census			[6]
1781	Convoy from Montevideo exported 432.000 leather to Europe. 6 mail frigates also carrying leather left from Montevideo.			[7]
1787	321.450 leather pieces exported from Montevideo			[7]
1792 – 1793	1.6 million leather pieces exported from Montevideo			[6]
1800	Leather export paralyzed in the country			
1806	English geologist mentions several ranches within 145 km east from Montevideo, ranging from 60.000 to 200.000 cattle each.	Large herds of pampa's deer. Peccaries also present in the south-central area of the country		[8]
1821	2.6 million cattle just in possession by Brazilians in southern Uruguay			[6]
1833		Pampa's deer and capybaras are very abundant		[9]
1867	English naturalist describes a standard ranch in central Uruguay and calculates a 64 cattle per square km density	Pampa's deer not uncommon but in lower numbers. Swamp deer absent at that time but was previously abundant. Jaguar and great anteaters were also absent by that time. Puma was still present, but already rare		[10]
1887	12 million cattle estimated in the country (5.2 million in possession by Brazilians in the whole country)			[6]
1892		Pampa's deer almost exterminated in some jurisdictions, but still abundant in the center of the country. Swamp deer only present in riparian forests of the Uruguay river.		[11]
1894		Figueira considers pampa's deer still abundant in the southeastern region. Swamp deer present only in the north	Only five species of bats recorded for Uruguay. *Desmodus* is not mentioned.	[12]
1908	8.2 million cattle, 21.5 million sheep. Official census data			[13]
1916	7.8 million cattle, 11.5 million sheep. Official census data			

1920 - 1930		Introduction of Axis deer, Dama deer, and wild boars in the southwest		[14]
1924	8.4 million cattle, 14.4 million sheep. Official census data			
1926			Sanborn visited Uruguay, at the Arequita cave he found only *Myotis levis*, but not *D. rotundus*	[15]
1930	7.1 million cattle, 20.5 million sheep. Official census data			
1933			First *Desmodus* collected in northern Uruguay	[16]
1935			Devincenzi cites the species based on the 1933's individual but consider it as a rare species in the country	[16]
1937	8.3 million cattle, 17.9 million sheep. Official census data			
1943	6.3 million cattle, 15.4 million sheep. Official census data			
1946	6.8 million cattle, 19.6 million sheep. Official census data			
1950			Following field work in northeastern Uruguay, Acosta y Lara considers the species to be "unexpectedly common", with large (hundreds of individuals) colonies.	
1959			Acosta y Lara publishes a list of 10 localities for the species in the north and eastern regions of the country	[17]
1969			First record of vampire bats in Arequita cave. This is the only cave where the species was recorded after the being visited several times by mammalogists.	[18]
1972			*Desmodus rotundus* considered to be abundant and widely distributed in the country.	[18]
1976 - 1978		9 populations of pampas deer with an estimated total of less than 1.000 individuals		
1980s		Introduction of Asian water buffalo		
1982		Wild boars declared agricultural pest		
1998		Estimated 1.000 pampa's deer, restricted to 2 populations		[19]
2003		Estimated 800 pampa's deer, restricted to 2 populations		[20]
2011	10.7 million cattle, 7.3 million sheep (Official Census)			[21]
2013		Wild boars abundant and widely distributed. Water buffalos restricted to two small populations. Dama deer restricted to the center of the country and dependent on conservation measures, because of hunting. Axis deer widely distributed in the country		[14]
2013 – 2018			The species is abundant and widely distributed. It appears very often in acoustic surveys regardless of habitat or location	Pers. Obs. Unpub. data.
2016	Estimated 12.1 million cattle. Estimated 2.5 million individuals removed annually from stock			[21]

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20. **Weber M, Gonzalez S**. Latin American deer diversity and conservation: A review of status and distribution. *Ecoscience* 2003; **10**: 443–454.
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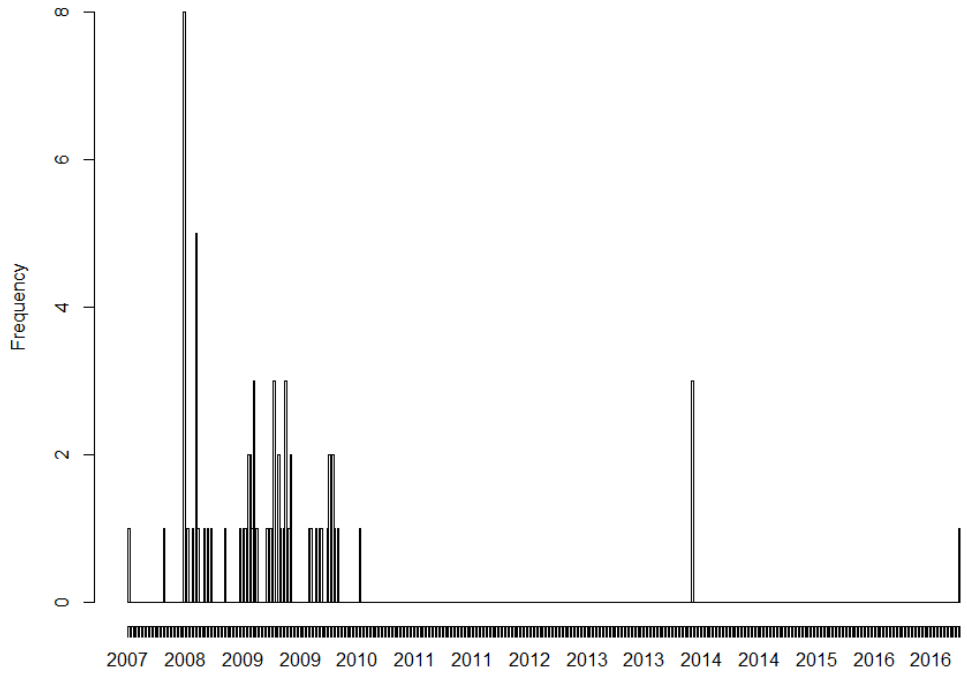
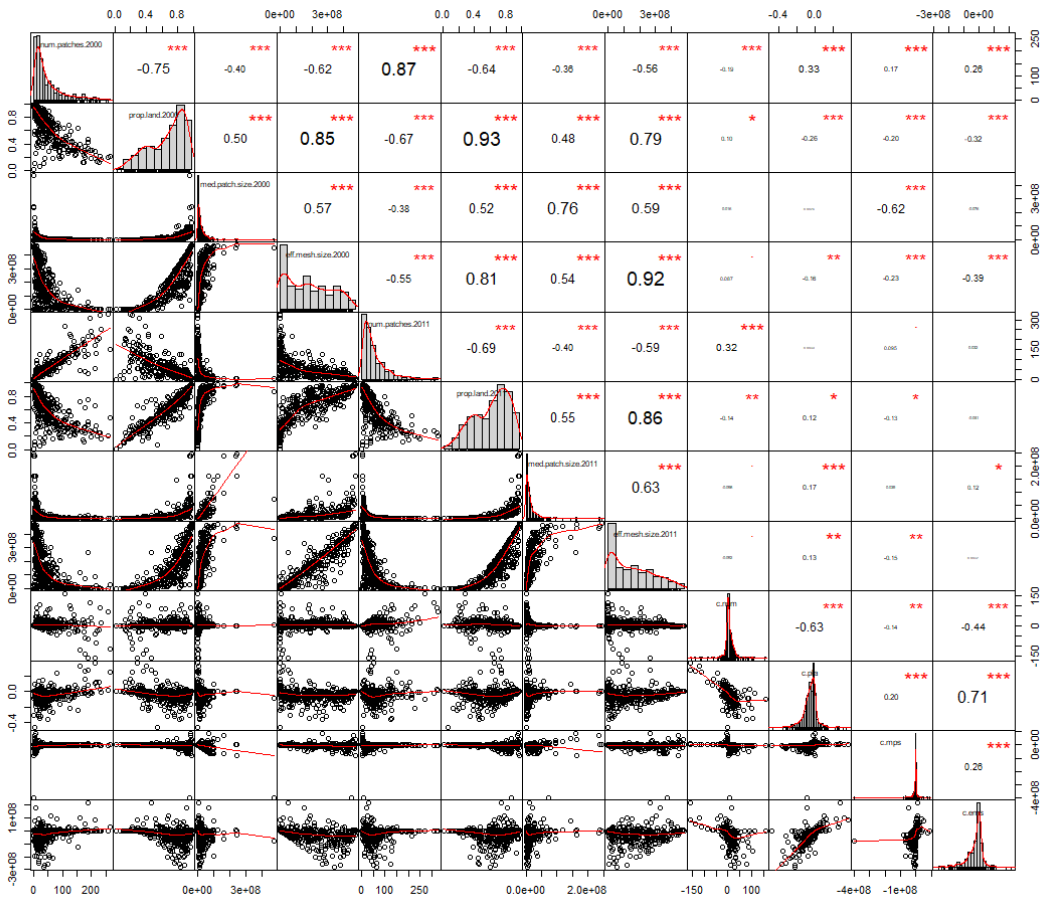
APPENDIX B

