

# Development of a dynamic model for the emergence of Lassa fever in West Africa: Appraisal 2021

## Contents

<b>1 Appraisal report</b>	<b>4</b>
1.1 Introduction . . . . .	4
1.1.1 Drivers of zoonotic disease emergence . . . . .	4
1.1.2 Hotspots for emerging zoonoses . . . . .	5
1.1.3 Rodents as zoonotic hosts . . . . .	7
1.1.4 Lassa fever as a model disease system . . . . .	8
1.2 Thesis outline . . . . .	12
1.2.1 Thesis aims and questions . . . . .	12
1.2.2 Chapter 1: General introduction . . . . .	12
1.2.3 Chapter 2: A scoping review of rodent trapping studies to understand the prevalence of zoonotic disease in West Africa. . . . .	12
1.2.4 Chapter 3: Rodent trapping to explore rodent assemblage structure in Eastern Sierra Leone. . . . .	13
1.2.5 Chapter 4: Serological investigation of <i>Lassa mammarenavirus</i> among rodent species in Eastern Sierra Leone. . . . .	14
1.2.6 Chapter 5: Serological studies investigating <i>Lassa mammarenavirus</i> seropositivity in peridomestic species. . . . .	16
1.2.7 Chapter 6: Development of a LIPS serological assay for <i>Lassa mammarenavirus</i> . . . . .	17

1.2.8	Chapter 7: Parameterisation of a mathematical model of viral transmission in rodent populations to investigate spillover risk. . . . .	18
1.2.9	Current progress . . . . .	18
1.2.10	Chapter 8: General discussion . . . . .	20
1.3	Project timeline . . . . .	20
1.4	Training and development . . . . .	21
<b>2</b>	<b>Appendix 1: A scoping review of rodent trapping studies to understand the prevalence of zoonotic disease in West Africa.</b>	<b>21</b>
2.1	Abstract . . . . .	21
2.2	Introduction . . . . .	22
2.3	Methods . . . . .	24
2.3.1	Literature search . . . . .	24
2.3.2	Data extraction . . . . .	24
2.3.3	Analysis . . . . .	25
2.4	Results . . . . .	26
2.4.1	Included studies . . . . .	26
2.4.2	Location and habitats of rodent trapping studies to investigate potential biases . . . . .	28
2.4.3	Rodent presence, absence, abundance . . . . .	28
2.4.4	Pathogen presence and absence . . . . .	31
2.4.5	Host-pathogen associations . . . . .	33
2.5	Discussion . . . . .	34
2.5.1	Limitations . . . . .	37
2.5.2	Implications . . . . .	37
2.6	Conclusion . . . . .	37
2.7	Acknowledgments . . . . .	38
2.8	Supplementary 1 . . . . .	38

2.9	Supplementary 2 . . . . .	38
2.10	Supplementary 3 . . . . .	38
<b>3</b>	<b>Appendix 2: Rodent trapping protocol</b>	<b>38</b>
3.1	Introduction . . . . .	38
3.2	Preparation for field work . . . . .	39
3.3	Locating the trap sites . . . . .	39
3.3.1	Lalehun . . . . .	40
3.3.2	Seilama . . . . .	42
3.3.3	Bambawo . . . . .	44
3.3.4	Lambayama . . . . .	44
3.3.5	Baiama . . . . .	45
3.4	Trap setup . . . . .	46
3.5	Trap check . . . . .	46
3.6	Rodent sampling . . . . .	46
3.7	Data collection process . . . . .	47
3.7.1	Direct ODK entry . . . . .	47
3.7.2	Data entry sheets . . . . .	48
<b>4</b>	<b>Appendix 3: Visit reports: Visit 1 to 3</b>	<b>48</b>
<b>5</b>	<b>Appendix 4: ICREID poster</b>	<b>55</b>
<b>6</b>	<b>Appendix 5: Training log</b>	<b>56</b>
<b>7</b>	<b>References</b>	<b>56</b>

# 1 Appraisal report

## 1.1 Introduction

The strong link between human health and the environment is particularly evident in the context of infectious diseases (Whitmee *et al.*, 2015; Sandifer, Sutton-Grier and Ward, 2015). Significant reductions in morbidity and mortality associated with infectious diseases have been achieved following improvements in hygiene, sanitation and the use of antimicrobials (Aiello and Larson, 2002). However, in 2019, 13.9% of annual deaths and 16.6% of disability adjusted life years lost globally were directly attributable to infectious diseases (Vos *et al.*, 2020). Up to 58% of these infectious diseases are considered zoonotic, either through direct transmission from zoonotic sources (e.g. Ebola virus disease, Lassa fever, Nipah virus) or emerging from a zoonotic origin (e.g. HIV, multiple influenza strains, SARS-CoV and MERS-CoV) (Woolhouse and Gowtage-Sequeria, 2005; Wit *et al.*, 2016). Global pandemics such as the ongoing HIV and SARS-CoV-2 outbreaks demonstrate the potential impact of zoonotic diseases on human mental and physical health in a highly connected world (Tatem, Rogers and Hay, 2006; Bogoch *et al.*, 2020).

### 1.1.1 Drivers of zoonotic disease emergence

separate spillover of known established pathogens and emergence of novel pathogens

The emergence of a novel zoonotic disease from animal hosts into human populations (*i.e. spillover events*) can usefully be conceptualised as a complex system (Wood *et al.*, 2012; Redding *et al.*, 2019). In such a complex system, multiple, interconnected components - e.g., the physical environment, the zoonotic host niche, the human populations - interact to modify the risk of zoonotic disease spillover (Figure 1.). First, with regards to the physical environment, changes such as anthropogenic climate change leading to increased temperature and altered precipitation can modify habitat suitability for animals (García-Peña *et al.*, 2021) (Figure 1. A). In addition, such changes can allow pathogens to viably persist in the environment for prolonged periods. For example, increased precipitation in Fiji has been associated with increased number of human cases of leptospirosis and an increased risk of infection (Lau *et al.*, 2016). Second, with regards to reservoir populations, land use modification including deforestation and subsequent conversion to agricultural or urban habitats (Figure 1. B) can lead to their increased abundance (Jones *et al.*, 2013) and the displacement of non-reservoir hosts with which they compete (Gibb *et al.*, 2020). These alterations to species' assemblages can lead to a reduction in the "dilution effect", defined as - increasing species diversity (species richness plus evenness) within the host community reducing zoonotic disease risk as measured by infection prevalence (Ostfeld and Keesing, 2000). Reduced infection prevalence is typically observed in species rich ecosystems (Civitello *et al.*, 2015), with reduced biodiversity increasing the prevalence of zoonotic pathogens within

host species (Bordes, Blasdell and Morand, 2015) (Figure 1. C). Third, translocation of species into new environments has resulted in range expansion of human commensal species (Tompkins *et al.*, 2015) that may transport pathogens into novel regions (Morand *et al.*, 2015; Guth *et al.*, 2020) or become novel hosts for zoonoses (Daszak, Cunningham and Hyatt, 2000). Further, introduction of domesticated livestock and agricultural intensification is an important driver of zoonotic risk, firstly, through food-borne zoonoses (e.g., *E. coli*) and secondly as novel pathogen host populations (e.g., *Nipah virus* and domesticated pig populations) (Karesh *et al.*, 2012). Fourth, global changes in human populations will lead to higher population density (Roser, 2013) and in some zoonotic disease systems, increased contact with reservoir species (Hassell *et al.*, 2017). Higher human population density and wildlife contact rates would increase density-dependent transmission of infectious diseases which may result in increased probability of zoonotic disease spillover events (Begon *et al.*, 1999; Wang *et al.*, 2021) (Figure 1. E). Future responses to physical environment change may also lead to large scale migration of human populations from non-endemic to endemic regions, this could increase the risk pathogen spillover further in certain regions (Cattaneo *et al.*, 2019). Fifth, drivers within these human societies such as access to healthcare (Cascio *et al.*, 2011; Walsh *et al.*, 2020) and food security (Brooks *et al.*, 2021) (Figure 1. H) moderate the risk of zoonotic disease spillover in non-linear ways (e.g. the banning of wild animal markets may drive these activities away from comprehensive monitoring systems (Roe *et al.*, 2020)). This highlights the complexity of the dynamic risk of zoonotic disease spillover (Figure 1. D). In addition, as is characteristic of complex systems, spillover events are not homogeneous across space and time, with specific regions and human populations proposed to be at risk of emerging or endemic zoonotic infectious diseases (Suzán *et al.*, 2015; Allen *et al.*, 2017).

### 1.1.2 Hotspots for emerging zoonoses

#### spillover and novel pathogen emergence events

The potential impact of zoonotic spillover on human health has led to significant research efforts dedicated to the production of “hotspot” maps (i.e., prediction maps) for likely **spillover events**. Various methodological approaches have been used, including literature reviews of previous emergence events, host-based species distribution mapping and machine learning approaches applied to data on known and predicted pathogens. First, a review of available data on emerging infectious disease events - defined as, the first temporal emergence of a pathogen in a human population which was related to the increase in distribution, increase in incidence or increase in virulence or other factor which led to that pathogen being classed as an emerging disease - explored their geographic location accounting for bias in research effort (Jones *et al.*, 2008). Approximately, 60% of these events were zoonotic, with Eastern Europe, Southern and South East Asia, Central and South America and West and East Africa identified as geographic regions at increased risk

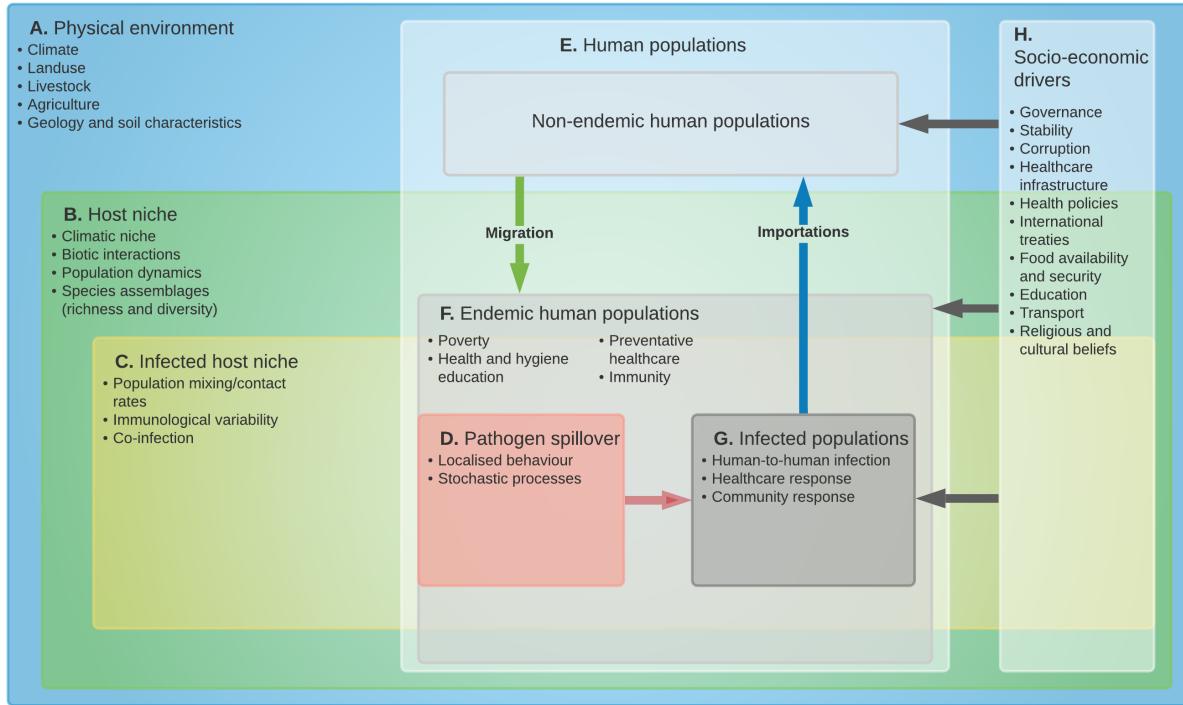


Figure 1: Conceptual map of zoonotic infectious disease emergence and spillover risk. Letters **A-G** are key causal system components leading to pathogen spillover into human populations. **H** represents potential socioeconomic drivers of risk of spillover and maintenance of an epidemic in human populations. Bulleted lists within the systems are examples of key sub-components with arrows showing direct links within nested systems. Within the physical environment (**A**), host niches (**B**), the infected host niche (**C**) and human populations (**E**) are nested with change occurring over prolonged time periods (i.e. years rather than days). Endemic human populations (**F**) are a nested subgroup of human populations (**E**) where populations at risk can be modified by migration or expansion of the host niche (**B**). Socio-economic drivers (**H**) affect all human populations and may also modify the physical environment (**A**), the host niche (**B**), the infected host niche (**C**) and pathogen spillover (**D**). Pathogen spillover occurs at the spatial and temporal interface of infected hosts (**C**) and human populations at risk (**F**). Infected human populations (**G**) can lead to wider transmission within endemic human populations in addition to importing infectious cases into non-endemic human populations. Systems dynamics model adapted from (Redding *et al.*, 2019).

for infectious disease emergence from wildlife. Second, analysis of the distribution of known and predicted hosts - based on taxonomy and traits - of zoonotic infectious diseases, identified Central and South America, Central East Africa, Europe and South East Asia as containing high reservoir richness (Han, Kramer and Drake, 2016). In addition, the two taxa were proposed to contribute to the greatest number of potential unique zoonotic pathogens were rodents (Rodentia) and bats (Chiroptera). This has been further supported by a study investigating the effect of land use change under different future scenarios, which identified West Africa as a region of increased hazard for rodent-borne diseases (García-Peña *et al.*, 2021). Third, applying machine learning to data on zoonotic host status and host life history traits, locations and species have been identified where pathogen diversity is currently under researched (Olival *et al.*, 2017; Albery *et al.*, 2020). Specifically, several of these studies have identified West Africa as a region at risk for zoonoses emergence. However, these studies were limited by not incorporating key information on healthcare infrastructure, disease surveillance processes, agricultural and livestock intensification and human population demographic change, which contribute to the risk of zoonotic spillover.

### 1.1.3 Rodents as zoonotic hosts

As highlighted above, many studies have identified rodents as particularly important for zoonotic spillover events in West Africa. Rodents are ubiquitous, representing more than 40% of all global mammal species and are found on all continents except Antarctica. Many of these species are commensal and thrive in human dominated ecosystems (D'Elía, Fabre and Lessa, 2019). Ongoing land use change can lead to replacement of more specialised rodent species by generalists that are better able to adapt to changing conditions (Gibb *et al.*, 2020). Generalists typically exhibit a cluster of traits - termed a "fast" life history - including high abundance, small body mass, high reproductive rate and a reliance on innate, constitutive immunity to protect against pathogens (Albery and Becker, 2021). In addition, rodents with traits conducive to range expansion into altered habitats are also more likely to host known zoonotic pathogens (Albery *et al.*, 2021). Human activity can artificially increase species' ranges through introduction into new habitats via trade-and transport networks (e.g. *Mus musculus* and *Rattus norvegicus*). Pathogens of these newly introduced species **are likely also** transported via the same routes (e.g. plague) (Vogler *et al.*, 2013).

Rodents play important roles in the provision of ecosystem services and disservices (Keesing *et al.*, 2010; Cunningham, Daszak and Wood, 2017). In West Africa these include services such as being seed predators, seed dispersers and controlling insect abundance (Fischer *et al.*, 2018), and disservices such as acting as pest species of agricultural crops (Makundi, 2011). Biodiverse rodent species assemblages can compound these services, as individual species often occupy different niches (Fischer *et al.*, 2018). The role that a

rodent species may have in the emergence of a zoonotic pathogen can be conceptualised as an ecosystem disservice (Ostfeld and Keesing, 2017). An estimated 244 of 2220 rodent species are hosts of at least 85 known zoonotic pathogens (Han *et al.*, 2015). Rodent borne diseases are distributed globally (Meerburg, Singleton and Kijlstra, 2009) and are typically associated with resource poor settings, low levels of education, poor sanitation, and limited access to healthcare (Iles *et al.*, 2006). Within resource poor countries the risk of disease is not uniform and follows a socio-economic gradient with diseases primarily affecting the poorest communities within endemic regions (Grace, 2014; Khalil *et al.*, 2021).

#### 1.1.4 Lassa fever as a model disease system

##### Spillover and novel pathogen emergence

In West Africa (suggested to be an at-risk region for emerging zoonoses), *Borrelia sp*, Schistomiasis and Toxoplasmosis are important rodent-borne diseases leading to significant human morbidity and mortality. Lassa fever is a particularly important and under explored rodent-borne zoonosis of humans (Meerburg, Singleton and Kijlstra, 2009). Caused by the Arenaviridae *Lassa mammarenavirus*, Lassa fever is a zoonosis first identified from a case series of infected patients originating near Jos, Nigeria in 1969 (Frame *et al.*, 1970). *Mastomys natalensis* was identified as the reservoir following an outbreak in Sierra Leone during 1970-72 (Monath *et al.*, 1974). However, several other rodent species have been found to carry the virus, potentially revealing producing a more complex pathogen transmission network (Olayemi *et al.*, 2016). Human infection is caused by spillover of the virus from infected rodents or their excreta (McCormick *et al.*, 1987). Viral infection in humans is thought to occur via the nasopharyngeal mucosa, with subsequent viral spread to the lymphatics before dissemination to virtually all tissues (Jahrling *et al.*, 1980). Necrosis can occur, particularly in the liver and spleen, severe disease is caused by vascular instability and impaired haemostasis resulting in a viral haemorrhagic fever syndrome (Walker *et al.*, 1982). Human-to-human transmission is less commonly observed. When reported, it is associated with nosocomial (healthcare) transmission through direct contact with an infected person's bodily fluids and secretions, with healthcare workers at greatest risk (Fisher-Hoch *et al.*, 1995). Risk factors for Lassa fever infection include hunting rodents for food, rodents residing in human habitation and insecure food storage (Meulen *et al.*, 1997). Among healthcare workers, specific risks include direct patient interactions, specifically surgery, and nursing care, although adequate personal-protective equipment can reduce this risk (Holt *et al.*, 2021). Lassa fever is currently considered endemic in 8 West African countries: Benin, Ghana, Guinea, Liberia, Mali, Nigeria, Sierra Leone and Togo by the World Health Organisation (WHO) (World Health Organisation, 2021b). Sporadic cases in Burkina Faso and Côte d'Ivoire have also been reported (Figure 2 A). Imported cases have been reported from non-West African countries such as the United Kingdom, Germany and the United States (Tuite *et al.*, 2019; Wolf *et*

*al.*, 2020).