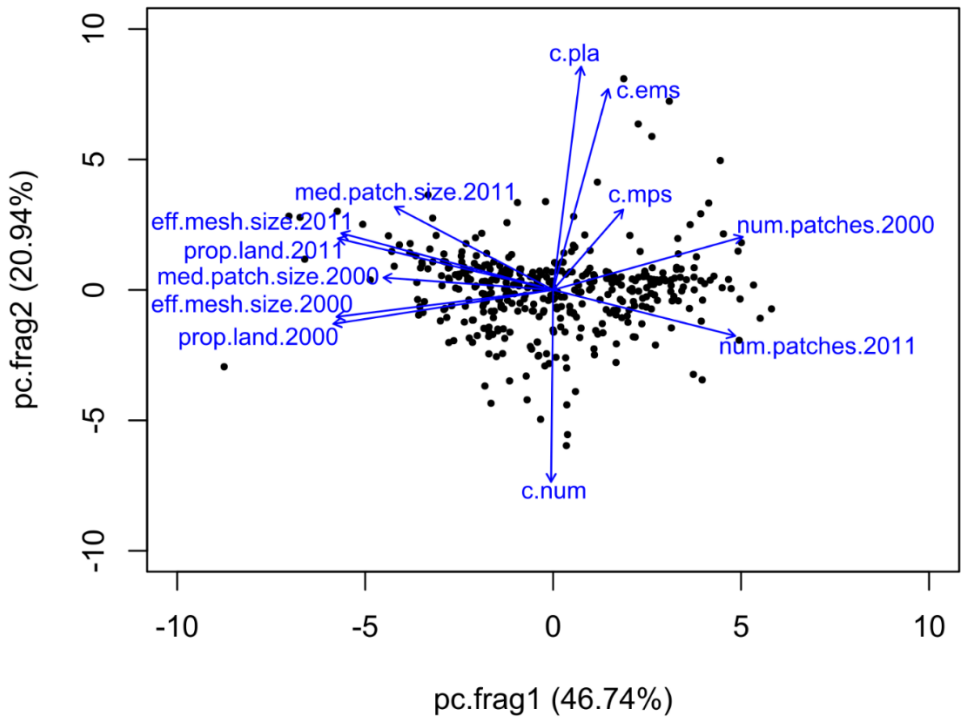
SUPPLEMENTARY MATERIAL TO CHAPTER 3: SYNERGISTIC EFFECTS OF GRASSLAND FRAGMENTATION AND TEMPERATURE ON BOVINE RABIES EMERGENCE



Supplementary Figure S1. Temporal distribution of rabies outbreaks as number of new quarantined ranches per week from 2007 to 2017.

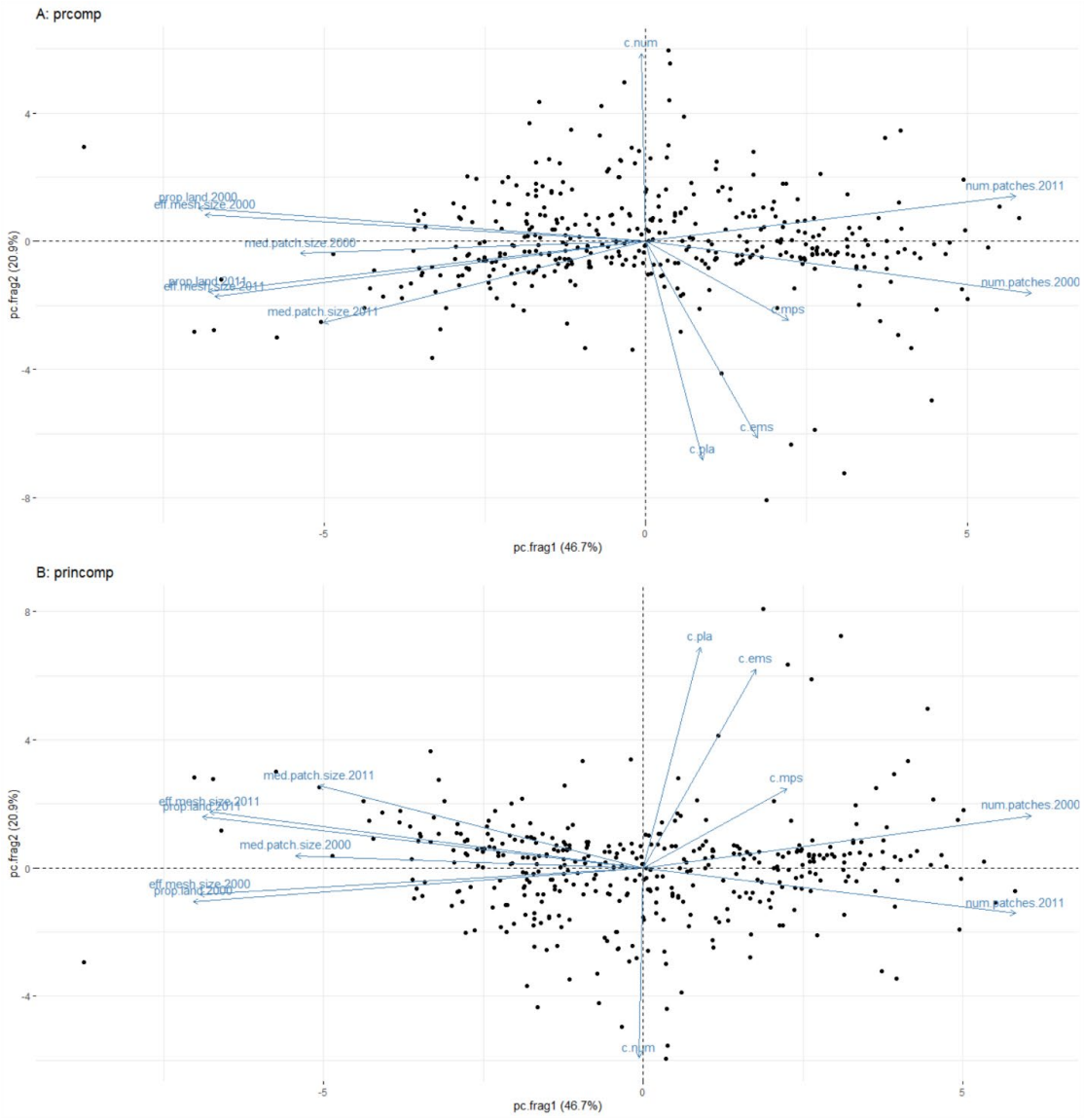


Supplementary Figure S2. Correlation matrix of grassland fragmentation metrics. Collinearity is observed for several pairs of variables, showing that variables provide similar information about the fragmentation process

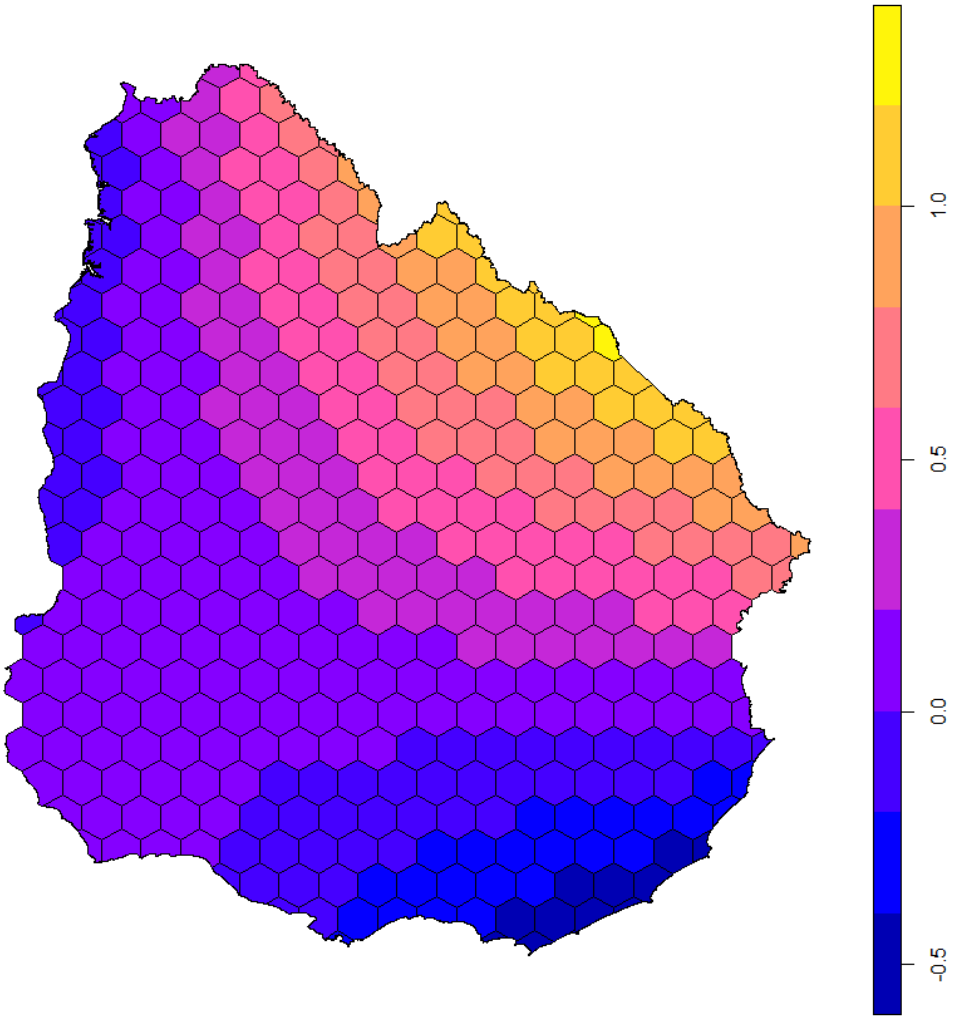


Supplementary Figure S3. Biplot for the Principal Component Analysis on the grassland fragmentation metrics. The first principal component (*pc.frag1*) explains the 46.74% of the total variance and is related to fragmentation status. *pc.frag1* increases with the increase in number of patches or with the decrease in proportion of landscape covered by livestock areas, the mean patch size and the effective mesh size. The second principal component (*pc.frag2*) explains the 20.94% of the total variance and is associated with recent change in fragmentation. Negative values in the second principal component are indicative of increased fragmentation from 2000 to 2011. Variables included in the analysis: *num.patches.2000*, *num.patches.2011* (number of grassland patches per cell in

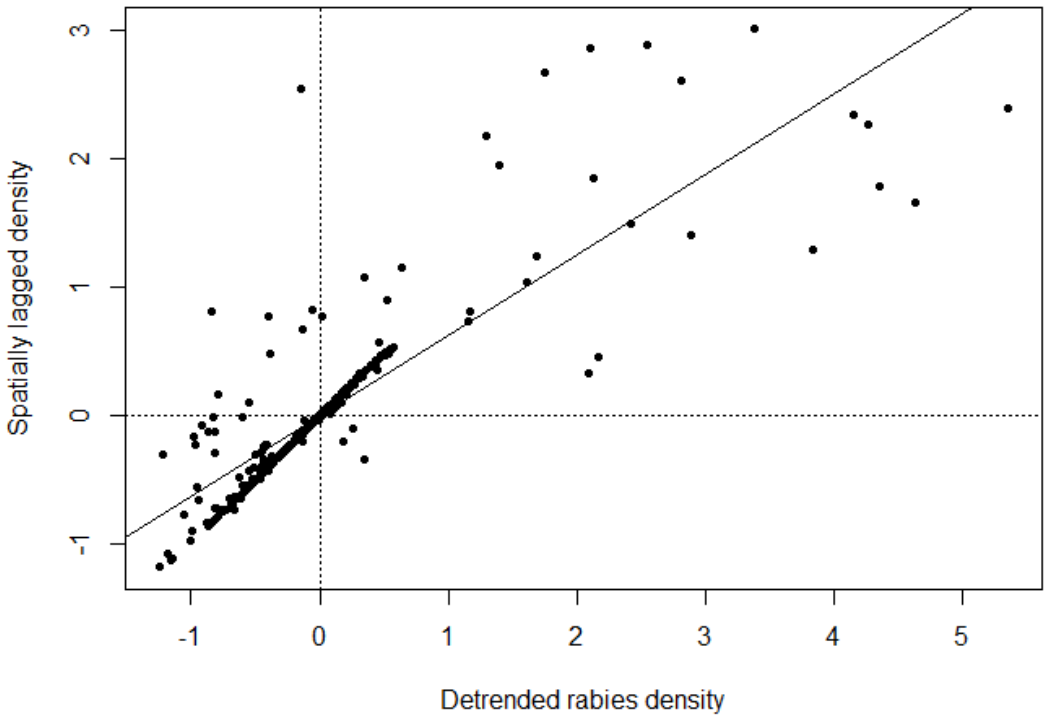
2000 and 2011), *prop.land.2000*, *prop.land.2011* (proportion of cell covered by grasslands in 2000 and 2011), *med.patch.size2000*, med.patch.size2011 (mean size of grassland patches per cell in 2000 and 2011), *eff.mesh.size2000*, *eff.mesh.size2011* (effective mesh size of grassland areas in 2000 and 2011), *c.num* (change in number of grassland patches from 2000 to 2011), *c.pla* (change in proportion of cell covered by grasslands patches from 2000 to 2011), *c.mps* (change in mean size of grassland patches per cell from 2000 to 2011), *c.ems* (change in effective mesh size of grassland patches per cell from 2000 to 2011). See text for calculation of grassland fragmentation metrics.



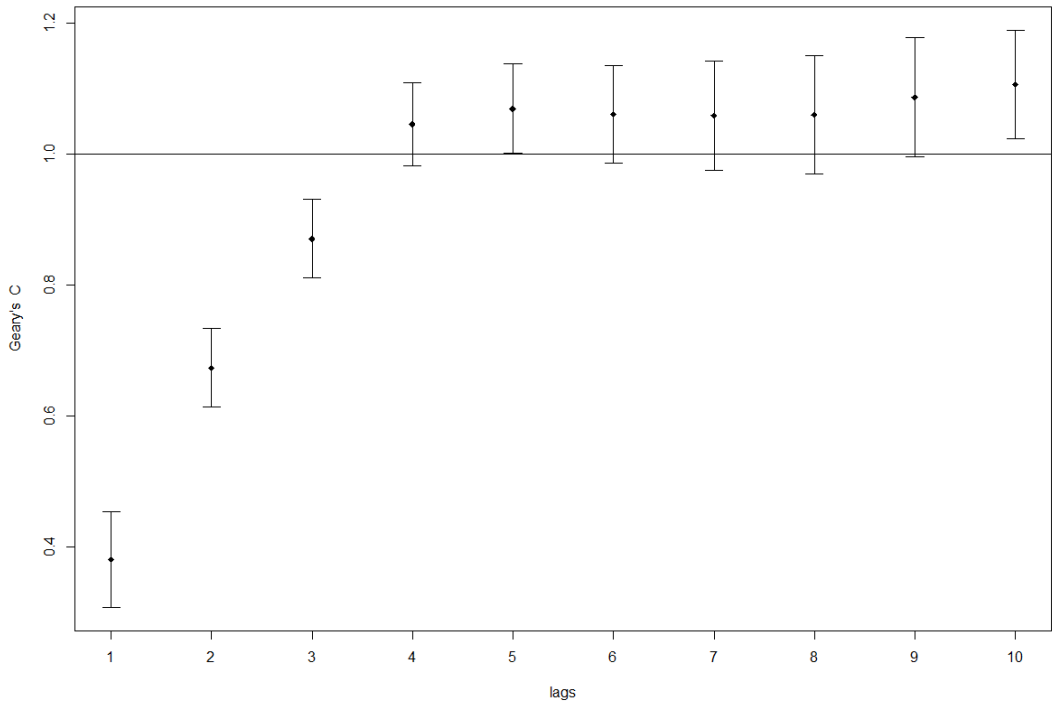
Supplementary Figure S4. Comparison of the results from the Principal Component Analyses using spectral decomposition (function prcomp, panel A) and singular decomposition (function princomp, panel B). Both methods gave similar results, with only inverted loadings on the second principal component.



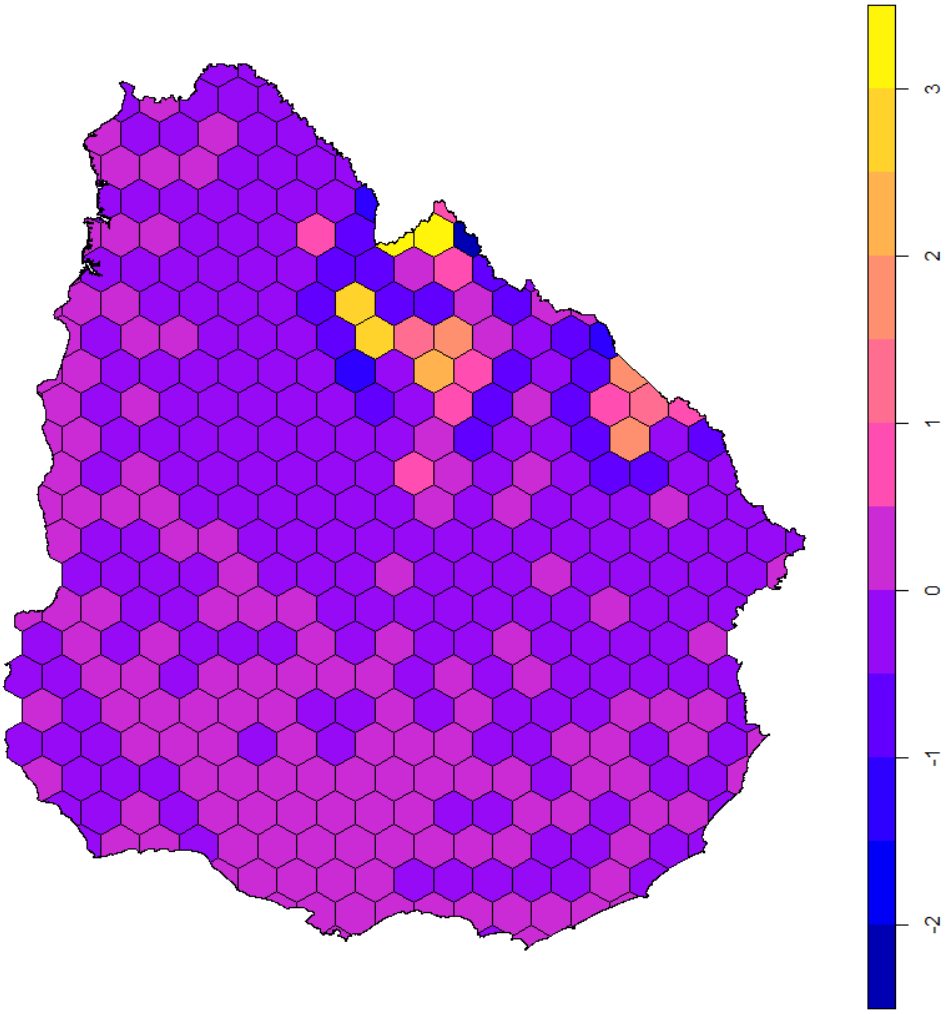
Supplementary Figure S5. Distribution of fitted values from the linear regression showing a northeast-to-southwest trend on the density of ranches with rabies cases. There are higher expected densities in the Northeastern region of the country and decreasing values in the northeast-to-southwest direction. See also Figure 3 A-B in the main text for a comparison of the predicted values with the observed density of quarantined ranches.