**1.1.4.1 Lassa fever epidemiology** Annual Lassa fever incidence is unknown, with estimates ranging between 150,000 (McCormick *et al.*, 1987) and 4,300,000 cases per year (Basinski *et al.*, 2021) cases annually. The uncertainty surrounding these estimates is due to a combination of few serological studies, limited disease surveillance and an overlap between the symptomatology of Lassa fever with other infectious diseases in these endemic regions (e.g. malaria). Mortality (case-fatality rate) is reported as 29.7% (Kenmoe *et al.*, 2020), although this varies by country and year. These mortality estimates are likely biased due to widespread under-reporting of pauci- or asymptomatic cases which make up at least 80% of infections (World Health Organisation, 2021b). Morbidity is poorly understood; however, significant long term sensori-neural hearing loss is the most important sequelae in survivors of acute infection (Ficenec, Schieffelin and Emmett, 2019). However, the incidence and severity of this has not been comprehensively studied (Ficenec *et al.*, 2020), hence illustrating the need for further research into this rodent-borne disease.

Due to the paucity of data, Lassa fever has been designated as a WHO priority disease for research and development to drive improvements in epidemiological understanding and clinical treatment. To date, the most comprehensive epidemiological data has been generated by the Nigerian Centres for Disease Control, with seasonal dynamics of outbreaks reported following the implementation of a mandatory notification processes and the creation of national reference laboratories for Lassa fever in several Nigerian states (Agbonlahor *et al.*, 2021). Since, 2017, weekly situation reports have been published (Figure 2. A). In Sierra Leone a partnership established between the US Centres for Disease Control and the Ministry of Health and Sanitation in 1976 led to the creation of a dedicated Lassa fever ward at Kenema General Hospital in the endemic Eastern Province, with testing of suspected and confirmed cases recorded since 2008 (Shaffer *et al.*, 2014). In Guinea, few cases have been reported since a study in 1999 (Bausch *et al.*, 2001), with seven cases reported since 2011. Data from Liberia is limited with cases intermittently recorded in WHO situation reports (World Health Organisation, 2021a). Benin, Ghana, Mali, and Togo do not make any data available on incident cases. However, cases from these countries have been recorded in academic journal articles and shared through the ProMED network (ProMED, 2021). A regional sero-epidemiological survey funded by the Coalition for Epidemic Preparedness Innovation (CEPI) has recently been implemented in Nigeria, Sierra Leone, Liberia and Guinea in preparation for phase 2 vaccine studies (Coalition for Epidemic Preparedness Innovations, 2020). Despite this increased focus on human epidemiology, few studies on the primary rodent hosts in this geographic region have been conducted.

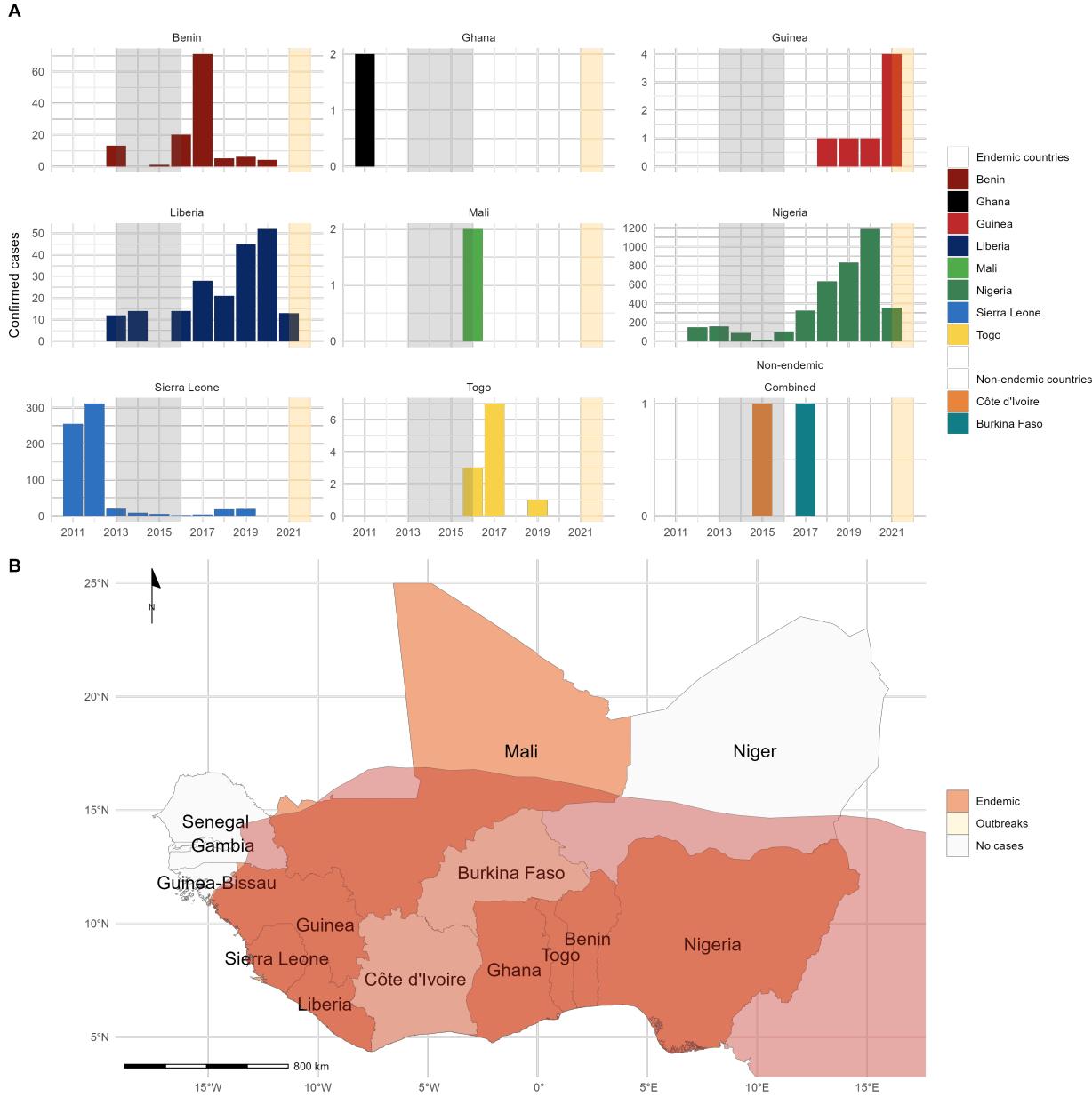


Figure 2: **A** Confirmed Lassa fever cases in West African countries since 2011. The grey shaded area represents the Ebola epidemic that lasted from December 2013 until June 2016. The orange shaded area represents incomplete case data from the current year. Annual confirmed and reported cases are shown for the countries considered endemic for Lassa fever. Ghana and Mali have only reported confirmed cases for a single year. Guinea and Togo reported cases for several of the years reviewed. Benin, Liberia, Nigeria and Sierra Leone reported cases during all or most years, however, case counts were much larger in Nigeria than elsewhere. Single cases have been reported for two non-endemic countries, Côte d'Ivoire and Burkina Faso. **B** Map of West Africa with the distribution of *Mastomys natalensis* (red shaded region) the primary reservoir host of *Lassa mammarenavirus* highlighted. All countries of the West African region are labelled with countries shaded by the status of Lassa fever in the country reported by the WHO.

**1.1.4.2 Zoonotic hosts of *Lassa mammarenavirus*** The primary host of *Lassa mammarenavirus* has been identified as the multimammate rat (*Mastomys natalensis*) a rodent species that is found across much of sub-Saharan Africa; however, outside of West Africa no individual rodents have been found to be infected with *Lassa mammarenavirus*. Other rodent species including *Hylomyscus pamfi* and *Mastomys erythroleucus* have been found to be acutely infected (through Polymerase Chain Reaction based assays) or have evidence of prior exposure to the virus (through serological assays) (Olayemi *et al.*, 2016). Some evidence exists for prior exposure to *Lassa mammarenavirus* in non-rodent species, including domestic dogs, non-human primates and shrews (Kenmoe *et al.*, 2020). However, comprehensive studies in diverse Lassa fever endemic regions have not been performed and the role these species may have in *Lassa mammarenavirus* transmission dynamics is unclear. Most studies to date have focussed on the known host with fewer studies investigating wider rodent assemblages in endemic regions (Fichet-Calvet and Rogers, 2009). Research examining rodent assemblages is therefore urgently needed, as the interaction of different within an ecosystem can modify pathogen spillover risk (as outlined in the conceptual map in Figure 1.).

Dynamic models of Lassa fever spillover risk have been produced to investigate seasonal dynamics of infection in Nigeria (Akhmetzhanov, Asai and Nishiura, 2019 ; Musa *et al.*, 2020). These mechanistic models incorporate *Mastomys natalensis* populations, however, they are limited by sparse data to parameterise rodent populations and viral transmission dynamics within the hosts. Further, these models do not utilise a model structure that reflects the host species existing within wider rodent assemblages that moderate pathogen spillover risk. Similar limitations exist for a stochastic individual based model developed to explore the effect of rodent control on Lassa fever spillover risk in Guinea (Marien *et al.*, 2019). A dynamic model derived from the conceptual map (Figure 1.) will incorporate important factors that are missing from these earlier models.

The number of reported cases of Lassa fever is increasing (Figure 2. B), likely due to increased surveillance. However, growing human populations at risk and land use change in the endemic region likely also contribute to increased cases (Agbonlahor *et al.*, 2021). The disentangling of the factors that drive increased cases is vital for understanding the potential future risk of Lassa fever in the endemic region and beyond. Therefore, comprehensive baseline data of human cases and viral transmission among rodent assemblages, in addition to dynamic modelling of the disease system, are required. This would also help understand factors driving novel disease spillover from rodent populations under changing environmental conditions.

## 1.2 Thesis outline

### 1.2.1 Thesis aims and questions

In this thesis, I aim to explore the effect of the composition of rodent assemblages and the environment they reside within on the risk of Lassa fever spillover in Sierra Leone, West Africa. First, I will trap rodents in a Lassa fever endemic region Sierra Leone. Second, I will use the data to parameterise a dynamic model of *Lassa mammarenavirus* transmission. I expect my thesis to contribute to a greater understanding of the ecological conditions supporting viral transmission in dynamic populations of rodents and humans, with the dynamic model used as a framework for other host-pathogen systems in similar contexts. Below, I outline the planned chapters of this thesis and the questions I seek to address.

The primary objective will be to describe Lassa fever spillover risk although I expect the methodological approach adopted will be applicable to understanding novel pathogen emergence.

### 1.2.2 Chapter 1: General introduction

### 1.2.3 Chapter 2: A scoping review of rodent trapping studies to understand the prevalence of zoonotic disease in West Africa.

This chapter includes a scoping review of rodent trapping studies in West Africa to synthesise current evidence for the presence and absence of potential zoonotic infectious diseases in rodents and small mammals. I proceed to investigate the effect of sampling bias and spatial heterogeneity on the identification of regions at potential risk of zoonotic infectious disease emergence. Using this framework I ask whether the current evidence shapes our understanding of risk and I will identify locations and host-pathogen systems in need of further targeted research.

I aim to address the following questions:

1. What information do rodent trapping studies provide to inform zoonotic spillover risk maps and what disease systems require further research?
2. To what extent does sampling bias influence the prediction of spillover risk?
3. Which diseases endemic to West Africa have been investigated using rodent trapping?

Check abstract is the same

**1.2.3.1 Abstract** Rodents are important reservoirs of zoonotic infectious diseases; 255 species are known hosts of 85 zoonotic pathogens. These reservoir species are globally distributed with West Africa containing multiple known and several further predicted reservoir species. Typically, International Union for Conservation of Nature (IUCN) species distribution maps and Global Biodiversity Information Facility (GBIF)

presence maps are used to determine regions at risk for zoonotic spillover events, however, these datasets are biased by incomplete sampling. To investigate the impact of rodent species sampling heterogeneity on zoonotic spillover risk we have systematically reviewed rodent trapping studies to produce a contextually rich dataset that can be used to explore this risk at finer spatial resolution. Here we show that sampling of rodents and their pathogens in the West African region are spatially biased by country and trapping habitat. We found that rodent trapping effort was associated with regional population density and was increased in habitats modified by human activity. We produce updated rodent species ranges compared to available IUCN maps and enrich GBIF data by including locations of rodent species absence and trapping effort. Furthermore, we report the spatial bias in the investigation of four important rodent zoonoses, Arenaviridae, *Borellia* sp., *Bartonella* sp. and *Toxoplasma* sp.. Our results highlight that incorporating sampling bias is important when assessing the risk for zoonotic spillover events from rodents. The synthesis of contextually rich rodent trapping data contributes important information that is lacking in IUCN distribution maps and GBIF species presence data. We anticipate this dataset can support the production of more complete spatial risk assessments of zoonotic spillover events. For example, the inclusion of absence data and trapping effort can help identify regions where data sparsity may produce inappropriately predicted risk. Furthermore, this data specifies regions in need of comprehensive study for four targeted rodent zoonoses, Arenaviridae, *Borellia* sp., *Bartonella* sp. and *Toxoplasma* sp..

**1.2.3.2 Current progress** A draft manuscript has been produced and is attached as appendix 1. A web based application has also been produced to provide curated data for re-use and to support further exploration of this dataset, the app is available [here](#).

#### **1.2.4 Chapter 3: Rodent trapping to explore rodent assemblage structure in Eastern Sierra Leone.**

In this chapter I report a longitudinal rodent trapping study at four study sites in a Lassa fever endemic region of Eastern Sierra Leone. I perform rodent trapping along socio-ecological gradients to understand the association of land use on rodent species assemblages and population structures. I will perform morphological and molecular speciation on sampled rodents to understand the spatial and temporal dynamics of rodent species at the selected study sites. Data obtained from this study will be used to inform species occupancy and distribution models in the Eastern province of Sierra Leone. These models can be used to identify regions of interest for further investigation of Lassa fever epidemiology.

I aim to address the following questions:

1. What rodent species are prevalent at the study sites and how are species assemblages structured?
2. How do these species assemblages vary over time and space and are population structures associated with land use?
3. What is the potential impact of climate and land use change on *Lassa mammarenavirus* spillover risk?

**1.2.4.1 Abstract** Lassa fever is endemic to Eastern Sierra Leone. The principal reservoir species, *Mastomys natalensis*, is considered abundant in human dominated habitats, although these rodent species assemblages are not well described. To investigate the structure of these assemblages in this heterogeneous landscape we have conducted four monthly rodent trapping surveys. We produce a comprehensive description of rodent assemblage structures through morphological and molecular speciation, age classification, reproductive status and habitat preference. We expect to find that *Mastomys natalensis* populations display seasonal dynamics in abundance and habitat choices with populations concentrated in areas of human habitation during the dry season. Further, we expect to find that rodent species within these assemblages diversify into habitat niches, and that during periods of increased competition for resources (i.e., the dry season), generalist species displace specialist species. These data will be used to inform species occupancy and distribution maps of Eastern Sierra Leone developed from remote sensing data. These will display the likely discontinuity in *Mastomys natalensis* populations. Potentially, these maps will go some way towards explaining the observed limited geographic radiation of outbreaks of Lassa fever in this region. We anticipate that this data will help inform higher resolution models of Lassa fever risk in Eastern Sierra Leone based on the distribution of reservoir and non-reservoir species. Furthermore, this data will highlight the need for comprehensive studies of rodent species assemblages when producing hotspot maps for zoonotic pathogen spillover risk, alongside providing a methodological framework to explore this in other host-pathogen systems.

**1.2.4.2 Current progress** The study protocol has been uploaded to the Open Science Framework and is attached as appendix 2. Additional material including data collection sheets in .xlsx format for use with the Open Data Kit are available here. An initial report from the first three study visits has been produced and is attached as appendix 3, it is also available here.

## **1.2.5 Chapter 4: Serological investigation of *Lassa mammarenavirus* among rodent species in Eastern Sierra Leone.**

In this chapter I perform serological analysis investigating the prevalence of antibodies against *Lassa mammarenavirus* in rodents sampled in Chapter 3 to investigate the associations of species, rodent age, seasonality

and land use on infection status. I will use two serological assays, an Enzyme Linked Immunosorbent Assay (ELISA), developed by collaborators at the Bernard Nocht Institute of Tropical Medicine, and a novel Luciferase Immunoprecipitation Systems (LIPS), developed with collaborators at University College London (UCL) and the International Atomic Energy Association (IAEA) (Chapter 6), to identify prior infection. This work will be used to develop a model to identify spatial and temporal associations of *Lassa mammarenavirus* prevalence in rodent populations.

I aim to address the following questions:

1. Which rodent species in a Lassa fever endemic region display evidence of prior infection with *Lassa mammarenavirus*?
2. What is the role of diverse or depauperate rodent assemblages on risk an individual rodents risk of prior infection with *Lassa mammarenavirus*?
3. Is there any additional benefit to utilising a species agnostic LIPS assay alongside established rodent ELISAs?

**1.2.5.1 Abstract** The primary host of *Lassa mammarenavirus*, the causative pathogen of Lassa fever, is *Mastomys natalensis*. However, evidence of prior infection has been observed in six further rodent species in West Africa: *Hylomyscus pamfi*, *Lemniscomys striatus*, *Mus minutoides*, *Mastomys erythroleucus*, *Praomys daltoni* and *Rattus rattus*. To investigate the maintenance of *Lassa mammarenavirus* in rodent species assemblages we have conducted four monthly rodent trapping surveys in a Lassa fever endemic region of Sierra Leone. We conducted two serological assays on samples obtained from trapped rodents. First, a novel LIPS assay developed by the study team was performed, second, a previously developed and validated rodent ELISA. We report seroprevalence to *Lassa mammarenavirus* within these rodent species assemblages investigating the association with rodent age and habitat location on serological status. We expect to find that the majority of seropositive individuals are from the expected reservoir species and that a cyclical pattern of seropositivity is observed, associated with periods of high reservoir species abundance, low species diversity and concentration within human dominated habitats. Further, we expect that non-reservoir species will show peaks in seropositivity associated with peaks in the reservoir species, potentially representing spillover infections. These data will be used to inform a model of viral transmission among reservoir and non-reservoir species and will be compared to seasonal data in human Lassa fever infections obtained from hospital data available in the literature. Further, we expect to show the concordance between the novel LIPS assay and ELISA in detecting prior exposure to *Lassa mammarenavirus* providing another tool to investigate viral transmission dynamics among rodent species. Finally, this data may highlight the importance of surveying

the entire rodent species assemblage rather than limiting sample analysis to the known reservoir when investigating the risk of pathogen spillover.