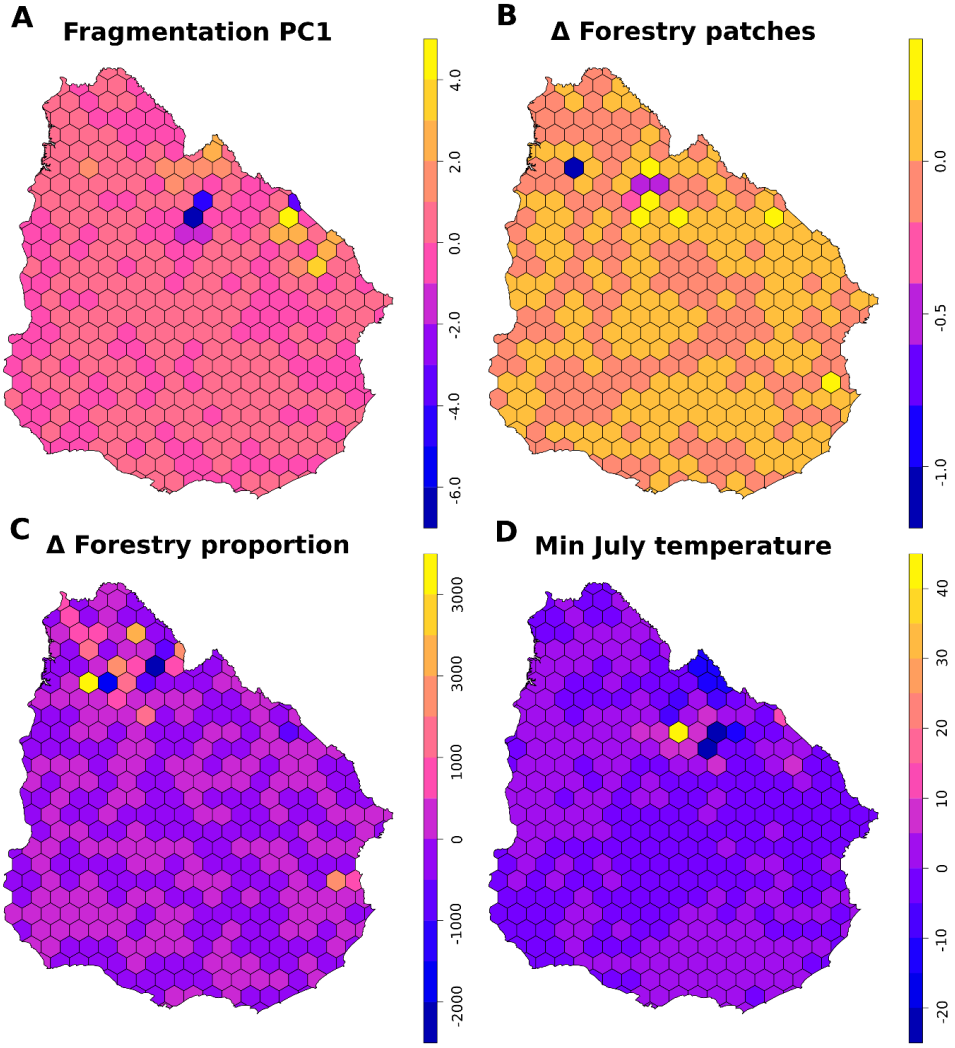
Supplementary Figure S6. Moran plot for the residuals from the linear regression analysis. Even after taking out the first order effect, the detrended residuals still show a positive spatial autocorrelation ($C=0.38$, p-value<0.01), showing that there is an underlying spatial structure of the rabies outbreaks not explained by the invasion-wave-like effect.



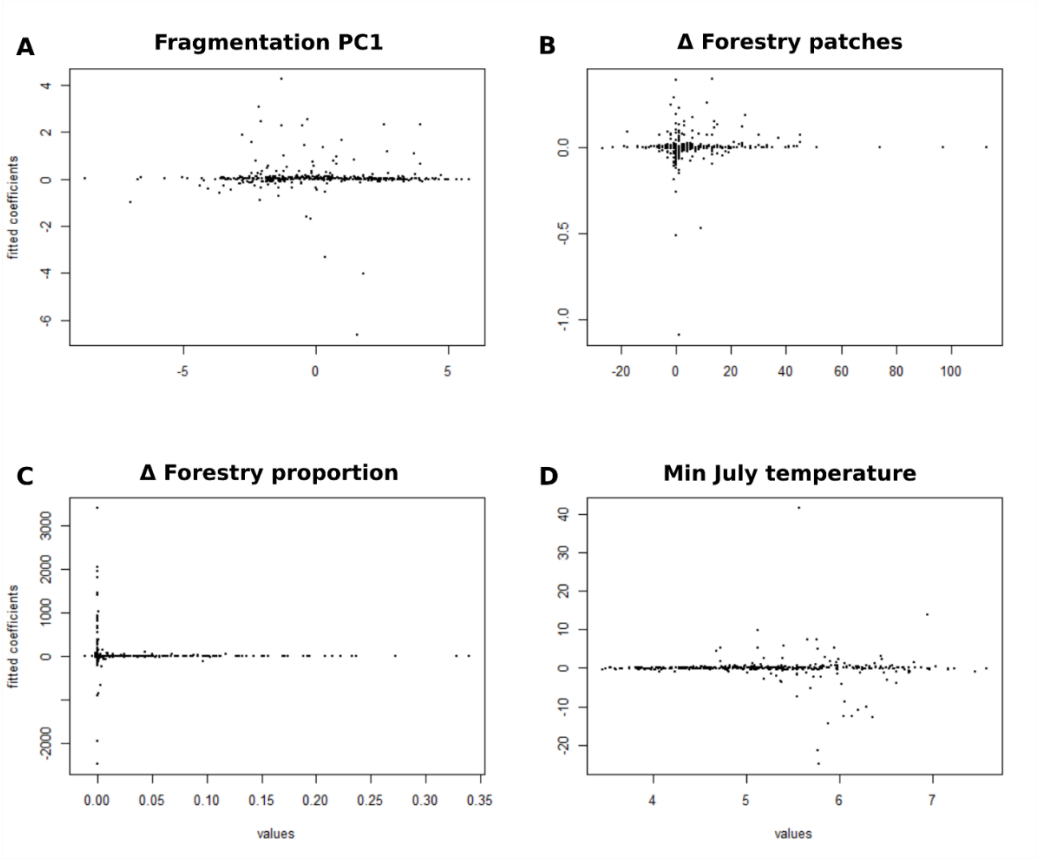
Supplementary Figure S7. Correlogram for the residuals from the linear model. A positive significant autocorrelation (Geary's C value lower than 1) is observed only for the first three lags, whereas for the rest of the lags, no significant autocorrelations are observed. The spatial structure of density of outbreaks is only observed up to the third order of neighboring in the regular hexagonal-cell grid