### **1.2.6 Chapter 5: Serological studies investigating *Lassa mammarenavirus* seropositivity in peridomestic species.**

The role of non-rodent, peri-domestic species on *Lassa mammarenavirus* transmission dynamics is unknown. Previous studies have been limited to viral culture assays as no host species agnostic serological assays exist. Following the development of the LIPS assay (Chapter 6) obtained samples (Chapter 3) will be investigated for the prevalence of antibodies to *Lassa mammarenavirus* in peridomestic species including dogs (*Canis*), cats (*Felis*) and goats (*Capra*) in Lassa fever endemic regions of Eastern Sierra Leone. This chapter will provide initial evidence for the involvement or lack of involvement of these species in *Lassa mammarenavirus* transmission pathways.

I aim to address the following questions:

1. Is there any evidence of non-rodent peri-domestic species being involved in *Lassa mammarenavirus* transmission in a Lassa fever endemic region of Eastern Sierra Leone?
2. Is a species agnostic serological assay able to contribute to our knowledge of the factors driving Lassa fever spillover risk?

**1.2.6.1 Abstract** The role of non-rodent species in *Lassa mammarenavirus* transmission dynamics and pathogen spillover into human populations is unknown. Evidence of prior infection has been reported from dogs (*Canis*), monkeys and baboons (Primates), however, few systematic studies have been conducted in Lassa fever endemic regions. To investigate the potential role on non-rodent peri-domestic species on the maintenance of *Lassa mammarenavirus* within an ecosystem we conducted a pilot serological survey of cats (*Felis*), dogs (*Canis*) and goats (*Capra*) in the Eastern province of Sierra Leone. We report seroprevalence obtained through a novel, species agnostic, LIPS assay against *Lassa mammarenavirus*. This study will contribute to the limited evidence base for non-rodent, non-human involvement in *Lassa mammarenavirus* transmission. Further, this study will act as a proof-of-concept for this methodological approach to be conducted more representatively across the study region and in further Lassa fever endemic regions outside of Eastern Sierra Leone.

### **1.2.7 Chapter 6: Development of a LIPS serological assay for *Lassa mammarenavirus***

In this chapter I will describe the development of a novel serological assay to investigate the prevalence of immune correlates of prior infection with *Lassa mammarenavirus* for use in rodents and peri-domestic species. I will describe the initial bioinformatic approach to identify potential epitopes to target for investigation. I will describe work with collaborators at UCL and IAEA to develop a stable production system of epitope-luciferase complexes for use in the assay. I will conduct an assessment of the sensitivity and specificity of this novel test in rodents for serological evidence of infection using ELISA. There is no current comparator for evidence of prior infection in non-rodent species. This assay will be of use to the research community in the investigation of other potential hosts of *Lassa mammarenavirus* as this will be the first species agnostic serological assay. Further, the development of an antigen based serological assay may produce useful triangulation to currently used antibody based serological assays. This section of the thesis will also produce evidence for the utility of a LIPS based approach in investigating infections where multiple host species may be involved.

I aim to address the following questions:

1. Is LIPS a suitable approach to develop a species agnostic serological assay for *Lassa mammarenavirus*?
2. How does the sensitivity and specificity of the developed assay compare to ELISA in rodent samples?

**1.2.7.1 Abstract** *Lassa mammarenavirus*, the causative agent of Lassa fever, has high phylogenetic diversity. Several clades exist which causes difficulty in developing diagnostic tests. Further, few assays are validated for non-human sera. Here we show the development of a novel, species agnostic LIPS assay with acceptable sensitivity and specificity for application in testing non-human sera to investigate viral transmission dynamics. First, we conducted epitope analysis of *Lassa mammarenavirus* nucleoprotein and glycoprotein sequences available on NCBI genbank we compare predicted epitope regions to regions of low sequence diversity. Second, we compared epitope binding in these regions between different *Lassa mammarenavirus* strains to understand the impact of phylogenetic diversity on potential antigen-antibody binding regions. Third, we demonstrate proof-of-concept for this *Lassa mammarenavirus* assay by expressing plasmids producing the Luciferase-antigen complex, purification of the product and subsequent binding to a positive control sample. We present the sensitivity and specificity of this assay in relation to a previously validated ELISA in rodent sera. We expect this assay to offer an important tool in assessing the contribution of non-rodent species to Lassa fever transmission.

**1.2.7.2 Current progress** Analysis has been performed on available sequences on the NCBI GenBank dataset for *Lassa mammarenavirus* we have identified multiple suitable epitope candidates using bioinformatic approaches and are currently working on expressing these epitopes within plasmids conjugated with the Luciferase enzyme. The initial bioinformatic process has been presented at the International Conference on (Re-)Emerging Infectious Diseases 2020, the poster is attached as appendix 4, it is also available here.

### **1.2.8 Chapter 7: Parameterisation of a mathematical model of viral transmission in rodent populations to investigate spillover risk.**

In this chapter I will use empirical data from Chapters 3, 4 and 5 to parameterise a dynamic model of *Lassa mammarenavirus* transmission in rodents and peri-domestic species. This model will progress currently developed models by incorporating land use data and rodent species assemblage data which is currently lacking. The model will be constrained to Eastern Sierra Leone but the structure could be adapted with further data from additional studies to generalise to other Lassa fever endemic regions. This model will be used to explore the effect of land use change on rodent population assemblages and prevalence of *Lassa mammarenavirus* in these populations.

I aim to address the following question:

1. Does the incorporation of land use type and the structure of rodent species assemblage contribute to our understanding of *Lassa mammarenavirus* transmission dynamics in a Lassa fever endemic region of Sierra Leone?

### **1.2.9 Current progress**

A draft model framework has been produced. A comprehensive review of dynamic models for rodent borne zoonoses will be conducted to further refine this model.

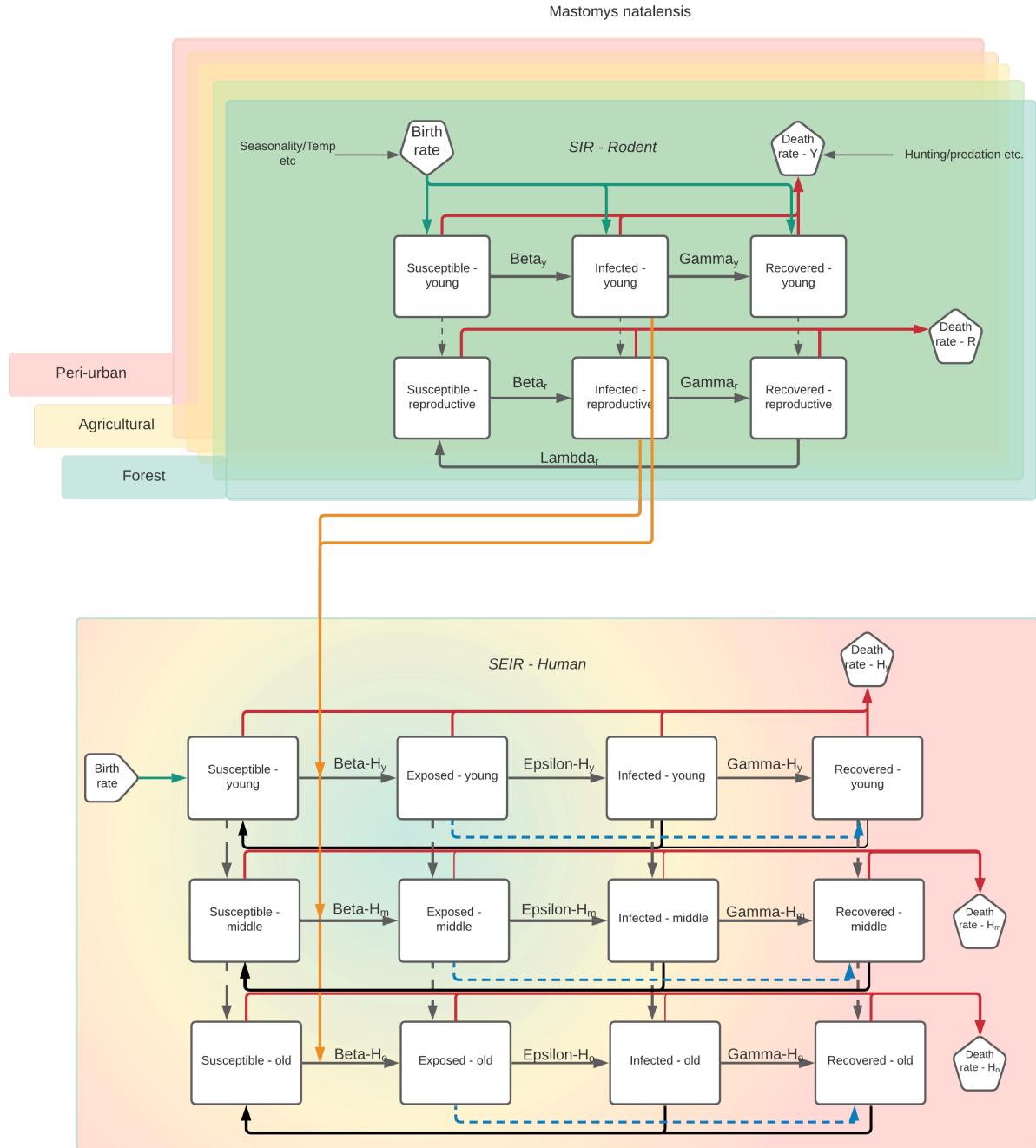


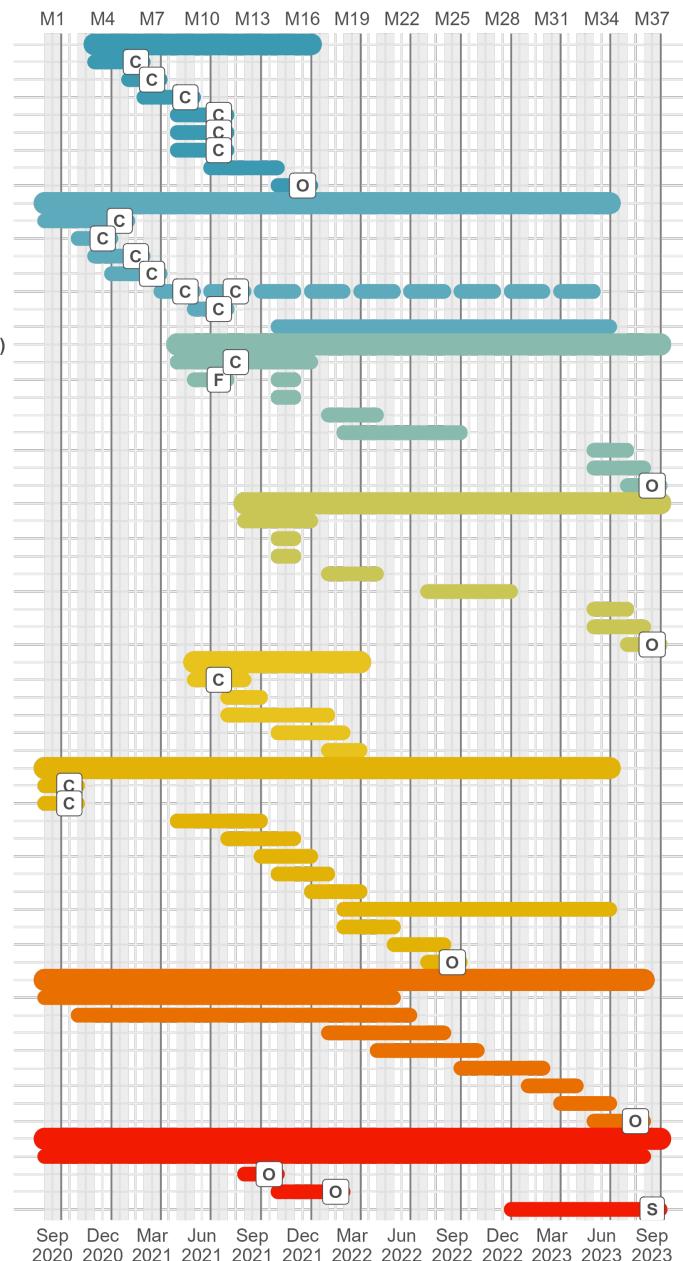
Figure 3: Draft dynamic transmission model structure. *Mastomys natalensis* population dynamics are modelled in different habitats based on their interaction with abiotic and biotic factors including rainfall, food availability and intra-species competition (top section). Viral transmission dynamics are modelled in these populations with spillover into human populations simulated (bottom section). Within the age structured rodent and human population infections are allowed to occur as a function of conspecific contacts or exposures to infected individuals.

### 1.2.10 Chapter 8: General discussion

## 1.3 Project timeline

PhD project timeline, 2020-2023

- 1. Scoping review (Rodent trapping)**
  - 1.1. Study protocol
  - 1.2. Search completion
  - 1.3. Data extraction
  - 1.4. Double checking
  - 1.5. Analysis
  - 1.6. Draft manuscript
  - 1.7. Revise manuscript
  - 1.8. Submit manuscript
- 2.0. Rodent trapping**
  - 2.0.1. Study protocol
  - 2.0.2. Pilot visit - SL
  - 2.0.3. Pilot data analysis and report
  - 2.0.4. Revise protocol
  - 2.0.6. Study visit
  - 2.0.5. Additional site setup - SL
  - 2.0.7. Rodent sample analysis (lab)
- 2.1. Rodent trapping (Population dynamics and distribution)**
  - 2.1.1. Study protocol
  - 2.1.2. Lab work - SL
  - 2.1.3. Pilot protocol
  - 2.1.4. Analysis plan (speciation)
  - 2.1.5. Draft manuscript (interim analysis)
  - 2.1.6. Final analysis
  - 2.1.7. Final manuscript
  - 2.1.8. Submit manuscript
- 2.2. Rodent trapping (Lassa serology)**
  - 2.2.1. Study protocol
  - 2.2.2. Pilot protocol
  - 2.2.3. Lab work - SL
  - 2.2.4. Analysis plan
  - 2.2.5. Draft manuscript (interim analysis)
  - 2.2.6. Final analysis
  - 2.2.7. Final manuscript
  - 2.2.8. Submit manuscript
- 3. Peridomestic species (Lassa serology)**
  - 3.1. Study protocol
  - 3.2. Pilot protocol
  - 3.3. Sample collection
  - 3.4. Peridomestic species sample analysis
  - 3.5. Analysis plan
- 4. LIPS development**
  - 4.1. Bioinformatics (epitope discovery)
  - 4.2. Literature review (assays)
  - 4.3. Plasmid production
  - 4.4. Assay development
  - 4.5. Assay validation
  - 4.6. Benchmarking against ELISA
  - 4.7. Deployment in SL
  - 4.8. Sample analysis
  - 4.9. Draft manuscript
  - 4.10. Final manuscript
  - 4.11. Submit manuscript
- 5. Socio-ecological model of LF**
  - 5.1. Conceptual model
  - 5.2. Data acquisition and pre-processing
  - 5.3. Model testing and refinement
  - 5.4. Model parameterisation
  - 5.5. Model application
  - 5.6. Draft manuscript
  - 5.7. Final manuscript
  - 5.8. Submit manuscript
- PhD timeline**
  - 6.1. Funding
  - 6.2. Upgrade appraisal
  - 6.3. PIPS
  - 6.4. PhD write up



C = Completed, F = Failed to achieve, O = PhD output, S = PhD submission deadline

The current status of each chapter in relation to the above timeline is highlighted.

- Chapter 2: Scoping review is at the manuscript draft stage. This project will also significantly contribute to the general introduction of the final thesis.
- Chapter 3 and 4: Rodent trapping will produce the majority of empirical data collection for the thesis. It was initiated in November 2019, data collection from this arm of the project is expected to be completed in May 2023. However, the projects (2.1 and 2.2) the data will be used in will be initially conducted using interim results to increase the feasibility of the timeline.
  - Lab work was intended to start in July 2021, however, failure to obtain adequate reagents in country have pushed this back to November/December 2021.
  - Lab work will begin in November/December 2021.
- Chapter 5: A field team has been deployed to obtain non-rodent, peridomestic species samples for Lassa serology and LIPS development.
- Chapter 6: LIPS development is ongoing; several posters have been presented on the methodology used to reach the current stage of development (expression within *vero* cells). Optimisation is ongoing and will be followed up with validation and benchmarking.
- Chapter 7: A conceptual model has been drafted which will undergo refinement and testing prior to parameterisation with empirical data.

## 1.4 Training and development

My training and development log is attached as appendix 5

## 2 Appendix 1: A scoping review of rodent trapping studies to understand the prevalence of zoonotic disease in West Africa.

### 2.1 Abstract

Rodents are important reservoirs of zoonotic infectious diseases; 255 species are known hosts of 85 zoonotic pathogens. These reservoir species are globally distributed with West Africa containing multiple known and several further predicted reservoir species. Typically International Union for Conservation of Nature (IUCN) species distribution maps and Global Biodiversity Information Facility (GBIF) presence maps are used to determine regions at risk for zoonotic spillover events, however, these datasets are biased by incomplete sampling. To investigate the impact of rodent species sampling heterogeneity on zoonotic spillover risk we

have systematically reviewed rodent trapping studies to produce a contextually rich dataset that can be used to explore this risk at finer spatial resolution. Here we show that sampling of rodents and their pathogens in the West African region are spatially biased by country and trapping habitat. We found that rodent trapping effort was associated with regional population density and was increased in habitats modified by human activity. We produce updated rodent species ranges compared to available IUCN maps and enrich GBIF data by including locations of rodent species absence and trapping effort. Furthermore, we report the spatial bias in the investigation of four important rodent zoonoses, Arenaviridae, *Borellia* sp., *Bartonella* sp. and *Toxoplasma* sp.. Our results highlight that incorporating sampling bias is important when assessing the risk for zoonotic spillover events from rodents. The synthesis of contextually rich rodent trapping data contributes important information that is lacking in IUCN distribution maps and GBIF species presence data. We anticipate this dataset can support the production of more complete spatial risk assessments of zoonotic spillover events. For example, the inclusion of absence data and trapping effort can help identify regions where data sparsity may produce inappropriately predicted risk. Furthermore, these data identify regions in need of comprehensive study for our four targeted rodent zoonoses, Arenaviridae, *Borellia* sp., *Bartonella* sp. and *Toxoplasma* sp..

## 2.2 Introduction

The potential effect of zoonotic infectious diseases outbreaks on human health and societies has been dramatically highlighted through the ongoing SARS-CoV-2 pandemic and recent Ebola virus outbreaks. The number of zoonotic spillover events are projected to increase under intensifying anthropogenic pressure such as, increased human populations (Allen *et al.*, 2017), increasing urbanisation (Hassell *et al.*, 2017), and global climate change (Morse *et al.*, 2012). In addition, two taxa - rodents (Rodentia) and bats (Chiroptera) - are proposed to contribute to the greatest number of zoonotic pathogens and the greatest risk of novel pathogen emergence (Han *et al.*, 2015). Of 2,220 extant rodent species, 244 (10.7%) are described as being reservoirs of 85 zoonoses (Han, Kramer and Drake, 2016). Specifically, West Africa has previously been identified as region at increased hazard for rodent-borne zoonotic spillover events under different projected scenarios (García-Peña *et al.*, 2021). Rodents are implicated in the transmission pathways of several human diseases in this region, including, Lassa fever, Schistosomiasis and Leptospirosis (Meerburg, Singleton and Kijlstra, 2009).

Rodent species form diverse assemblages, which provide important and beneficial ecosystem services including pest regulation and seed dispersal (Fischer *et al.*, 2018). The role of rodent species' in zoonotic infectious disease spillover or novel pathogen emergence can be viewed as an ecosystem disservice. Rodents typically

demonstrate “fast” life histories (Dobson and Oli, 2007), these traits are also associated with a species being a reservoir of zoonotic pathogens (Han *et al.*, 2015; Albery and Becker, 2021). Further, these traits are prevalent in species that thrive in human dominated landscapes where they displace species that are less likely to be reservoirs of zoonotic pathogen (Gibb *et al.*, 2020). The widespread occurrence of reservoir species and their proximity to human activity make the description of rodent species assemblages and host-pathogen interactions vitally important to understanding the risk of zoonotic spillover and novel pathogen emergence (Han, Kramer and Drake, 2016).

Rodent trapping studies provide a useful method to describe rodent population structures and survey for potential zoonoses. Studies have been conducted in West Africa to both identify novel potential zoonotic pathogens within rodents (USAID, 2021) and to investigate the prevalence and burden of known pathogens within known rodent hosts (e.g. for Lassa fever (Fichet-Calvet *et al.*, 2009) and Schistosomiasis (Catalano *et al.*, 2020)). These studies provide contextually rich information on when, where and under what conditions rodents were trapped that are typically missing from the global datasets (Bovendorp, McCleery and Galetti, 2017). Despite this, these global datasets (e.g. IUCN, GBIF and GIDEON) are typically used to inform species distribution maps and zoonotic disease spillover predictions (Smith *et al.*, 2014; Pigott *et al.*, 2014; Han, Kramer and Drake, 2016).

These studies have been used to identify potential geographic hotspots where virus and host species diversity may be expected to be at its greatest to predict regional zoonotic disease spillover risks. However, there remains the potential for important confounding in these spatial distributions through bias generated by study design and selection of sampling sites (Plowright *et al.*, 2019). For example, systematically increased sampling (e.g. more intensive studies over longer time periods) or over-representation of certain habitats (e.g. peri-urban landscapes) could lead to an apparent association between locations and risk that is driven by these factors rather than an underlying host and virus association (Wille, Geoghegan and Holmes, 2021; Gibb *et al.*, 2021). Conversely some regions may not be sampled adequately and therefore under-represented in these datasets due to sparse human populations or inaccessible habitats this may result in these regions being reported inappropriately as at low risk of novel pathogen emergence or zoonotic disease spillover events.

Here, we identify rodent trapping studies performed across West Africa and identify the location and habitat types in which they have been conducted, the pathogens assessed and the host-pathogen associations that have been reported in order to quantify the potential bias and to identify regions requiring further focussed investigation.

## 2.3 Methods

### 2.3.1 Literature search

We conducted a search in Ovid MEDLINE, Web of Science (Core collection and Zoological Record), JSTOR, BioOne, African Journals Online, Global Health and the pre-print servers, BioRxiv and EcoEvoRxiv for the following terms as keywords, no date limits were set:

1. Rodent OR Rodent trap\*
2. West Africa (or the individual countries)
3. 1. AND 2.

We searched other resources including the UN Official Documents System, Open Grey, AGRIS FAO and Google Scholar using combinations of the above terms. Searches were run on 2021-03-01.

We included studies if they met all of the following inclusion criteria; i) reported findings from trapping studies where the target was a small mammal, ii) described the type of trap used or the length of trapping activity or the location of the trapping activity, iii) included trapping activity from at least one West African country, iv) recorded the genus or species of trapped individuals, v) were published in a peer-reviewed journal or as a pre-print on a digital platform or as a report by a credible organisation. We excluded studies if they met any of the following exclusion criteria: i) reported data that were duplicated from a previously included study, ii) no full text available, iii) not available in English. One reviewer screened titles, abstracts and full texts against the inclusion and exclusion criteria. At each stage, a random subset (10%) was reviewed by a second reviewer.

### 2.3.2 Data extraction

We extracted data from eligible studies into a Google sheets document. Extracted variables included i) study identifiers; ii) study aims; iii) trapping methodology; iv) geolocation data; v) method of speciation; vi) trapping locations and dates; vii) trapped species; viii) number of trap-nights and ix) microorganisms/pathogens of interest. The data extraction tool is archived and available in Supplementary 1 or at this link.

**2.3.2.1 Location of rodent trapping studies and habitats studied** We extracted GPS locations for the most precise location presented (i.e. trap, trap-line, study site or study region). We extracted coordinates

in the format reported and converted them to decimal degrees. We recorded the habitat classification scheme used by a study (e.g. IUCN Habitat Classification Scheme (Version 3.1)). For studies not using standardised habitat recording, the explicit description of the habitat in which the trap was placed were extracted. For studies reporting multiple habitat types (e.g. rice field, corn field and vegetable garden) for a single trap, trap-line or trapping grid, a higher order classification of habitat type was recorded (e.g. agricultural land).

**2.3.2.2 Rodent presence, absence, abundance** We mapped genus and species names to the species names used in the Global Biodiversity Information Facility (GBIF) taxonomy (GBIF: The Global Biodiversity Information Facility, 2021b). We extracted information on the presence, absence and number of trapped individuals. For studies reporting on all trapped individuals (i.e. not those only reporting on the presence of a specific species of interest), the pseudo-absence of a species reported as present elsewhere in the study was explicitly recorded as an absence at that trap location.

**2.3.2.3 Pathogen presence and absence** We extracted data on all pathogens assayed in studies investigating rodents for potential zoonoses. The number of rodents tested and the number of positive or negative samples were recorded alongside the type of assay used (e.g. Polymerase Chain Reaction (PCR), Enzyme Linked ImmunoSorbent Assay (ELISA) or viral culture). If studies reported indeterminate results this was noted. Where possible, pathogens were identified to species level. However, where an assay only allowed for attribution to a family of viruses or bacteria, the higher order grouping was used (e.g. Arenaviridae for a PCR using a non-specific arenavirus primer).

### 2.3.3 Analysis

**2.3.3.1 Location and habitats of rodent trapping to investigate potential biases** We summarised the number of studies, the year in which trapping occurred and the country in which they were conducted. We used the GPS coordinates of single trap, trapping grid/line or study site and the number of trap nights to calculate trapping effort (trap night density) within level 2 administrative areas in West Africa. The median trap success rate for all rodents at a defined trap site was calculated separately for trap sites which included built environments and non-built environments due to important differences in trap success between these different environments. The number of rodents trapped at a trap site was then used in combination with these values of trap success to impute the number of trap nights for trap sites where there was no record of the number of trap nights. We summarised the habitat types of trap sites based on information reported in the study, the dictionary used to match habitat types is available in Supplementary

2.

For studies investigating rodent zoonoses we compared the location of trapping sites with SEDAC Global Population Density estimates for 2005 - the median year studies were started (Socioeconomic Data and Applications Center, 2021). We used a Generalised Additive Model (GAM) incorporating a spatial interaction term to investigate the association of number of trap nights and human population density (Pedersen *et al.*, 2019). The model structure was specified as:

$$\text{Trap night density} \sim \text{Tweedie}(\log(\text{Populationdensity}_{2005}) + (\text{Longitude} * \text{Latitude}))$$

We obtained land cover classifications from the European Space Agency Copernicus dataset at 300m<sup>2</sup> spatial resolution generated using Copernicus Climate Change Service information (2005) we extracted the proportion of land cover classes within all regions of West Africa and all regions in which rodent trapping occurred for investigation of zoonotic diseases. We compared the proportion of possible land cover classes with those of regions where trapping occurred.

Finally, we mapped the presence and absence of rodent species and compared this to the presence and absence produced from GBIF (GBIF: The Global Biodiversity Information Facility, 2021a) and IUCN (IUCN, 2021) datasets to give a measure of the extent of each species range in which they have been sampled.

**2.3.3.2 Rodent pathogen associations** We summarised the presence and absence of microorganisms, the assays used, their host species and the locations from where the samples were obtained. We investigated the association between rodent species and the detection of potential pathogens and report the proportion of positive and negative tests for each species and pathogen pair.

## 2.4 Results

### 2.4.1 Included studies

We identified 4,282 relevant citations, with 126 rodent trapping studies included. The list of included studies in available in Supplementary 3 or through the interactive web based application produced to explore this dataset, available here. The earliest included studies were published in 1974 with increasing numbers of studies being performed annually since 2005. The median length of rodent trapping activity was one year (IQR 0-2 years). The median time from completion of rodent trapping to publication is 3 years (IQR 2-5 years) (Figure 1.).

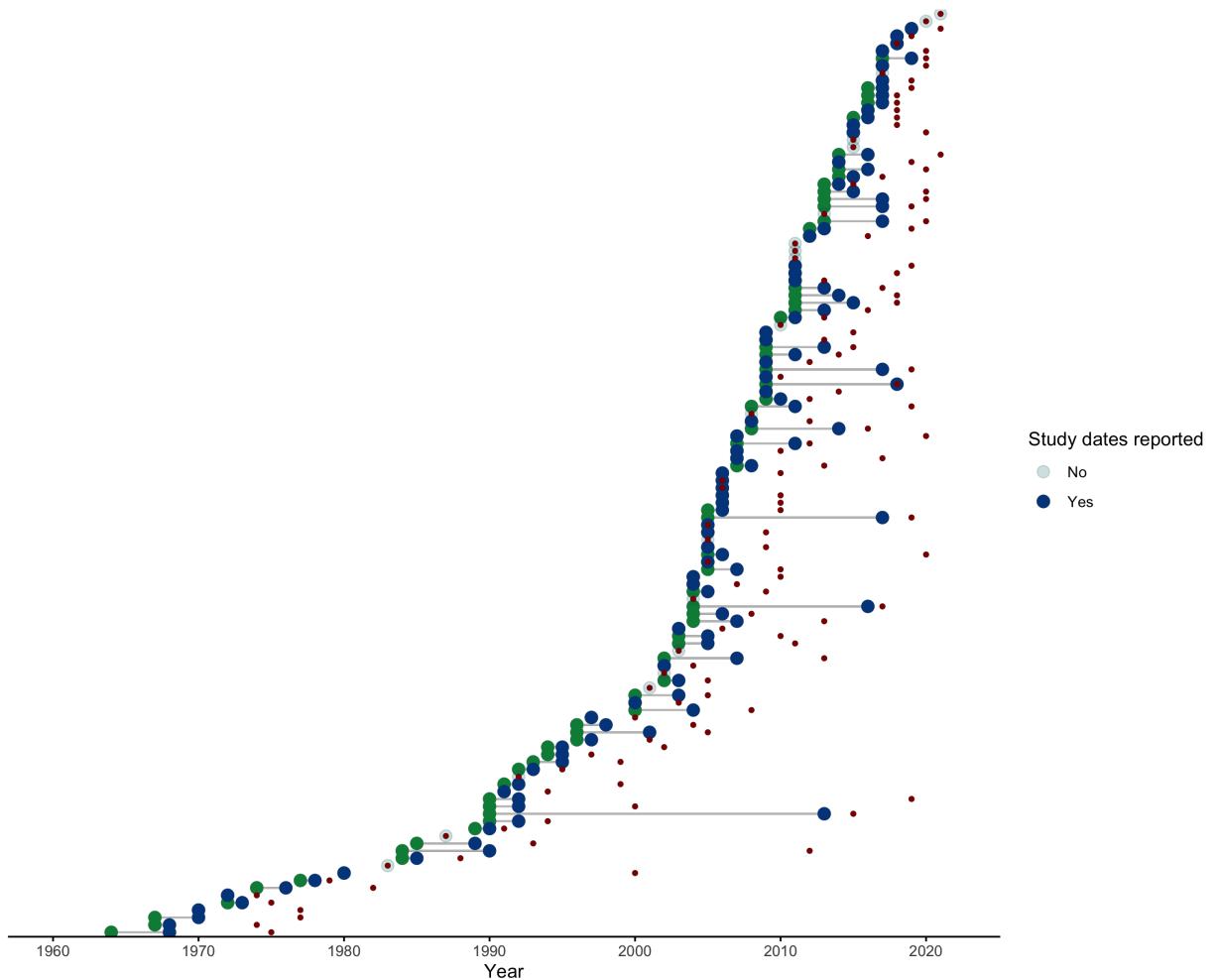


Figure 4: Each row represents one of the 126 included studies, green points designate the first year of data collection, blue points designate the end of data collection. For studies completed within one year the blue point completely overlies the green. Studies with a transparent grey point did not report the year in which trapping was conducted. The year of publication is shown by a red point.

#### **2.4.2 Location and habitats of rodent trapping studies to investigate potential biases**

Rodent trapping took place at 1,331 trap sites with at least one trap site recorded from 14 West African countries. No rodent trapping studies were identified from Gambia or Togo. The most frequently studied countries were Senegal, Ghana, Guinea and Nigeria (more than 10 studies each). Fewer than 5 studies were conducted in Liberia, Burkina Faso and Guinea-Bissau. Thirty-one (25%) studies reported trapping at a single study site, 46 (37%) studies trapped at between two and five study sites, the remaining 49 studies trapped at between six and 93 study sites. Trap sites were situated in 273 (19.3%) of 1414 level 2 administrative regions in the 14 West African nations. The areas with highest trap night density included the capital cities of Niger (Niamey), Sierra Leone (Freetown), Senegal (Dakar), Mali (Bamako) and Ghana (Accra) and the largest cities of Côte d'Ivoire (Abidjan) and Benin (Cotonou). Outside of these cities, Northern Senegal (Fatique, Thies, Saint-Louis and Kedougou), Southern Guinea (Kindia and Nzerejore), Edo and Osun States in Nigeria and Eastern Sierra Leone had the highest density of trap nights (Figure 2. A).

Greater numbers of trap nights were conducted in areas with higher population densities ( $R_2 = 0.18$ ) adjusting for spatial autocorrelation. In particular Mauritania, Northern Senegal, Eastern Sierra Leone, Eastern Guinea and South West Nigeria were trapped at higher rates than would be expected based on their population densities. South West Nigeria, Northern Nigeria, Liberia, Côte d'Ivoire, Ghana, Niger and Burkina Faso were trapped at lower rates than would be expected.

No studies reported trap habitats with reference to a standardised habitat classification scheme. Extracted habitat types were grouped into 30 categories (see Supplementary 2 for the habitat dictionary). At least one habitat was recorded for 17,122 trap sites (95%), with two or more habitats for a single trap site recorded for 4,403 (24%) sites. Single trap sites could span multiple habitat types resulting in 22,202 distinct habitat and trap sites. The most commonly trapped sites were in or around buildings (29%), in areas described as the rodents “natural habitat” (24%), in agricultural areas (13%) (e.g. rice fields, palm plantations), forests (8%) and in the area surrounding buildings (4%).