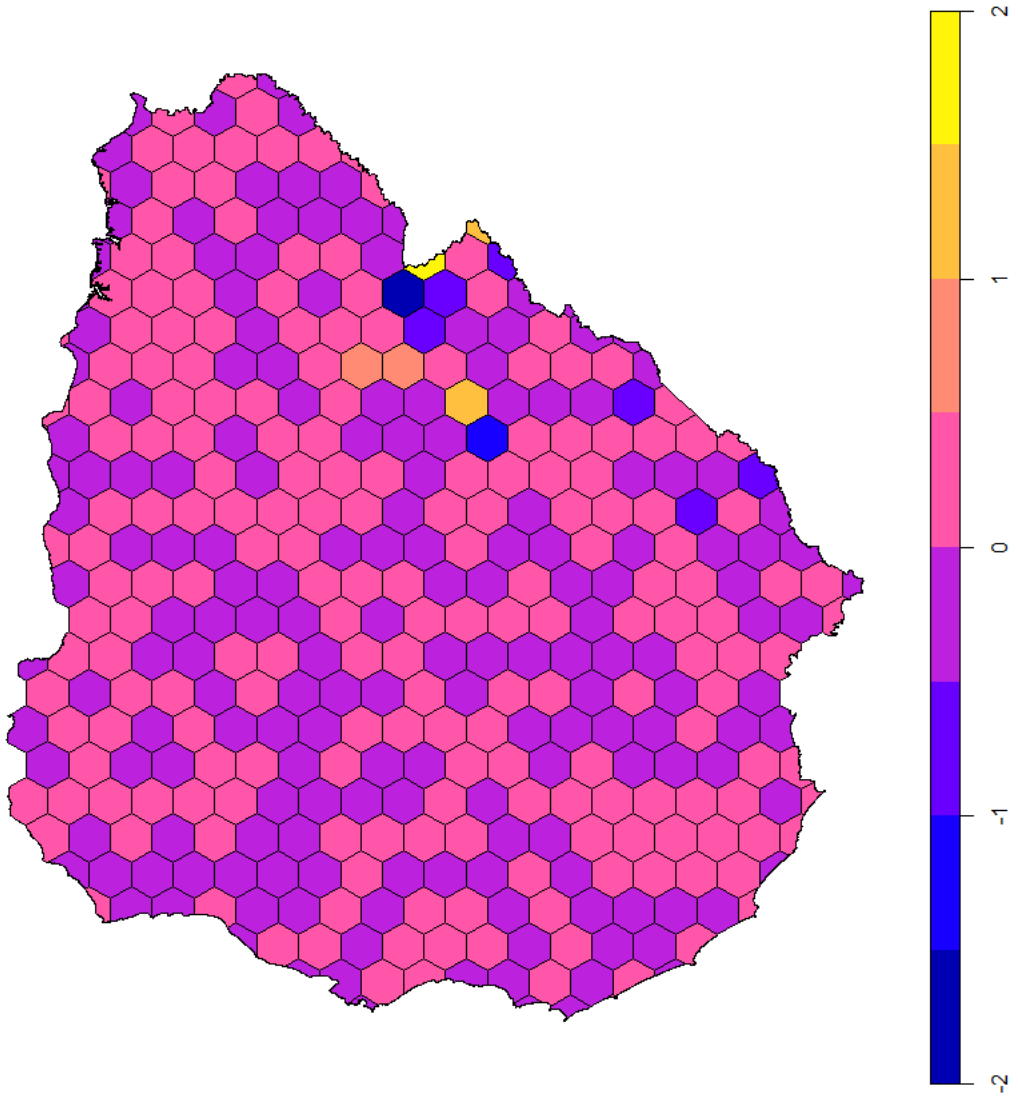
Supplementary Figure S8. Distribution of the residuals from the final Spatial Autoregressive (SAR) Model (*detrended_residuals ~ c.fnp * temp_jul*). The autocorrelation analysis for the residuals, using Geary's C, shows no significant autocorrelation (Geary's C=0.96, p-value=0.15).


A
Fragmentation PC1
4.0
2.0
0.0
-2.0
-4.0
-6.0
B
Δ Forestry patches
0.0
-0.5
-1.0
C
Δ Forestry proportion
3000
2000
1000
0
-1000
-2000
D
Min July temperature
40
30
20
10
0
-10
-20

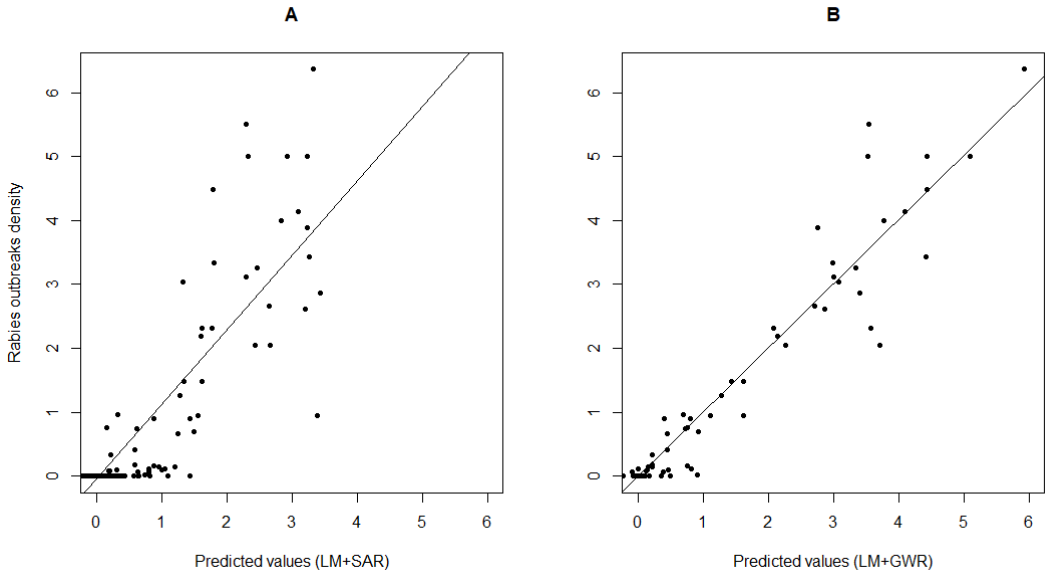
Supplementary Figure S9. Results from the Geographically Weighted Regression model: geographic distribution of fitted coefficients from the final model (*detrended_residuals ~ pc.frag1 + c.fnp + c.fpl + temp_jul*). A. Fragmentation PC1 (*pc.frag1*), positive values indicate higher fragmentation of livestock areas B. Δ forestry patches (*c.fnp*), change in number of exotic forestry patches between 2000 and 2011 C. Δ forestry proportion (c.fpl), change in proportion of landscape covered by exotic forestry between 2000 and 2011 D. Minimum Jul temperature (*temp_jul*), average minimum temperature of the coldest month.



Supplementary Figure S10. Distribution of the fitted coefficient values for the final GWR model (*detrended_residuals ~ pc.frag1 + c.fnp + c.fpl + temp_jul*), in relation to the original values of each variable: A. Fragmentation PC1 (*pc.frag1*), positive values indicate higher fragmentation of livestock areas B. Δ forestry patches (*c.fnp*), change in number of exotic forestry patches between 2000 and 2011 C. Δ forestry proportion (c.fpl), change in proportion of landscape covered by exotic forestry between 2000 and 2011 D. Minimum Jul temperature (*temp_jul*), average minimum temperature of the coldest month.



Supplementary Figure S11. Distribution of the residuals from the final Geographically Weighted Regression (GWR): *detrended_residuals ~ pc.frag1 + c.fnp + c.fpl + temp_jul*. Residuals from the final model showed no significant spatial autocorrelation (Geary's C=1.13, p-value=0.99).



Supplementary Figure S12. Linear correlation of rabies outbreak density with predicted values by combination of linear model and Simultaneous Autoregressive model (A), and combination of linear model and Geographically weighted regression (B). The plotted lines in both cases represent the simple linear regression between the rabies outbreaks density and the predicted values. In both cases there is a positive and significative linear association (R^2=0.76, $p<0.01$ and R^2=0.94, $p<0.01$ respectively), showing good prediction power of both methods. Not only there is a strong correlation, but also in both examples, the intercept is not different from zero ($p>0.05$) and the regression coefficients for the predicted values are close to 1 (β=1.16 for SAR and β=1.00 for GWR), showing that there almost no bias in the prediction. In B, the GWR shows better accuracy and less dispersion of predicted values for each observed value. Non-stationary coefficients allow the model to perform better than SAR in predicting the zero values for rabies density.

Supplementary Table S1. Legend of the Land Cover Classification System used in Uruguay (LCCS-Uy, modified from Álvarez et al. 2015). The four classes considered for livestock areas (i.e. He, Ar, Pa and ANi, by their original codes in Spanish) and the class for forestry (PF) are highlighted. We selected the classes to include at the 17-classes level, as the 46-classes legend is not conserved across different versions of the LCCS-Uy products.

Main Groups	17-classes legend	46-classes legend
A11 - Cultivated and managed terrestrial areas	Irrigated crops >4-5 ha	Irrigated crops >4-5 ha
		Sugar cane
		Rice field >4-5 ha
		Sugar cane or rice > 4-5 ha
	Rainfed crops > 4-5 ha	Rainfed crops > 4-5 ha
	Small crops <4-5 ha	Rainfed crops < 4-5 ha
		Irrigated crops < 4-5 ha
	Forestry Plantations (PF)	Forestry venture > 5 ha
		Coastal implanted forest
		Eucalyptus forest > 5 ha
		Pine trees forest > 5 ha
		Shelter and shadow forest <5 ha
		Urban park
	Orchards	Citrus orchards
		Fruit orchards
A12 - Natural and semi-natural vegetation	**Natural Herbaceous (He)**	Natural grassland
		Sandplain herbaceous
		Natural grassland with sparse palm groves (1-15%)
		Natural grassland with rocks
	Shrublands (Ar)	Shrublands and natural grassland
	Native Forests	Native hillside forests
		Native riparian forest
		Native forests
		Native savannah forest
	Palm Groves (Pa)	Palm Groves
A24 - Natural and semi-natural aquatic or regularly flooded vegetation	**Natural Flooded Areas (ANi)**	Permanently flooded herbaceous coverage
		Temporarily flooded herbaceous coverage
B15 - Artificial surfaces and associated areas	Urban equipment	Airport
		Airfield
		Sport facilities
		Industrial facilities
		Port areas
	Urban areas	Urban areas
	Sparse Urban Areas	Sparse urban area and croplands
		Sparse urban area and natural grasslands
		Sparse urban area and forestry
B16 - Bare soil areas	Quarries, sand pits, open-pit mines	Quarries, sand pits, open-pit mines
	Bare soil areas	Sand beaches
		Sand dunes