#### **2.4.3 Rodent presence, absence, abundance**

73,164 small mammals were trapped (592 were trapped outside of West African countries), 2,830 (4%) trapped individuals were identified to order level (Rodentia), 7,760 (11%) were identified to genus level, the remaining 62,574 (85%) were identified to species level. In studies reporting the number of trap nights the median trap success rate of traps placed in or around buildings was 13% (IQR 6-24%), this compares to a

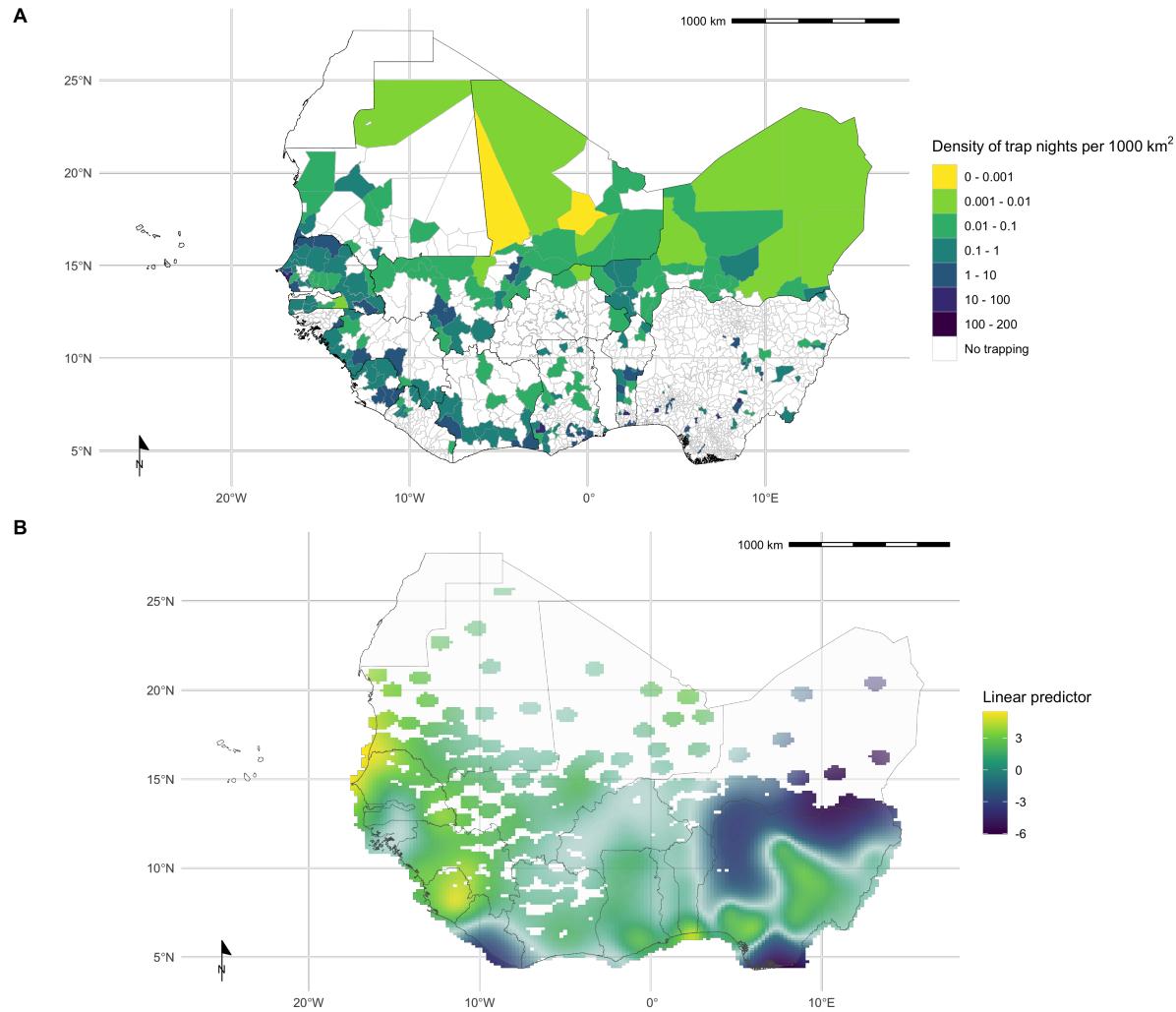


Figure 5: **A:** Map of West Africa, countries where rodent trapping has occurred are mapped to level 2 administrative areas. The colour of the region corresponds to the trap night density per 1000 km<sup>2</sup>. Colourless regions reported no trapping. **B:** This shows the linear predictor from obtained from the GAM. Predictions are limited to coordinates are trap sites (coloured areas), uncertainty in the linear predictor is represented by the transparency of the colour. Yellow regions represent areas with higher than expected trapping effort for the regional population and purple regions represent areas trapped at lower than expected rates based on their populations

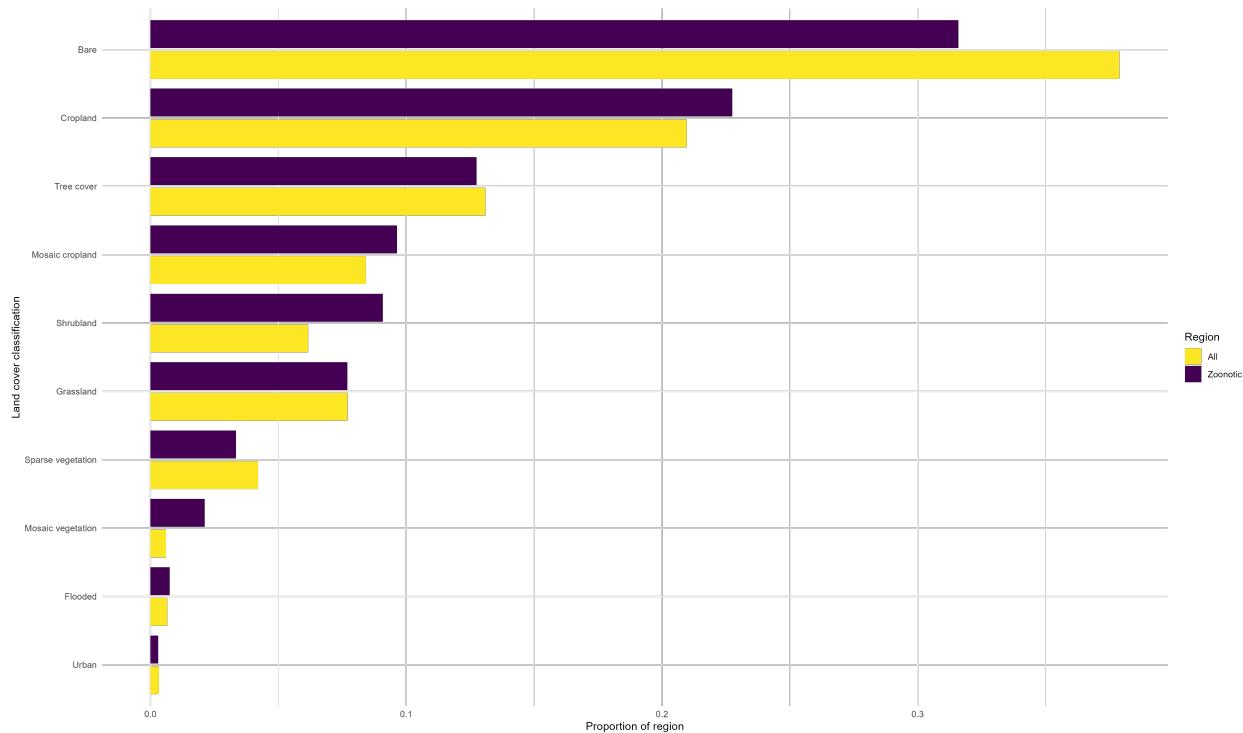


Figure 6: The proportion of the 10 land cover classes reported by the ESA Copernicus dataset at 300m<sup>2</sup> resolution are shown for all regions of West Africa (yellow) and for regions in which trapping occurred (purple). Rodent trapping occurred in regions over-representative for cropland, mosaic landscapes and shrubland while occurring in regions under-representative for bare- and sparse vegetation land cover classes.

median trap success rate of 3% (IQR 1-9%) in other habitats. The majority of the 147 species trapped were Rodentia (112) (Supplementary 3), of these Muridae (82) were the largest family of rodents, followed by, Sciuridae (10), Gliridae and Nesomyidae (both 6), Ctenodactylidae, Anomaluridae, Dipodidae, Hystricidae and Thryonomidae (2 or fewer). The remaining 34 species were from the orders of Soricomorpha (30), Erinaceomorpha (2) and Afrosoricida (1).

*Mastomys* sp. were the most commonly trapped genus (27,079, 37.7%), followed by, *Rattus* sp. (11,472, 16.1%), *Mus* sp. (8,624, 12.0%), *Arvicanthis* sp. (5,821, 8.1%) and *Praomys* sp. (5,409, 7.5%). *Mastomys natalensis* were the most commonly trapped species (11,116, 17.4%), followed by, *Rattus rattus* (9,959, 15.6%), *Mastomys erythroleucus* (7,386, 11.6%), *Mus musculus* (6,245, 9.8%), *Arvicanthis niloticus* (5,497, 8.6%), *Mastomys huberti* (4,699, 7.4%) and *Praomys daltoni* (1,854, 2.9%).

Locations of points for the presence or absence of the seven most commonly trapped species are shown in Figure 3 (left column). Absence include true absences and pseudo-absences dependent on the trapping effort at the site. This are shown compared to species presence obtained from GBIF (centre column) and IUCN species range data (left column) (Figure 3).

#### 2.4.4 Pathogen presence and absence

Sixty-two studies presented data on microorganisms that infect or are carried by small mammal species in West Africa. Seven studies solely investigated pathogens of rodents, including Hydatigera species (previously Taenia species) and Trichuris species. Zoonotic pathogens and potentially zoonotic micro-organisms were investigated in 55 studies. Thirty-two microorganisms were tested for, 8 of these were specified to species level, the remaining 24 were specified at higher taxonomic classifications.

Polymerase Chain Reaction (PCR) was used in 32 studies to detect the presence of 22 different species or families of microorganisms from 21,953 rodent samples. The most common microorganisms assayed for using PCR were *Lassa mammarenavirus* or other Arenaviridae (31%) the bacteria *Borrelia* sp. (11.3%) and *Bartonella* sp. (6.5%), followed by Usutu virus (6.4%) and Hantaviridae (5.6%). Molecular tests (antibody or antigen based) were used in 11 studies to detect the presence of 9 different species or families of microorganisms from 11,430 rodent samples. The most common pathogens assessed for with serology were *Lassa mammarenavirus* or other Arenaviridae (78%), *Toxoplasma gondii* (10.6%), *Borrelia* sp. (6.3%) and *Leptospirosis* sp. (2.2%). Histological or direct visualisation assays were used in 8 studies investigating 11 parasitic or bacterial species from 11,229 rodent samples. The most common pathogens assessed for with histology or direct visualisation were *Borrelia* sp. (48.7%), *Schistosoma* sp. (20.4%) and other parasites.

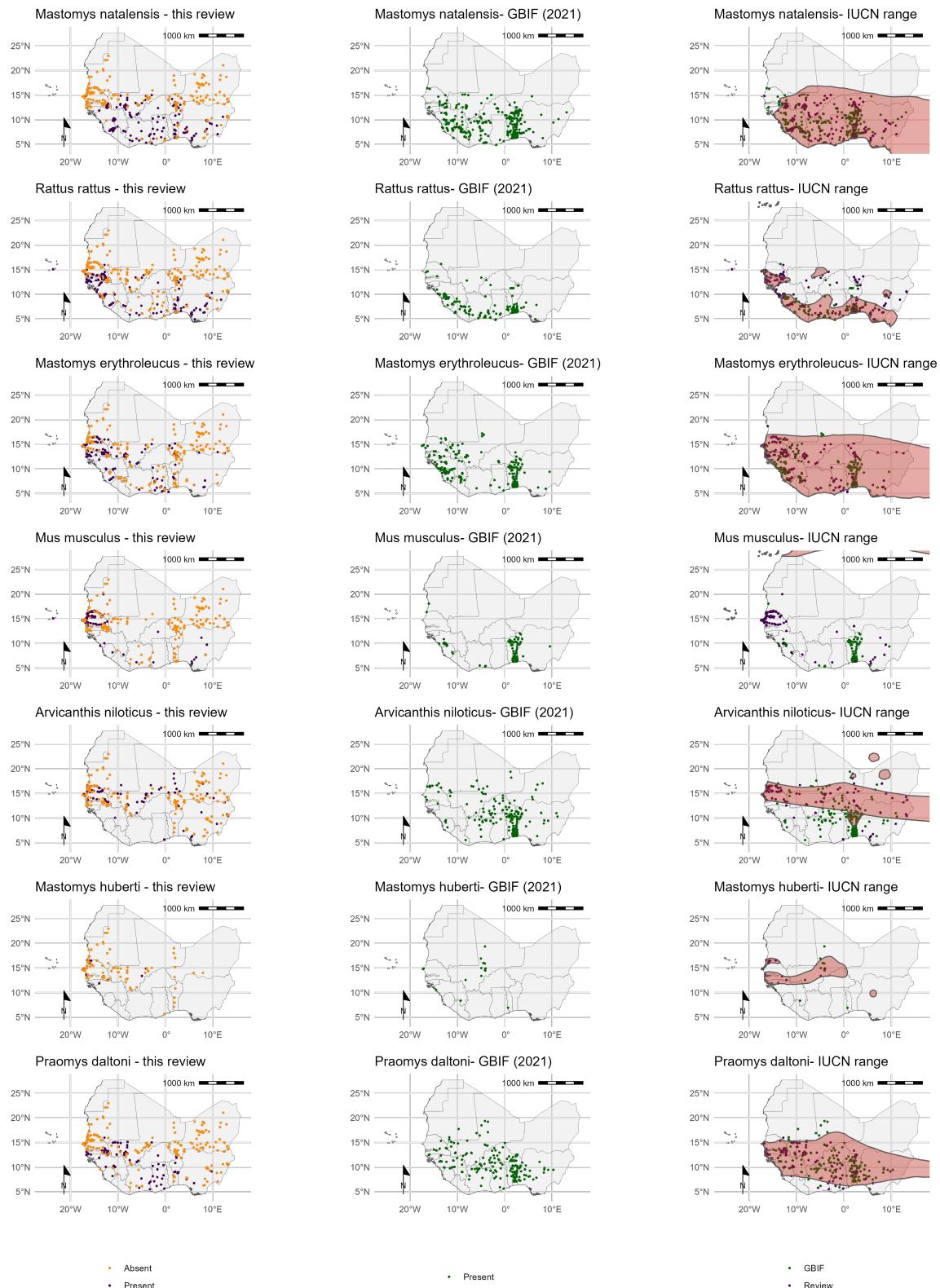


Figure 7: Each row corresponds to a single rodent species. The column on the left shows the presence and absence of a rodent species from the individual studies included in this review. The centre column shows the presence of a rodent species obtained from GBIF (September 2021) for records where longitude and latitude have been provided. The right column shows the range of rodent species as proposed by the IUCN (2021) (red shaded area), overlaid are the presence points from both this review and GBIF records.

Direct culture of *Lassa mammarenavirus* or Leishmania species were reported from 3 studies to detect the presence of these pathogen in 643 rodent samples.

Most studies tested for a single microorganisms (39), 16 studies tested for two or more microorganisms. The most frequently investigated microorganisms were *Lassa mammarenavirus* (17 studies) or members of the Arenaviridae family (10), the spirochete bacteria *Borrelia* sp. (8), *Bartonella* sp. and *Toxoplasma gondii* (4), the remaining 25 microorganisms were reported in three or fewer studies.

The location of trapped rodents that were tested for the four most commonly assayed microorganisms did not encompass the entire range of these hosts. The presence and absence of Arenaviridae, predominantly *Lassa mammarenavirus*, in rodents was investigated in Benin, Côte d'Ivoire, Guinea, Mali, Nigeria and Sierra Leone. No studies investigated the prevalence of these pathogens in hosts from Burkina-Faso, Guinea-Bissau, Liberia, Niger, Senegal and Togo. Arenaviridae were detected in rodents from 17 of 54 species or genera assayed in all the countries it was investigated except Benin (Figure 4 top row). The presence and absence of *Bartonella* sp. was investigated in Mali and Nigeria and Senegal with positive samples detected in 11 of 11 species or genera of rodents obtained at most sites from these countries (Figure 4 second row). The presence and absence of *Borrelia* sp. was investigated in Benin, Mali, Mauritania, Niger and Senegal. *Borrelia* sp. were detected in 8 of 44 species or genera of rodents assayed in Mali with negative samples obtained from Benin, Mauritania, Niger and Senegal (Figure 4 third row). The presence of *Toxoplasma gondii* was only investigated in Senegal and was detected in 6 of 8 rodent species assayed (Figure 4 fourth row).

#### **2.4.5 Host-pathogen associations**

Ninety-seven rodent species were investigated for the presence of a zoonotic pathogen, 42,940 assays were performed on 32,014 individual rodent samples. The rodent species most commonly assayed for zoonotic pathogens were, *Rattus rattus* (n = 2,977) assessed for 24 pathogens, *Mus musculus* (n = 3,402) for 23, *Mastomys natalensis* (n = 7,189) and *Mastomys erythroleucus* (n = 3,013) both 19 and *Arvicanthis niloticus* (n = 3,840) for 18. All remaining species were investigated for 10 or fewer pathogens.

*Lassa mammarenavirus* was detected in 14 species of rodents and Crocidura genera. *Mastomys natalensis* was the most common species to test positive (Figure 4.). Fifty-four species and genera were tested for arenaviruses, with 37 testing negative. *Bartonella* sp. infections were identified in all 11 species of rodents and shrews that were tested. *Mastomys erythroleucus* was the most common species to test positive. *Borrelia* sp. infections were identified in 13 species of rodents and shrews. *Mastomys natalensis* was the most common species to test positive. Forty-four species and genera were tested for *Borrelia* sp. with 36 testing negative.

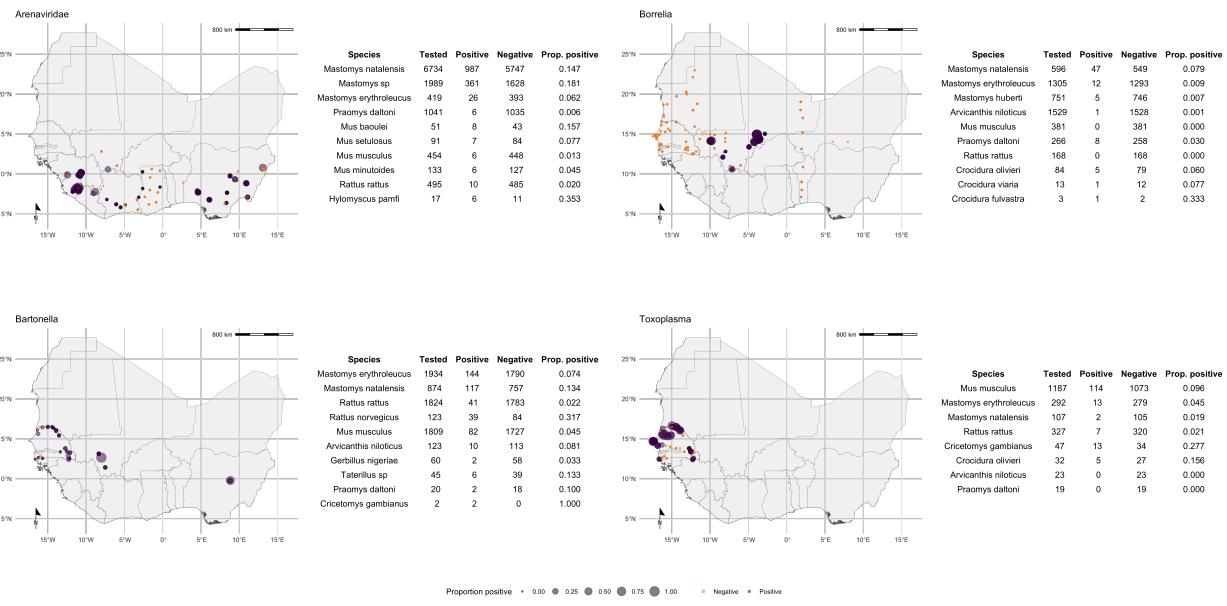


Figure 8: Presence/absence plots at each unique trapping site for the four most commonly assayed microorganisms Arenaviridae (top), *Bartonella* sp. (second row), *Borrelia* sp. (third row) and *Toxoplasma gondii* (fourth row). The tables to the right of each map highlight the 10 (or number applicable) most commonly positive and tested rodent species and genera assayed.

*Toxoplasma gondii* was detected in 6 species of rodents and shrews, with all individuals from two species *Arvicanthis niloticus* and *Praomys daltoni* testing negative. *Mus musculus* was the most common species to test positive. Finally, 38 species of Rodentia, 3 species of Soricomorpha and 2 species of Erinaceomorpha that were tested for pathogens had entirely negative results.