		Consolidated rock
		Bare soil
B27 - Artificial ice, snow or water bodies	Artificial water bodies	Channels
		Lakes, reservoirs
B28 - Natural ice, snow or water bodies	Natural water bodies	Lagoons
		Water courses
		Humid and seasonally flooded soils

Supplementary Table S2. Variables of livestock areas fragmentation included in the Principal Component Analysis and loading scores for first two principal components (*pc.frag1* and *pc.frag2*, very small loadings are not printed, and substituted by a star). Number of grassland patches per cell (in 2000, 2011 and the difference 2011-2000: *num.patches.2000*, *num.patches.2011*, *c.num*), Proportion of grassland landscape (in 2000, 2011 and the difference 2011-2000: *prop.land.2000*, *prop.land.2011*, *c.pla*), Mean grassland patch size (in 2000, 2011 and the difference 2011-2000: *med.patch.size.2000*, *med.patch.size.2011*, *c.mps*), and Effective grassland mesh size (in 2000, 2011 and the difference 2011-2000: *eff.mesh.size.2000*, *eff.mesh.size.2011*, *c.ems*)

		Loading scores	
Variable description	**Variable name**	***pc.frag1***	***pc.frag2***
Number of grassland patches per cell 2000	*num.patches.2000*	0.336	0.136
Number of grassland patches per cell 2011	*num.patches.2011*	0.323	-0.117
Change in number of grassland patches per cell (2011-2000)	*c.num*	*	-0.490
Proportion of grassland landscape 2000	*prop.land.2000*	-0.390	*
Proportion of grassland landscape 2011	*prop.land.2011*	-0.382	0.132
Change in the proportion of grassland landscape (2011-2000)	*c.pla*	*	0.570
Mean grassland patch size 2000	*med.patch.size.2000*	-0.301	*
Mean grassland patch size 2011	*med.patch.size.2011*	-0.281	-0.281
Change in mean grassland patch size (2011-2000)	*c.mps*	0.124	0.205

Effective grassland mesh size 2000	*eff.mesh.size.2000*	-0.384	*
Effective grassland mesh size 2011	*eff.mesh.size.2011*	-0.376	0.145
Change in effective grassland mesh size (2011-2000)	*c.ems*	*	0.513

Supplementary Table S3. Results from the linear model using the latitude (Y), longitude (X) and the interaction between both (Y * X). Estimated coefficients for the two variables and the double interaction are presented, along with the p-values (starred p-values are significant at 0.05 level).

Variables	**Estimate**	**p-value**
Intercept	5.16e+01	
Y	-8.21e-06	* <0.01
X	-1.14e-04	* <0.01
*Y * X*	1.82e-11	* <0.01

Supplementary Table S4. Descriptive statistics for the values from the coefficients in the last GWR model (*detrended_residuals ~ pc.frag1 + c.fnp + c.fpl + temp_jul*). The spatial structure of the coefficients is presented in the values of Geary's C autocorrelation index and the correspondent p-values (starred p-values are significant at 0.05 level).

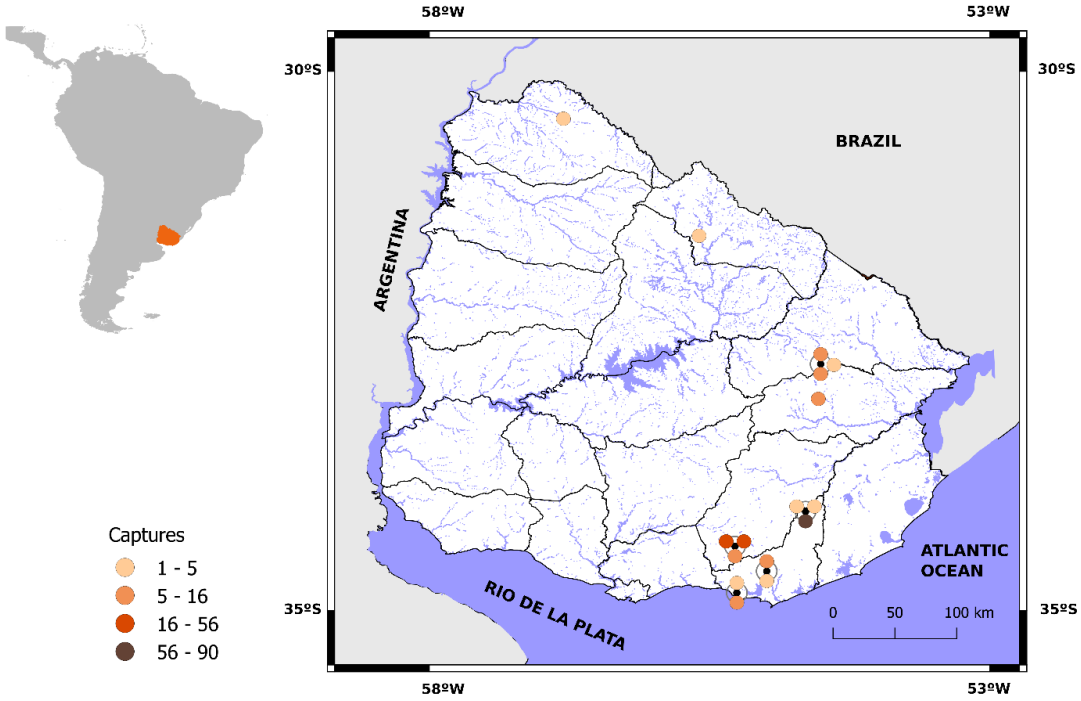
Variables	**Min**	**Median**	**Max**	**Geary's C**	**p-value**
pc.frag1	-6.64	0.01	4.26	0.75	*<0.01
c.fnp	-1.09	0.00	0.40	0.99	0.35
c.fpl	-2473.10	0.00	3400.60	1.02	0.68
temp_jul	-0.25	-0.01	41.56	0.76	*<0.01

Supplementary table S5. Results from the linear models to assess concordance among observed rabies outbreaks density and combined predictions from linear model (trend) and either Simultaneous Autoregressive model (SAR) or Geographically Weighted Regression (GWR). Estimated coefficients for the predicted values in each model combination are presented, along with the p-values (starred p-values are significant at 0.05 level). The second model show better fitting (R^2=0.94), concordance (coefficient estimate=1) and no bias (intercept estimate = 0). Figure S9 shows graphically the perform of both analyses.

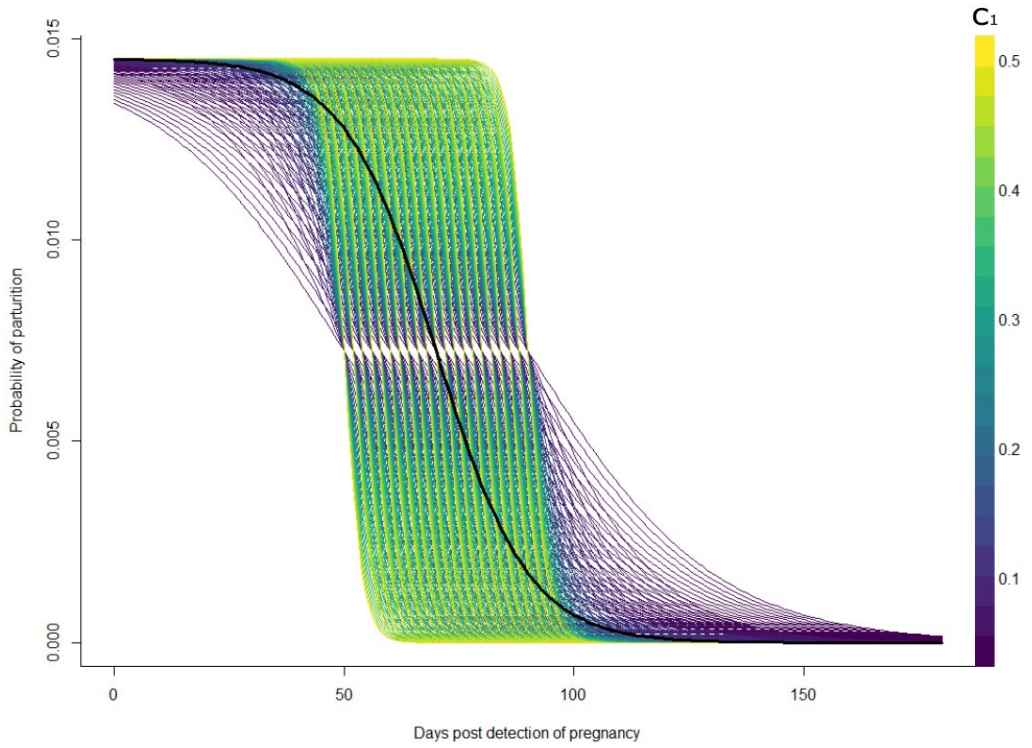
Variables	**Estimate**	**p-value**	**R^2**
Model 1: ***rabies density ~ (LM + SAR predicted values)***			0.76
Intercept	-0.04	0.09	
LM + SAR predicted values	1.16	*<0.01	
Model 2: ***rabies density ~ (LM + GWR predicted values)***			0.94
Intercept	0.00	0.66	
LM + GWR predicted values	1.00	*<0.01	