#### Page break

## 2.5 Discussion

We identified 124 rodent trapping studies conducted in the West African region since 1965. These studies were conducted in 14 countries with 54 studies providing information about zoonotic pathogens in trapped rodents. Studies were conducted in all West African countries except Gambia and Togo with between- and observed within-country variability in the number of trap nights conducted. Over 70,000 rodents identified to genus or species level were trapped from 112 species of Rodentia. Thirty-two potential pathogens were investigated across all studies, 5 rodent species were assayed for at least 18 potential pathogens. We identify several important limitations to available data, potentially biasing previous zoonotic risk studies, first, in the timing of studies and the locations in which they've been conducted, second, the locations where a species was recorded as present and third, the limited selection of pathogens assayed at these trapping sites.

First, the time period and location of trapping studies, when not systematically conducted, will introduce

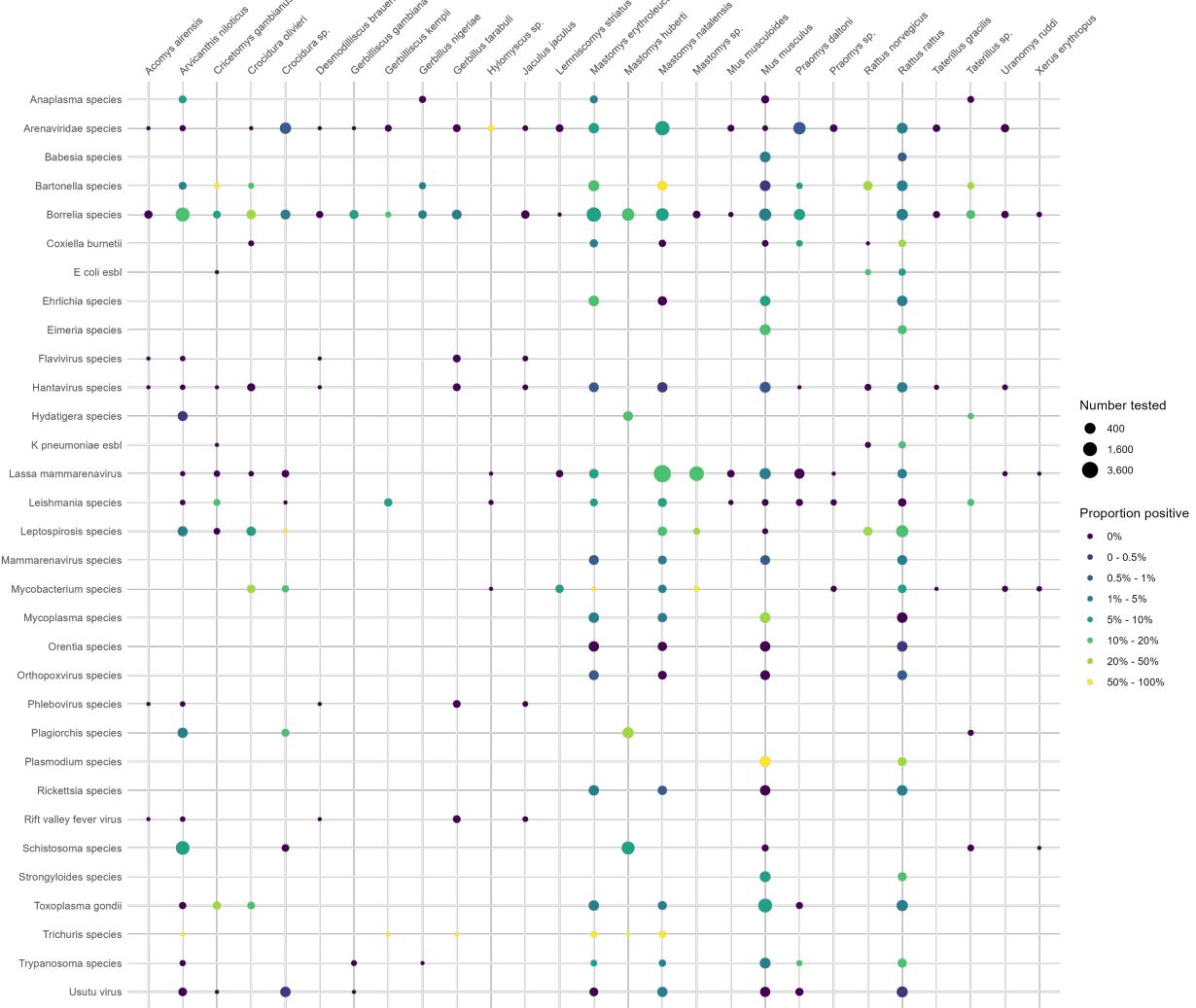


Figure 9: For the 27 most commonly assayed rodent species and genera, the percentage of positive assays against each of the 32 microorganisms tested for is shown. The size of a point is proportional to the **the** number of individuals assayed for that microorganism.

bias that must be accounted for when attempting to infer the risk of zoonotic disease spillover events or novel pathogen emergence. Most studies were conducted in a relatively short period since 2000. During this period human populations, agricultural farming and livestock intensification and urbanisation have progressed dramatically across West Africa (Acheampong *et al.*, 2019; Connolly, Keil and Ali, 2021). These changes alter the availability of habitats for rodent species, potentially increasing the prevalence of commensal species which are more likely to be reservoirs of zoonoses. Thus, incorporating the time period in which data were collected is important when modelling risk of zoonotic spillover or novel pathogen emergence based on known reservoir species distributions. In addition, several regions, namely North West Senegal, Eastern Sierra Leone, Southern Ghana, Southern Benin, South West and Central Nigeria have been sampled at much higher intensity than elsewhere in West Africa. This sampling heterogeneity will again introduce bias when inferring current or projected risk of zoonotic spillover or novel pathogen emergence.

plowright becker

Second, rodent species were found to have greater species ranges than those reported by IUCN data. Trapping studies were conducted from more regions across West Africa than were available from GBIF records. This increased spatial resolution and the inclusion of absence data alongside presence data presents a much more complete representation of the spatial heterogeneity of rodent species' locations within their ranges. Failing to incorporate this heterogeneity in favour of using ranges or presence only data to produce rodent reservoir species' distributions can importantly bias estimations of risk of zoonotic spillover or novel pathogen emergence. For example, the apparent absence of *Mastomys natalensis* at several trapping sites in Nigeria may explain some of the observed spatial variation in human Lassa fever cases (Redding *et al.*, 2021) that would not be expected based on this species IUCN range or presence only data from GBIF.

Third, while 32 potential zoonoses were investigated in at least one study, only four pathogen species or groups were investigated in more than three. Of these four pathogens, studies for *Lassa mammarenavirus* or other Arenaviridae were the most widely performed, both in absolute numbers but also by number of countries and number of study sites within countries. In contrast, *Toxoplasma* sp. were found to be prevalent in rodent hosts within Senegal - and despite a significant, known, burden of human disease - no studies investigated the prevalence of this pathogen in other West African countries (Bigna *et al.*, 2020). The limited geographic scope of sampling for these pathogens hampers efforts to understand the risk and human health burden of zoonotic spillover across this diverse region.

Finally, we found that only five of the 112 rodent species trapped had been tested for more than half of the potential pathogens. There is increasing emphasis on the role of co-infection and multi-species pathogen transmission systems on zoonotic disease spillover risk which depends on comprehensive testing of both known and un-known reservoir species (Sweeny *et al.*, 2021). More comprehensive testing of potential

reservoir species across West Africa is required to better understand the role of individual species in disease systems (Douglass and Vadell, 2016). This will also reduce the risk of bias in predictive risk modelling by better characterising host-pathogen associations in species rich environments.

### **2.5.1 Limitations**

We identified several limitations to our approach. We identified studies from a diverse range of sources, however, it remains possible that rodent surveys have been performed for additional purposes that would not be captured in a survey of the scientific and “grey” literature, for example, risk assessments or ecological assessments for development work may not have been identified through our search. We were limited by the reporting from included studies, particularly for measuring trapping effort and identifying habitat types trapped. We imputed trapping effort for studies that did not report any measure of effort, removing these studies had no meaningful effect on the association of human population density and trapping effort in sensitivity analysis. Habitat type and human population density were obtained from 2005, the median year in which studies were conducted. Changes in both human populations and land use in the studied period (1969-2020) have been large across West Africa, this introduces the possibility that these values were not appropriate for the region at the time when the rodent trapping study was conducted.

### **2.5.2 Implications**

Here we have identified important biases in the locations of studies, the trapping effort for different rodent species and the selection of zoonotic pathogens to assay across the West African region. The use of these data to infer risk of zoonotic spillover or novel disease emergence is likely inappropriate unless these systematic biases are addressed. We hope that this current synthesis of data will encourage further comprehensive ecological studies on rodent species assemblages and their pathogens. One priority would be to better understand the true distribution of known rodent reservoirs (particularly *Mastomys natalensis*) across the region with particular emphasis made to the habitat in which they reside and the structure of rodent assemblages.

## **2.6 Conclusion**

In conclusion we present a comprehensive synthesis of rodent trapping studies across West Africa, we identify important biases in the sampling of rodents and their pathogens that limits inference able to be drawn from

current evidence. We identify evidence gaps in the current literature and highlight geographic regions, habitat types and host-pathogen associations that would benefit from targeted research effort.

## **2.7 Acknowledgments**

The European Commission nor ECMWF are not responsible for any use that may be made of the Copernicus information or data it contains.

## **2.8 Supplementary 1**

**Data extraction tool**

## **2.9 Supplementary 2**

**Habitat dictionary**

## **2.10 Supplementary 3**

**Included studies table**

# **3 Appendix 2: Rodent trapping protocol**

## **3.1 Introduction**

Rodents are being trapped for an ongoing study of rodent population structures and assemblages in a Lassa fever endemic region of Eastern Sierra Leone. The study will continue for around 2 years. The primary outputs of the rodent trapping will be to identify to species level the small mammals in different habitat types at different times of the year in addition to the presence of antibodies to Lassa fever virus. Trapping will occur in pre-specified study site locations every 4 months. Study sites are villages, 5 have been trialled with 4 selected for ongoing work, the villages are Lalehun, Seilama, Lambayama and Baiama. At each study site up to 7 trap sites have been designated. A trap site contains a 7 x 7 grid of Sherman traps set up to 10 metres apart, the trap site may be entirely located within a single habitat type or may span multiple. The land use of the grid and its habitat structure will be noted at each study site visit. The specific location

and characteristics of the habitat of each trap are also recorded for each study site visit. Traps are checked each morning following rebaiting of the traps the evening before. Traps are set for four consecutive nights at each visit.

Trapped rodents are brought to the processing table within a trap following capture. The rodents are then euthanised following recommended procedures with halothene and cervical dislocation. Morphometric measurements are then obtained alongside biological samples for subsequent molecular speciation and assay for Lassa fever virus antibodies or acute infection. Rodent carcasses are then safely disposed of on site to reduce the risk of any infectious material remaining in the study environment. All data is collected in the field electronically using Open Data Kit questionnaires with photographic records collected as required. Samples are then returned to the study laboratory at Mercy Hospital, Bo, Sierra Leone where they remain in storage until analysis can be performed. Separate laboratory protocols describe the subsequent steps in the analysis (DNA Barcoding protocol and Lassa assay protocol).

### **3.2 Preparation for field work**

Before going to the field it's important the following are checked

1. Adequate number of clean and functioning Sherman traps are brought. You will need at least **324** to set up the correct number of grids.
2. Adequate sample pots for rodent specimens.
3. Spare batteries for GPS devices.
4. Battery packs for electronic devices.
5. Portable freezer for rodent specimens, to be stored at Panguma Hospital Lab.
6. Paper copies of the data entry forms.

### **3.3 Locating the trap sites**

The GPS devices can be used to locate the study sites. The coordinates of the corners of the study grids are below.

First turn on the device and press the find button on the front above mark. Use the direction buttons to select Coordinates and press enter. This takes to a page that asks you to enter the location. The left and right arrows at the bottom of the screen move across the numbers. Control what is highlighted using the directional buttons and enter to select. This will then produce a purple line that will guide you to the

coordinates. Tie some ribbon to a plant to identify this corner of the grid and then perform the same for the 3 other corners.

### 3.3.1 Lalehun

We have set up **six** sites in Lalehun. Each site represents a grid that we set lines of traps in. Each line is **7** traps.

For each of the below grids there are coordinates for the corners of the grid. Try and set up lines of traps within the grids so it would be easier if you find the corners first

1. **Grid 1:** Edge of the village

- Closest to the village = 8 11.801 N and 11 4.767 W
- Furthest from the village = 8 11.782 N and 11 4.742 W
- 8 11.781 N and 11 4.775 W
- 9 11.769 N and 11 4.758 W

2. **Grid 2:** Within and near a wet rice field

- Closest to the village = 8 11.921 N and 11 4.771 W
- 8 11.94 N and 11 4.758 W
- Furthest in the field = 8 11.923 N and 11 4.727 W
- 8 11.908 and 11 4.739 W

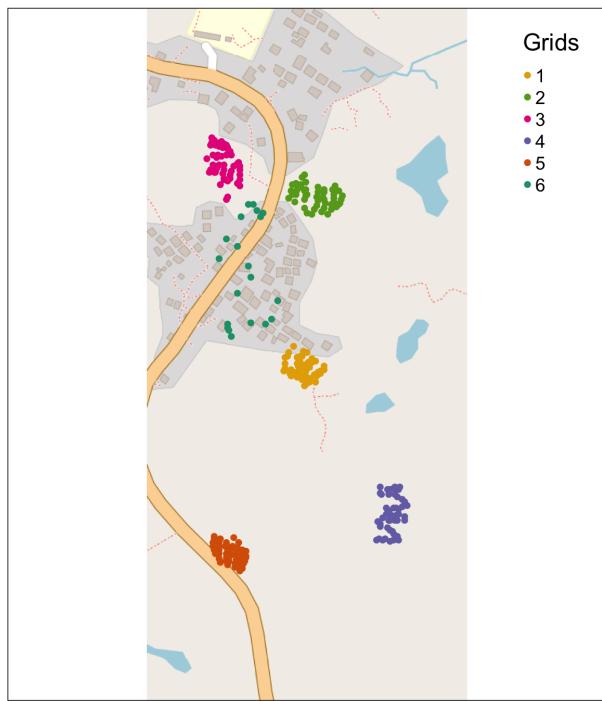
3. **Grid 3:** Split into two, one part on the banana field and fallow land, the other on the banana field and pineapple garden

- Near the compound = 8 11.9417 N and 11 4.811 W
- In the fallow land = 8 11.92 N and 11 4.822 W
- By the water store = 8 11.967 N and 11 4.826 W
- In the fallow land = 8 11.953 N and 11 4.838 W

4. **Grid 4:** Long term fallow land

- Closest to the road = 8 11.644 N and 11 4.699 W
- Across the hill = 8 11.687 N and 11 4.696 W
- Up the hill = 8 11.644 N and 11 4.681 W

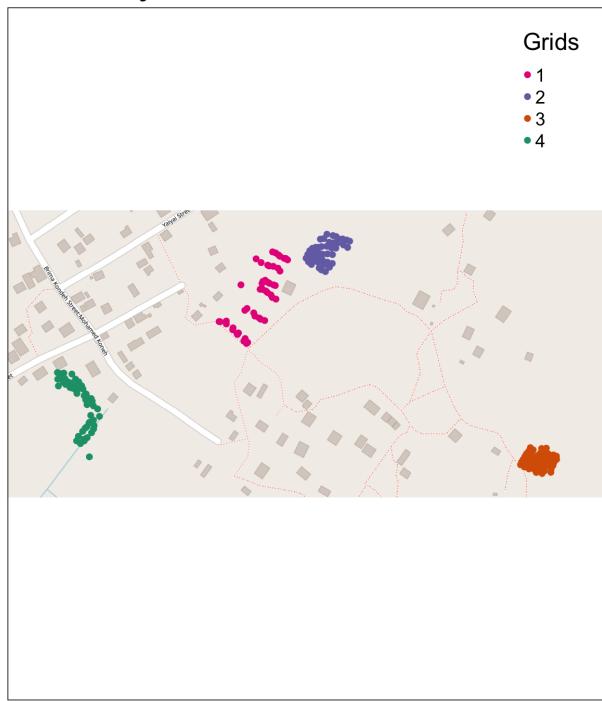
Lalehun



Seilama



Lambayama



Baiama

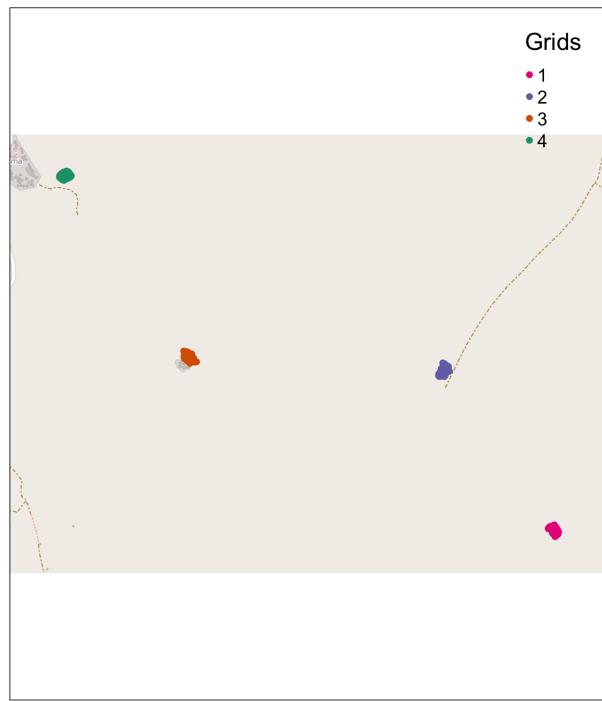


Figure 10: Trap locations in villages

- Up the hill furthest from the road = 8 11.687 N and 11 4.68 W

5. **Grid 5:** Cassava plantation

- Close to the road = 8 11.619 N and 11 4.811 W
- Down the hill along the road = 8 11.633 N and 11 4.831 W
- Into the field = 8 11.647 N and W 11 4.832 W
- 8 11.635 N and 11 4.806 W

6. In 3 lines through the village

- Line 1 beginning = 8 11.911 N and 11 4.797 W
- Line 1 end = 8 11.819 N and 11 4.79 W
- Line 2 beginning = 8 11.888 N and 11 4.822 W
- Line 2 end = 8 11.82 N and 11 4.802 W
- Line 3 beginning = 8 11.872 N and 11 4.828 W
- Line 3 end = 8 11.809 N and 11 4.818 W

7. **Within houses**

- 4 traps per home

We will add another “site” which will be traps within the houses. For the traps in the houses it is important to note what the room is used for, the type of material the house is made from and the type of roof.