APPENDIX C

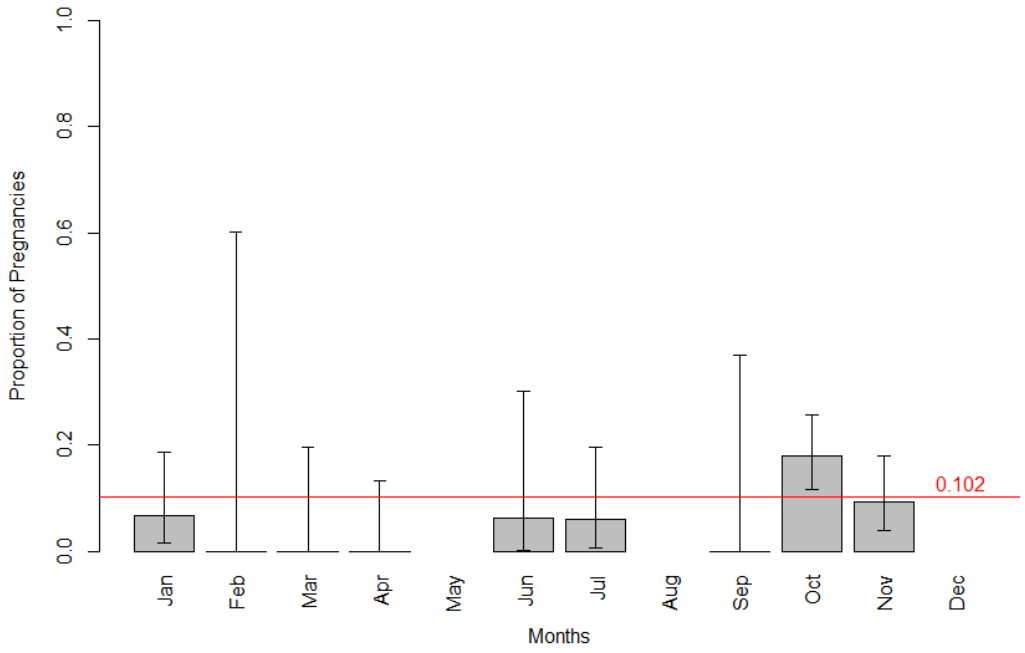
SUPPLEMENTARY MATERIAL TO CHAPTER 5: VAMPIRE BAT RABIES IN CHANGING LANDSCAPES: HOW LAND USE-INDUCED CHANGES IN POPULATION CONNECTIVITY AFFECT THE RISK OF RABIES VIRUS SPILLOVER



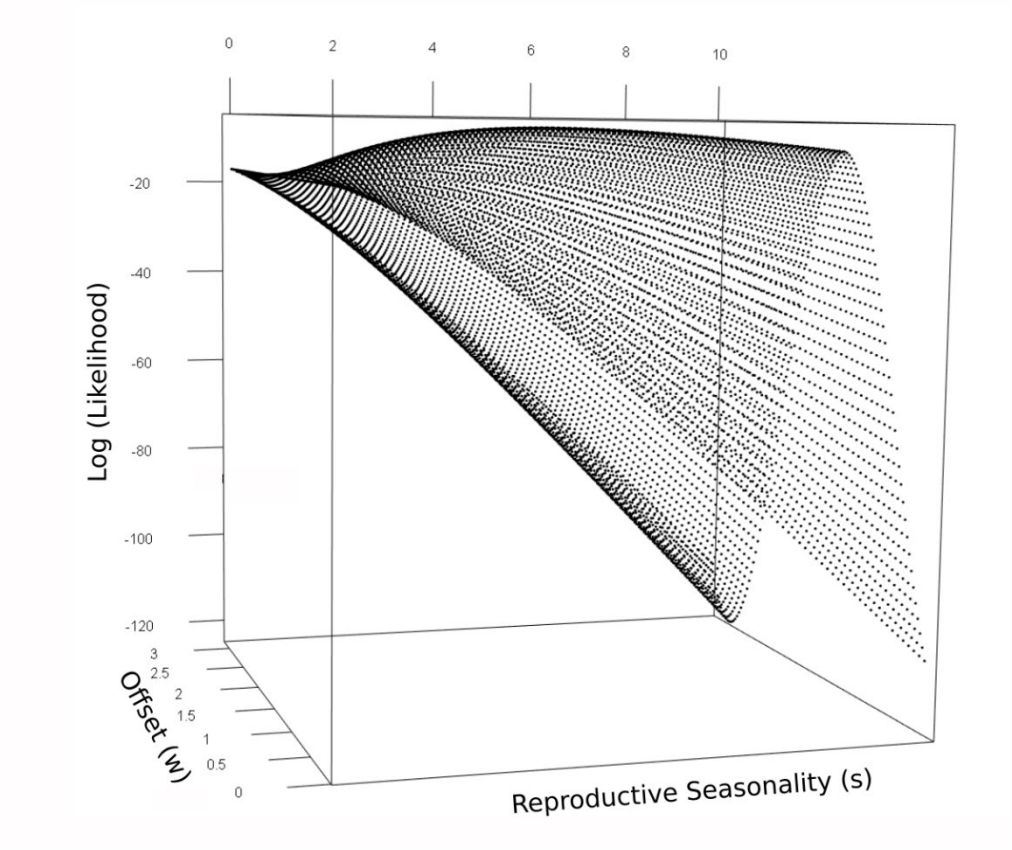
Supplementary Figure 1. Geographic distribution of sampled colonies in Uruguay, for estimation of birth rate and seasonality. Overlapping points are displaced for representation, around the geographic center of the cluster.



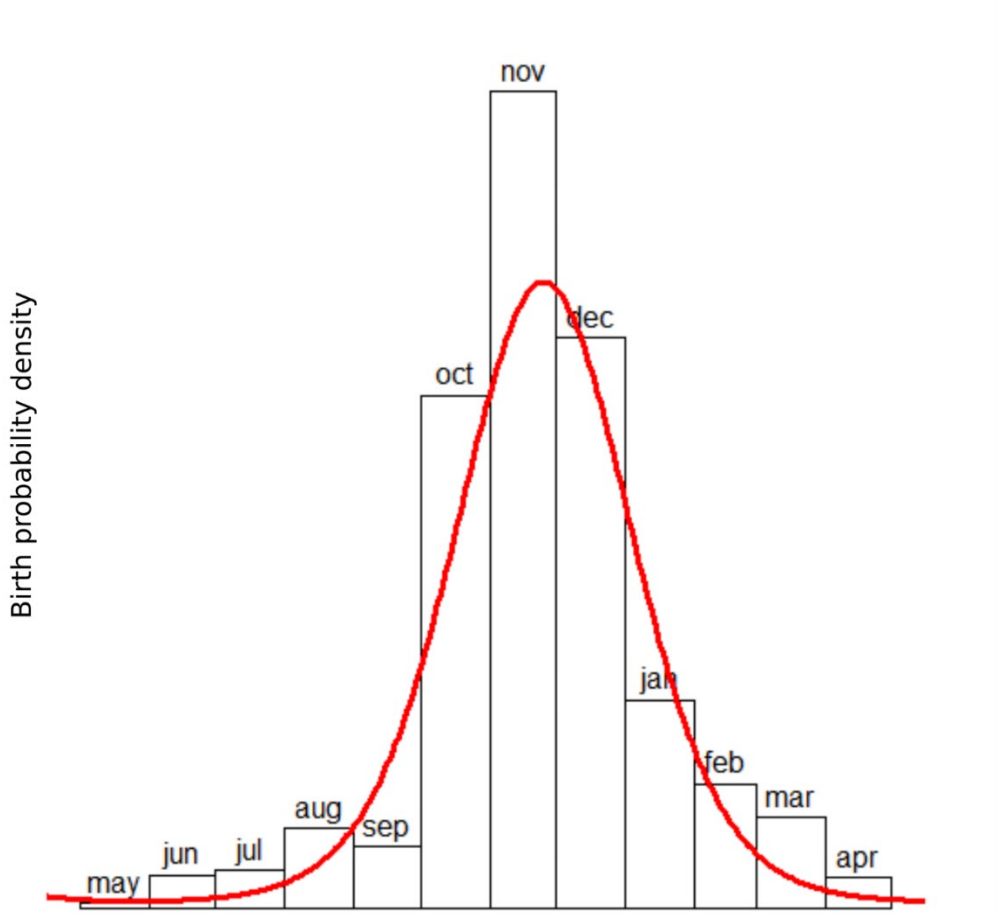
Supplementary Figure 2. Probability density functions for time to parturition given detection of pregnancy (see Methods: Reproductive seasonality estimation for details on the function), used in the sensitivity analyses. The graph depicts 420 curves corresponding to 20 values in c_1=[0.05; 0.50] and 21 values in c_2=[50; 90]. Values of c_1 control the slope and are shown in the color scale in the right. Values of c_2 control the position in time of the inflection point. The black curve shows the function used for the rest of the study: c_1=0.1; c_2=70.