For Lalehun we want to have traps in homes from across the village. Please try and make sure that traps are placed in homes from as many as the squares of **Figure 2** as possible. You will also need to record how many houses you asked to place traps in and how many said no. There is a separate **Indoor** sheet to record this on.

### 3.3.2 Seilama

Is positioned in a relatively forested area South West of Panguma. There is significant agricultural activity with fallow, clearance and burning practices used.

We have set up **six** sites in Seilama.

1. **Grid 1:** Palm plantation, near the village and main road

- Close to the main road = 8 7.325 N and 11 11.539 W
- Down the road away from the village = 8 7.375 N and 11 11.511 W
- 8 7.375 N and 11 11.535 W
- Set this corner yourself

2. **Grid 2:** Cacao and Coffee plantation

- Close to the village = 8 7.378 N and 11 11.649 W
- Along the stream = 8 7.4 N and 11 11.643 W
- 8 7.413 N and 11 11.653 W
- Away from the village = 8 7.384 N and 11 11.67 W

3. **Grid 3:** Recently harvested dry rice field

- Close to the village = 8 7.424 N and 11 11.657 W
- Along the ravine = 8 7.446 N and 11 11.66 W
- 8 7.467 N and 11 11.672 W
- 8 7.443 N and 11 11.685 W

4. **Grid 4:** Wet rice plantation

- Closest to the village = 8 7.234 N and 11 11.651 W
- Furthest from the village = 8 7.22 N and 11 11.669 W
- Into the field = 8 7.255 N and 11 11.673 W
- 8 7.234 N and 11 11.678 W
- Line outside of grid beginning = 8 7.258 N and 11 11.619 W
- Line outside of grid end = 8 7.258 N and 11 11.619 W

5. **Grid 5:** Disturbed forest, long term fallow

- Closest to the village = 8 7.413 N and 11 11.871 W
- Away from the village = 8 7.428 N and 11 11.884 W
- 8 7.441 N and 11 11.861 W
- 8 7.43 N and 11 11.849 W

6. Within the village, outside of houses, two lines of 7 within the village

- Set in a ring around the village

- Line 1 beginning = 8 7.307 N and 11 11.625 W
- Line 1 end = 8 7.357 N and 11 11.624 W
- Line 2 beginning = 8 7.31 N and 11 11.6 W
- Line 2 end = 8 7.362 N and 11 11.611 W

## 7. Within houses

- 4 traps per home

Seilama is a much smaller village so just try and make sure that you have good coverage of the different areas of the village when setting the traps in houses.

### 3.3.3 Bambawo

Bambawo was selected due to its proximity to the national park and relatively heavily forested areas of Eastern Province while being on the outskirts of Kenema. This site is no longer being used.

Four sites have been established in the village. We were missing traps for the first visit so two further trap sites will be established on the next visit.

### 3.3.4 Lambayama

Lambayama village.

#### 1. Grid 1: Wet rice fields along the side of the raised walkways.

- 51.0376 N and 11 11.7345 W
- 51.0254 N and 11 11.7181 W
- 51.0787 N and 11 11.7033 W
- 51.074 N and 11 11.6944 W

#### 2. Grid 2: Fallow land on the hill above the rice fields

- Closest corner to the rice field = 7 51.075 N and 11 11.6834 W
- 7 51.0891 N and 11 11.671 W
- Up the hill away from the field = 7 51.085 N and 11 11.6575 W
- 7 51.0671 N and 11 11.6719 W

**3. Grid 3:** Fallow land towards the reservoir

- Corner closest to the village = 7 50.9534 N and 11 11.5559 W
- On the same level towards the reservoir = 7 50.949 N and 11 11.5379 W
- Up the hill from corner 1 = 7 50.9635 N and 11 11.5501 W
- Top right corner = 7 50.9585 N and 11 11.5346 W

**4. Grid 4:** Gardens on the edge of the village towards the marsh

- This area is not set up as a true grid and is in parallel lines along the edge of the marsh and in the village gardens.
- 7 51.0077 N and 11 11.8312 W
- 7 50.9863 N and 11 11.8073 W
- 7 50.9659 N and 11 11.8175 W

### 3.3.5 Baiama

We have currently set 4 trap sites in Baiama, 1 will be added alongside the house site on the next visit.

**1. Grid 1:** Forested site

- Closest to the store house = 7 49.4867 N and 11 15.0235 W
- Towards the path = 7 49.4867 N and 11 15.0235 W
- Into the forest from the house = 7 49.4708 N and 11 15.0098 W

**2. Grid 2:** Fallow land, new rice field

- 49.8157 N and 11 15.235 W
- 49.8086 N and 11 15.2235 W
- 49.7903 N and 11 15.2436 W
- 49.7885 N and 11 15.2318 W

**3. Grid 3:** This site lies near the old Baiama village

- Closest to the village centre by the store house = 7 49.8151 N and 11 15.7421 W
- Away from the village into the forest = 7 49.8179 N and 11 15.729 W
- In the vegetable garden at the edge of the village = 7 49.8245 N and 11 15.7529 W
- Into the forest = 7 49.8386 N and 11 15.7509 W

#### 4. Grid 4: Banana plantation

- 7 50.1875 N and 11 15.9803 W
- 7 50.1968 N and 11 15.9904 W
- 7 50.1785 N and 11 15.9953 W
- 7 50.1844 N and 11 16.0042 W

### 3.4 Trap setup

The trap is placed in the habitat. The coordinates are entered into the ODK questionnaire. The trap is baited with locally produced fish, meat, oat and palm oil based bait in the evening prior to the trapping night. During the trap check in the morning traps are closed prior to refreshing the bait in the evening.

### 3.5 Trap check

Each morning following trapping each trap is checked for the presence/absence of bait, whether the trap has snapped shut over night and whether a rodent has been trapped. The weather overnight is also collected on this data entry form.

### 3.6 Rodent sampling

Trapped rodents are located at the trap check. The traps are placed in plastic bags and brought to the autopsy site. Rodents are euthanised prior to morphological measurements and sampling.

#### 0. Identification

- Rodent unique number
- Trap number
- Trap night
- Initial species/genus identification

#### 1. Morphological measures

- Weight in grams
- Length of head to base of tail (head body) in mm
- Length of the tail in (note whether tail is cut) mm

- Length of the hind foot (not including claws) in mm
- Diameter of the ear measured from the pinna to the edge of the ear in mm
- Length of the skull from the occiput to the tip of the nose, for shrews measure to the end of the projecting teeth in mm

## 2. Autopsy measures

- Rodent sex (M/F)
- Presence of internal or external testes for males
- Development of seminal vesicles for males
- The identification of a perforate vagina for females
- The presence of visible teats for females and the number of pairs of nipples
- The number of developing embryos for females

## 3. Sample collection

- Document whether the following samples have been successfully obtained
  - Photo of rodent
  - Serum sample, in vial and on filter paper
  - Tissue sample of liver and spleen
  - Tissue sample of ear
  - Eye of rodent

## **3.7 Data collection process**

### **3.7.1 Direct ODK entry**

There are three forms you can access through ODK connect on your mobile phone or the study team tablets. The forms once saved will automatically be sent to the ODK server once they can connect to the internet. There is a sim card in the tablet that can be loaded with credit.

1. site\_setup\_v2: This sheet is completed for each site on the first day of trapping. It is important to ensure you correctly write the trap number and its coordinates. If you make any errors you can edit the file or notify Dianah/David and they can amend it. You will describe each site, the habitat and surroundings of each trap and the coordinates for each trap. Photos can be taken if you are having difficulty completing the questions.

2. trap\_check\_v1: This sheet is used to collect information about the number of traps missing **bait**, **have been sprung shut** or contain **rodents** the next morning. It may be easier to note the traps on a piece of paper first and then to enter the data into ODK.
3. rodent\_v1: This sheet is used to collect information about the trapped rodent. The most important parts of this are to ensure that the trap number and rodent number are correct. The trap number is important to know where the rodent came from. The rodent number should be made by putting the number of the visit, then the 3 letters of the village and then the number this rodent is for this visit.

For example:

- The 12th rodent trapped in the 2nd visit in Seilama would be 2SEI-012
- The 3rd rodent trapped on the 1st visit in Bambawo would be 1BAM-003

### **3.7.2 Data entry sheets**

The **Trap site setup** sheet needs to be completed once for each trap site (the grid of 49 traps) once during the study visit for all of the sites that are setup (so 7 including the indoor traps). I expect 7 completed forms from each village. Please try and be as accurate as possible with the GPS coordinates.

The **Trap check** sheet needs to be completed for each trap site for each study night.

The **Rodent** sheet needs to be completed for each rodent that has been trapped.

The **Indoor** sheet only needs to be completed once for each set of traps placed indoors, so once per village.

## **4 Appendix 3: Visit reports: Visit 1 to 3**

Five villages have been enrolled, one will not be carried forward due to concerns from the local team. The village are:

- Bambawo (coordinates 8.009 N, -11.1303 E)
  - Visit 1 - 2021-06-23 to 2021-06-27
  - Visit 2 - 2021-10
- Lalehun (coordinates 8.1975 N, -11.0803 E)
  - Visit 1 - 2020-11-30 to 2020-12-03

- Visit 2 - 2021-04-01 to 2021-04-04
  - Visit 3 - 2021-07-08 to 2021-07-12
  - Visit 4 - 2021-10
- Lambayama (coordinates 7.8544 N, -11.1897 E)
  - Visit 1 - 2021-06-28 to 2021-07-02
  - Visit 2 - 2021-10
- Seilama (coordinates 8.1222 N, -11.1936 E)
  - Visit 1 - 2020-12-05 to 2020-12-09
  - Visit 2 - 2021-04-12 to 2021-04-15
  - Visit 3 - 2021-07-13 to 2021-07-17
  - Visit 4 - 2021-10
- Baiama (coordinates 7.83708 N, -11.26845 E)
  - Visit 1 - 2021-07-03 to 2021-07-07
  - Visit 2 - 2021-10

Rodents are trapped at up to 6 distinct trap sites per village with up to 49 traps per site. Data is collected on this group of data collection forms or on the digitised versions using electronic pads in the field using ODK all digital forms are encrypted locally on the device and sent to a server based at LSHTM. The .xlsx versions of the ODK forms are available on the OpenScience Framework project page.

We have currently obtained 7,270 trap nights across 3 visits (including the pilot). There will ideally be study visits 4 times per year (every 3 months), dates will be confirmed with the study team.

The land use predominantly obtained from Jung et al. and ground truthed through discussion with local communities guided the selection of the trapping sites at study villages.

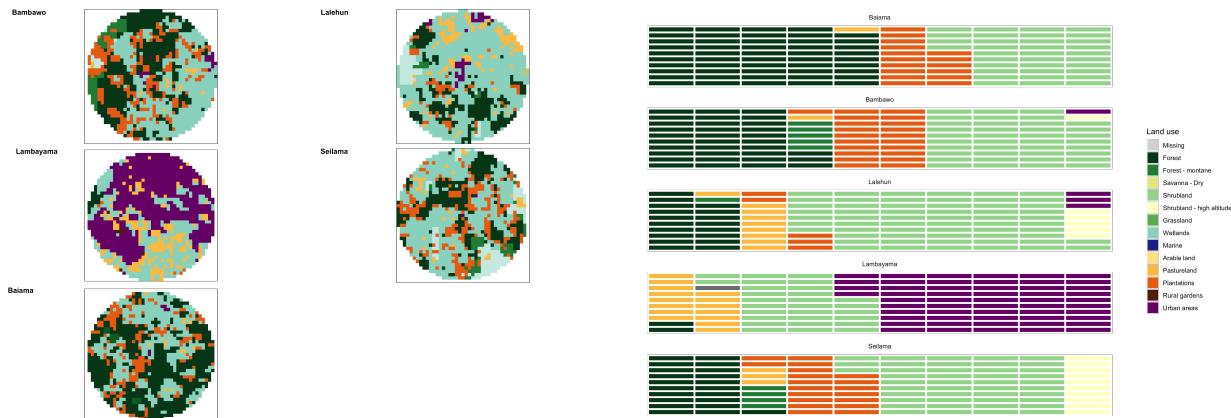


Figure 11: Land use at the study habitats (left) and a representation of the proportion of different land use classes (right)

The locations of traps within these areas are shown with the white points.

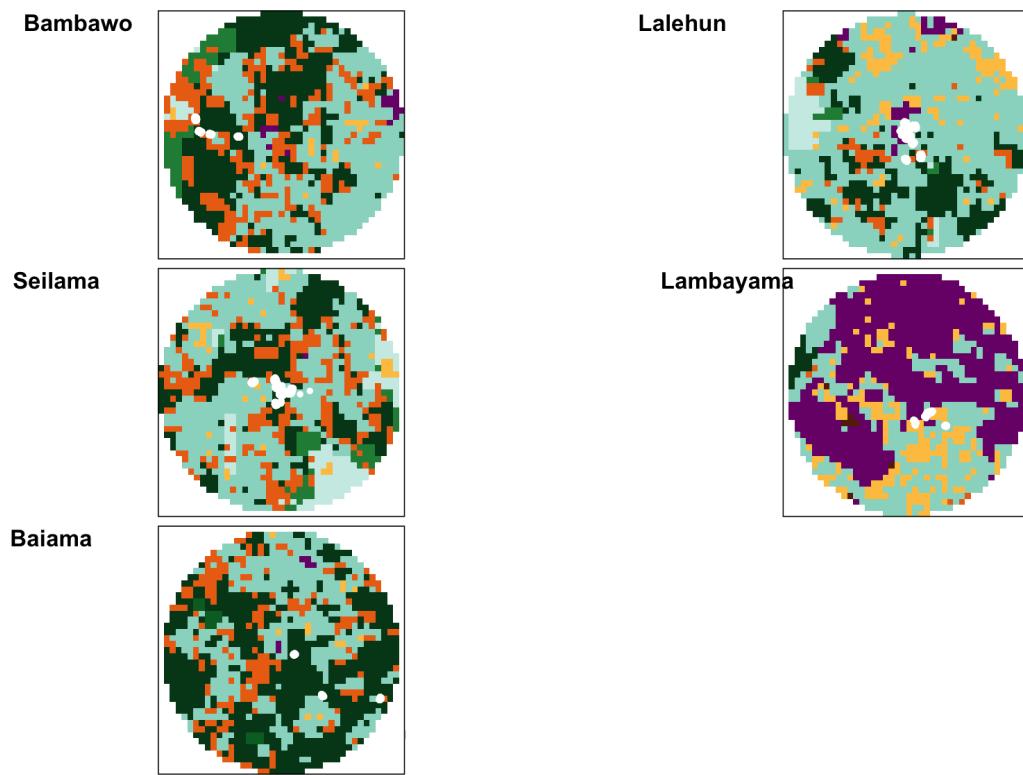


Figure 12: The trap locations are shown overlayed on these habitat maps.

The proportion of nights trapping in each habitat gave good coverage when compared to the different land use classes identified in Eastern Sierra Leone.

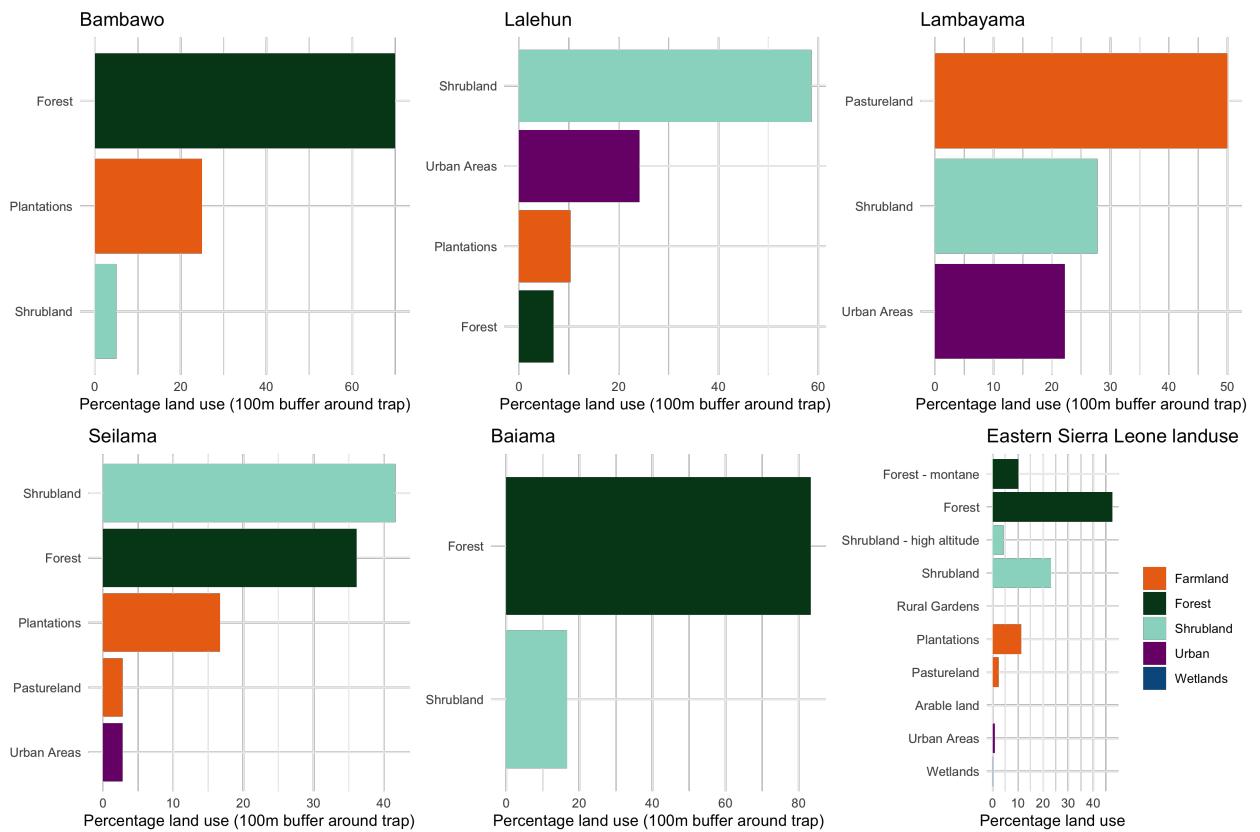


Figure 13: Comparison between trapping habitats and land use classes in Eastern Sierra Leone

The trap success rate is around 4% this is fairly acceptable based on the review I did. We have trapped 137 rodents from at least 13 species. The majority of trapped individuals have been shrews (crocidura), *Mastomys* sp., *Praomys* sp., *Mus minutoides* and *Lophuromys* sp.. Data from the most recent trapping activities from visit 3 during the rainy season have so far shown a dramatic drop in trap success.