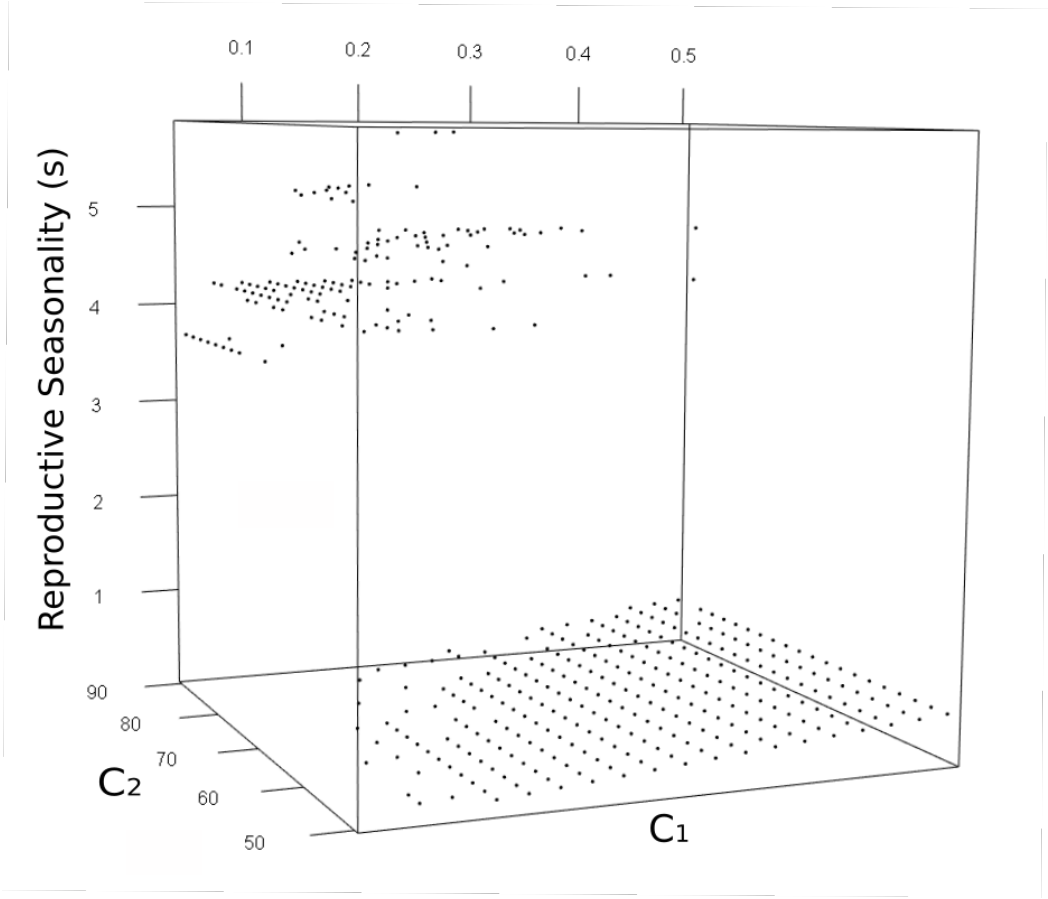
Supplementary Figure 3. Observed monthly proportion of pregnant females over total captured individuals. Error bars show exact binomial 95% confidence intervals, red horizontal line represents the global annual fecundity: 0.102 $years^{-1}$.



Supplementary Figure 4. Maximum likelihood estimation of the Synchrony parameter (s) of the seasonality equation in function of c_1 and c_2 values of the probability density function. The synchrony is dependent on the assumption about the shape of the of probability function for waiting time to parturition given the pregnancy is detected. However, the response is not continuous but rather binomial: the births are either not seasonal at all (values of s close to 0) or highly seasonal (higher values of s).

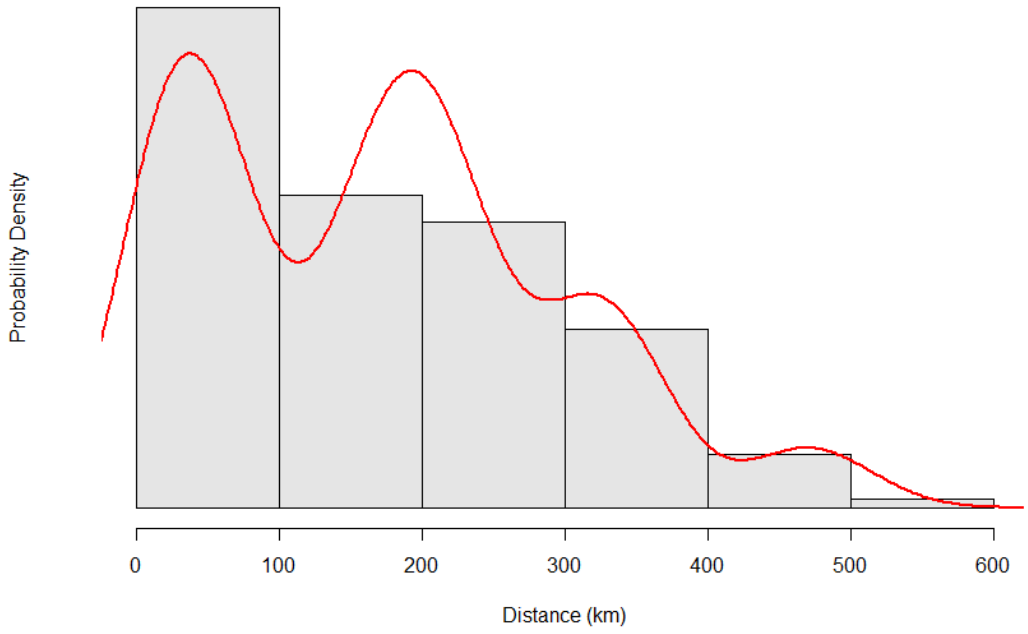


Supplementary Figure 5. Annual distribution of simulated birthdates, accounting for capture effort and sex bias. The peak of births occurs on Nov 23rd.

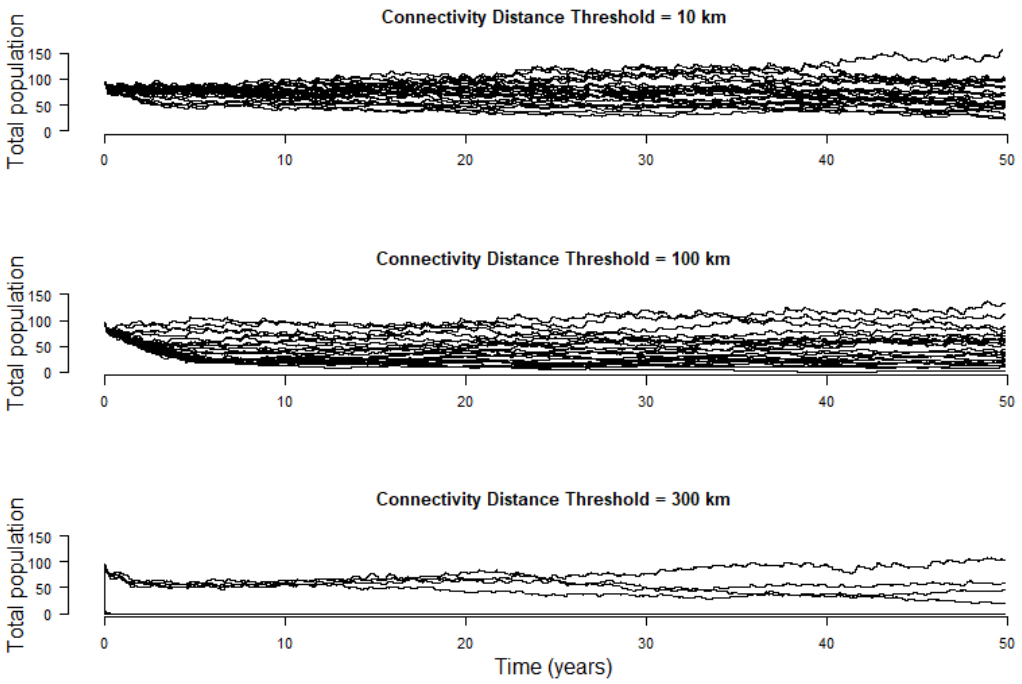


Supplementary Figure 6. Sensitivity analysis of the effect of c1 and c2 over the estimated reproductive seasonality (represented by synchrony s parameter). Most combinations of c1 and c2 over the explored parametric space render no relevant differences in the estimated seasonality. For combinations of c1 and c2 that result in a seasonal reproduction ($s \neq 0$), most combinations produce synchrony values between 4 and 5 (the maximum likelihood estimator used in the analysis was 4.55)

Supplementary Figure 7. Geographic distribution of the 25 colonies used for the metapopulation modeling. See supp. fig. 8 for the distribution of pairwise distances among colonies.



Supplementary Figure 8. Distribution of pairwise distances among colonies used for the metapopulation model simulations. A right-skewed bimodal distribution is observed, where the first density peak corresponds to the intra-cluster distances and the second peak account for inter-cluster distances.



Supplementary Figure 9. Dynamic of the total 25 subpopulations under different connectivity distance thresholds (10, 100 and 300 km). Each line represents the total number of individuals in one subpopulation, over 50 years simulation.