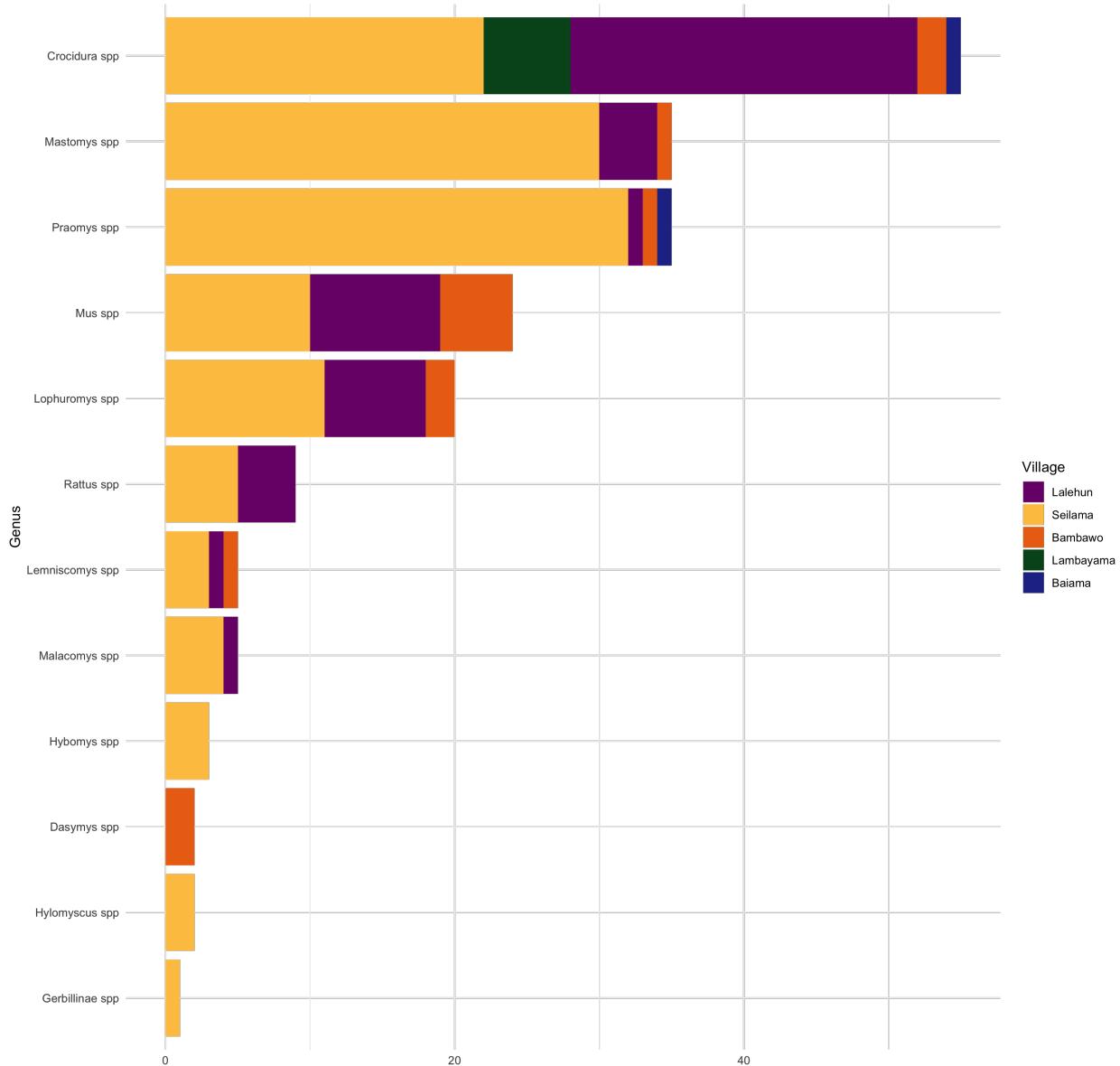


Figure 14: Species caught in all visits

Species accumulation curves have been produced for the first two visits from Lalehun and Seilama. It has not been possible to produce equivalent plots for Baiama and Lambayama due to the low number of individuals/species trapped in those locations.

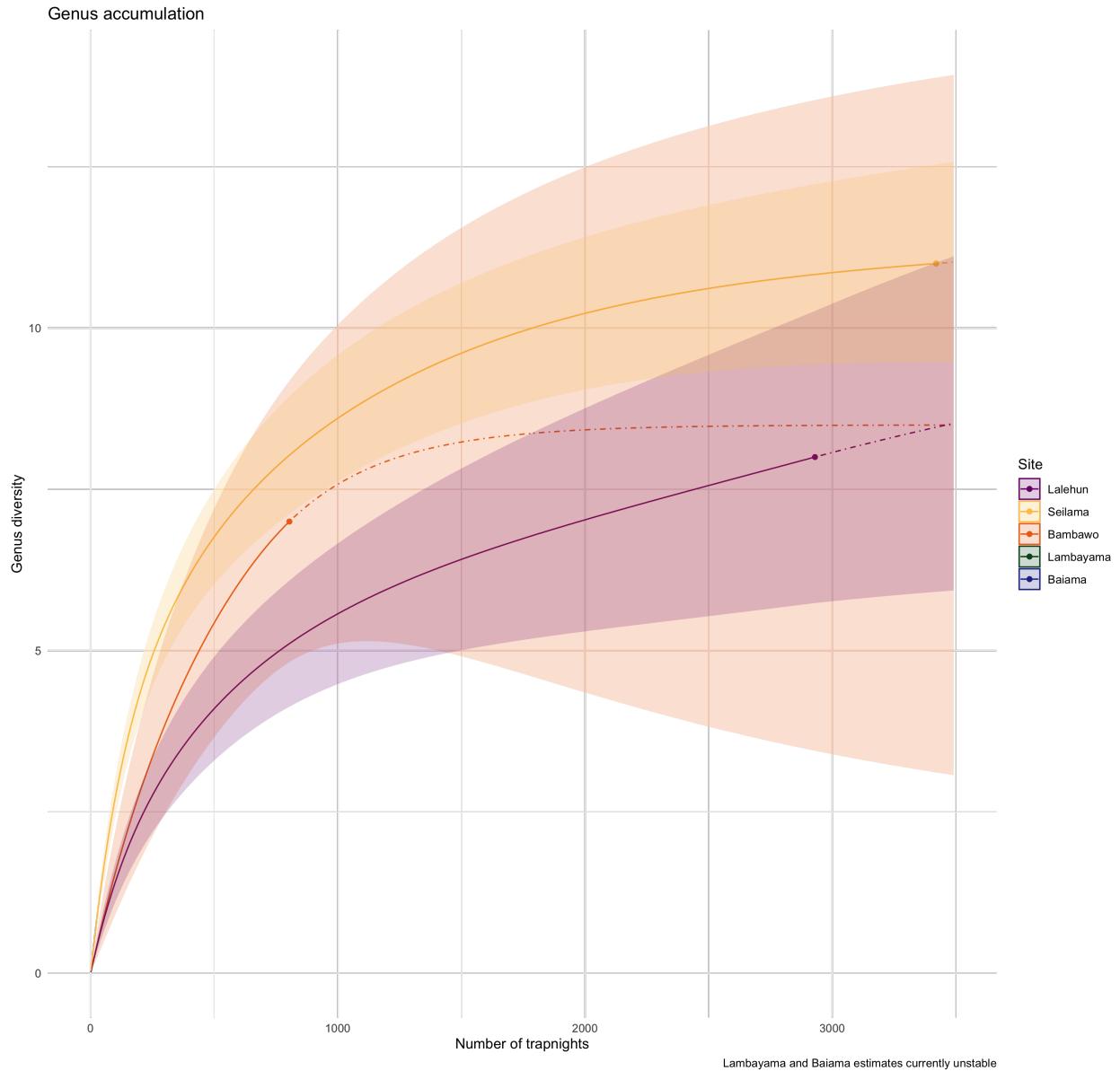


Figure 15: Species accumulation curves for Lalehun, Salaima and Bambawo

As we catch an increasing number of individuals we are seeing them clustering within expected habitats.

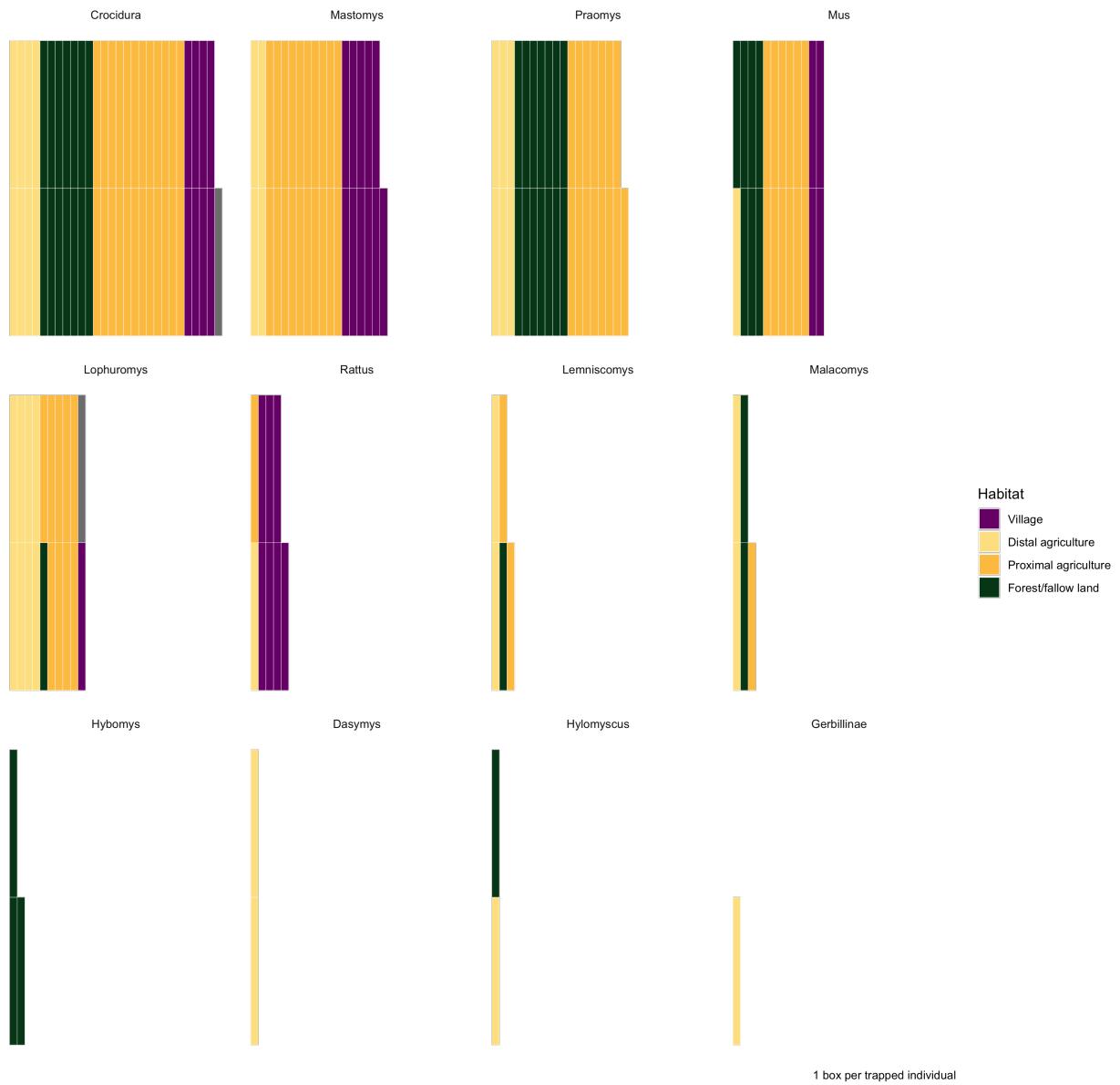


Figure 16: Clustering of species within preferred habitat classes

## 5 Appendix 4: ICREID poster

This poster was produced for the International Conference on (Re-)Emerging Infectious Diseases conference. This poster documents the workflow and initial results of our bioinformatic pathway to identify potential epitopes of interest for our Luciferase Immunoprecipitation Assay that we are developing alongside collaborators at UCL and the International Atomic Energy Association. The poster is available at higher resolution

here

Page break

## 6 Appendix 5: Training log

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# Outline of an innovative cross-species assay to evaluate *Lassa mammarenavirus* (Lassa fever) serology

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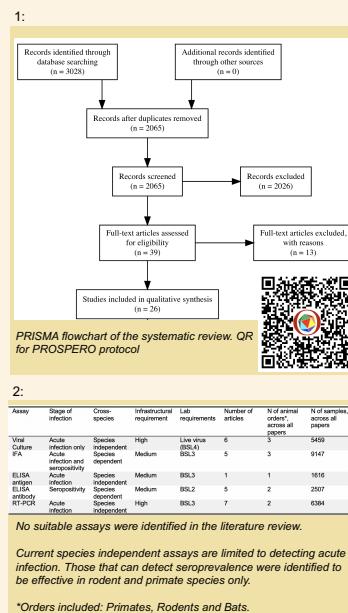


## Introduction

Lassa fever (LASV) is a zoonotic infectious disease endemic to West Africa that spills over to humans from the rodent species *Mastomys natalensis*. Additional rodent species in endemic regions demonstrate seropositivity for LASV but their role in its transmission remains unclear. The wider distribution of LASV in peri-domestic animals is unknown due to lack of suitable assays.

## Current diagnostic assays

A systematic review of Lassa fever assays in non-humans was conducted to identify assays feasible for use in peri-domestic animals (Panel 1). The assays and their suitability are shown in Panel 2.



Assay	Stage of infection	Cross-species	Infrastructure requirement	Lab requirements	Number of articles	N of animal orders across all species	N of samples across all species
Viral culture	Acute	Species independent	High	Live virus (BSL4)	6	2	5458
	Acute	Species specific	Medium	BSL3	5	3	9147
ELISA antigen	Acute	Species independent	Medium	BSL3	1	1	1616
ELISA antibody	Seroepidemiology	Species independent	Medium	BSL2	5	2	2507
RT-PCR	Acute	Species independent	High	BSL3	7	2	6364

*\*Orders included: Primates, Rodents and Bats.*

*Current species independent assays are limited to detecting acute infection. Those that can detect seroprevalence were identified to be effective in rodent and primate species only.*

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## Luciferase ImmunoPrecipitation System (LIPS)

No assays were suitable for cross-species seroprevalence studies. The LIPS assay has been used for other diseases that affect multiple animal species and would be viable for our planned research.

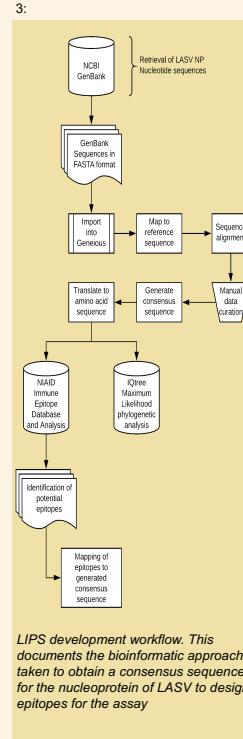
Our review identified that the nucleoprotein (NP) component of the virus was predominantly targeted by these assays. We focused our search for a suitable epitope on this region of the genome.

## Obtaining NP sequences

676 complete sequences were obtained from the NCBI GenBank (Panel 4). The sequences were aligned in Geneious using ClustalW. A consensus sequence was constructed. The workflow is demonstrated in Panel 3.

## Antigen screening

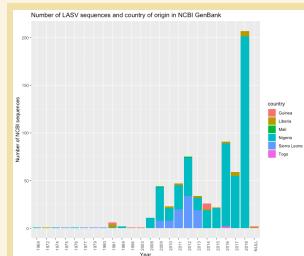
Potential antigens within the consensus sequence were identified using the NIAID immune epitope database and analysis resource. Bepipred, Bepipred-2 and Kolaskar-Tongoankar algorithms were used to score the antigenicity of different regions of the sequence. The analysis is presented in Panel 6.



## Plasmid construct

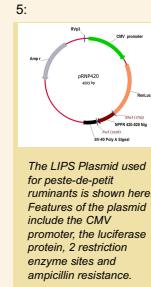
Six potential amino acid sequences were identified (Panel 7), chosen based on their sequence identity and scores using the specified algorithms. These plasmids will be constructed and purified for proof-of-concept testing within the LIPS. Panel 5 illustrates the structure of the proposed plasmid.

4:



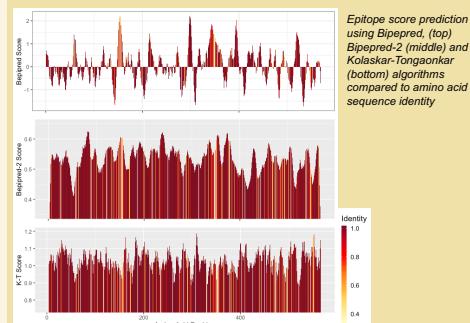
The origin of the LASV sequences by year of collection and country of origin is shown for the 676 sequences in the analysis.

24 of these were obtained from rodents (3.6%) with the remaining 652 samples obtained from humans.



The LIPS Plasmid used for pesto-de-poit ruminants is shown here. Features of the plasmid include the CMV promoter, the luciferase protein, 2 restriction enzyme sites and ampicillin resistance.

6:



7:

Epitope Start Number	End Residue	Method identifying suitable antigenicity	AA Identity
1	26	K-T & Bepipred-2	99%
2	116	K-T & Bepipred & Bepipred-2	94%
3	248	K-T & Bepipred	99%
4	305	K-T & Bepipred-2	100%
5	447	K-T & Bepipred-2	90%
6	500	K-T & Bepipred & Bepipred-2	96%

The 6 epitopes selected for further development within the LIPS LASV program

## Conclusions

We have identified a suitable assay to describe the seroprevalence of *Lassa mammarenavirus* in rodents and peridomestic animals in West Africa. The antigenic target is derived from publicly available viral sequences across the LASV endemic zone and across time. We have used established bioinformatic techniques to guide epitope selection.



Figure 17: ICRIED poster  
57

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