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TRANSMISSION ECOLOGY OF OLD WORLD ARENAVIRUSES IN NATURAL POPULATIONS OF THEIR RESERVOIR HOSTS

TRANSMISSIE-ECOLOGIE VAN OUDE WERELD ARENAVIRUSSEN IN NATUURLIJKE POPULATIES VAN HUN PRIMAIRE GASTHEREN

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"Since the soldier did not speak English, the accompanying medic translated his story for us. Four days prior, the soldier had been sleeping in his tent when he awoke to feel something crawling on his shoulder. He had grabbed what turned out to be a rat and flung it across the room, but not before the rodent managed to bite him. The soldier dutifully showed us the healing puncture wound on his hands.

The soldier had not thought much of the incident until he developed a fever and a cough three days later. The soldier had then talked to the medic of his battalion, who discussed the issue up the chain of command and eventually decided to bring the man to the Lassa ward. We had him describe the rat for us and lamented that he had thrown away the carcass. It sounded like a Mastomys natalensis (the species that transmits Lassa), but it was impossible to be sure. After some debate, Dr. Conteh decided to err on the side of safety and start the soldier on a prophylactic regimen of ribavirin.

We put the unfortunate man in his room, away from the other patients. His case was a reminder that in Africa, the majority of wartime deaths are not directly from bullets or bombs, but from the spread of infectious disease that historically accompany a society's breakdown."

Excerpt from the diary of Dr. Ross I. Donaldson
The Lassa ward (2003)

A Natal multimammate mouse (*Mastomys natalensis*)

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Summary

In West Africa, the multimammate mouse (*Mastomys natalensis*) is the primary reservoir of Lassa virus (LASV), an arenavirus that causes severe haemorrhagic fever in humans. It is estimated that this virus affects between 200.000 and 300.000 people yearly with a fatality rate of 1-2%. Humans get infected by close direct or indirect contact with the rodent or its excretions, which can be through contaminated food or water, direct consumption of the rodent, or inhalation of excretion particles. Because no vaccine for use in humans exists and therapeutic options are limited to the broad-spectrum antiviral ribavirin, rodent control is currently assumed to be the only feasible option to control Lassa fever. However, no solid information exists on how effective rodent control really is or which control strategies would be most effective to reduce spillover.

Mastomys natalensis also hosts several other arenaviruses such as Morogoro virus (MORV), which occurs in rodent populations in Tanzania. Because the ecology of Tanzanian *M. natalensis* is well known and MORV is not pathogenic for humans, MORV-*M. natalensis* is considered a suitable model for examining arenavirus-host interactions in general. The model is especially considered a safe substitute for studying closely related but pathogenic arenaviruses like LASV.

This thesis has two main objectives. The first is to improve our understanding of how arenaviruses can persist in rodent populations. Therefore, we will study the transmission dynamics of MORV in highly fluctuating populations of *M. natalensis* in Tanzania. The second is to explore which rodent control strategies are efficient to control LASV in Guinea. To achieve this, we will use data from different field experiments performed in Guinea, together with information derived from studying the first objective.

How can arenaviruses persist in populations of their reservoir host?

Seasonal variation in rainfall causes *M. natalensis* population densities to fluctuate heavily in Tanzania. When densities become very low, they may result in the extinction of parasites because transmission may cease due to lack of contacts between hosts. However, MORV seems to persist in such low densities regardlessly. As a first step to explore how this is possible we investigated the virulence of MORV in *M. natalensis*. This would indicate whether MORV causes adverse effects in its host, which if true, would be an additional factor that could limit the virus' persistence probability. In contrast, based on morphological characteristics (chapter

2) and survival data (chapter 3), we showed that MORV causes no or only mild adverse effects in its host. This could be an adaptation of the virus to persist during the yearly host population bottlenecks and is in line with the 'parasite trade-off hypothesis', which balances virulence and host survival so that transmission is maximized over the lifetime of infection.

Understanding parasite persistence in wildlife populations relies on a correct interpretation of the infection patterns. These patterns are well-investigated for MORV in laboratory conditions, where *M. natalensis* was inoculated intraperitoneally and followed for several weeks. However, important differences may occur when animals become infected in natural conditions due to circumstances that were not accounted for in the lab (e.g. due to differences in genetic strains, stress levels, infection dose or life histories). In order to validate these laboratory results, we compared MORV infection patterns between naturally and experimentally infected multimammate mice, using blood and excretion samples that were collected during a three-month capture-mark-recapture (CMR) study (chapter 4). We found a good match between the field and laboratory data for 52% of the naturally infected animals. Mismatches could be explained by infection patterns that were not yet tested in the lab, such as maternal antibodies or an antibody detection limit. An interesting result related to the persistence of MORV was that some animals in the field (16% of infected) showed signs of chronic or latent infections, while it was previously assumed that MORV infections in *M. natalensis* are predominantly acute.

The transmission dynamics of MORV in populations of *M. natalensis* were investigated by comparing model simulations to serological data of a ten-year CMR time series (chapter 5). In this dataset, we observed that seropositive animals were continuously present (indicating no extinction of the virus) and that seroprevalence peaks after the high-density season of the host. This seasonal pattern could be best approximated by mathematical models that included a combination of vertical and density-dependent horizontal transmission while a small number of animals had to be chronically infected to ensure viral persistence. To further investigate whether chronically infected individuals are indeed present in natural conditions, we performed a small lab experiment in which wild-caught rodents were caged for eight weeks, sampled each week and afterwards dissected. In agreement with the previous short CMR-study (chapter 4) and the model simulations, we observed that a low number of animals (8%) showed signs of chronic MORV infections.

How effective is rodent control to reduce LASV spillover risk in Guinea?

After investigating how MORV can persist in populations of *M. natalensis* in Tanzania, we moved our focus to the transmission of LASV in rodent populations in Guinea. Little attention has been paid to the ecology of *M. natalensis* in West Africa and in order to better understand how LASV spreads on a local scale, we first investigated the movement patterns of *M. natalensis* in Lassa fever-endemic villages (chapter 6). By performing several CMR and dyed bait (Rhodamine B) experiments in different villages in Guinea, we observed that *M. natalensis* easily moves between the fields and the houses in the village. This observation supports the hypothesis that rodents move from the fields to the houses to search for food during the dry season, which might explain the higher incidence of human Lassa fever cases during this period. Information derived from these experiments was also used to inform a mathematical model for LASV transmission (discussed in the next chapter), as it provided an estimate of the population density and the action radius of *M. natalensis* in these villages.

Last, we analysed data from a rodent control experiment performed in rural villages in Guinea (chapter 7). We found that an annual rodent control treatment could decrease LASV transmission in the rodent populations, although the effect was small (5% reduction in seroprevalence per year) given the effort. Furthermore, as the rodent populations recovered rapidly after rodenticide application, we conclude that annual rodent control is an unsustainable approach to limit LASV spillover risk. Nevertheless, data obtained from this experiment (together with the data discussed previously) proved to be useful to parametrize a mathematical model for LASV transmission. Simulations of this model suggest that year-round elimination or vaccination of rodents are more sustainable approaches to control LASV than annual elimination, and should be considered by policymakers.

Samenvatting

De Natal veeltepelmuis (*Mastomys natalensis*) is de primaire gastheer van Lassavirus (LASV), een arenavirus dat hemorragische koorts veroorzaakt bij mensen in West-Afrika. Ieder jaar infecteert dit virus tussen de 200.000 en 300.000 mensen waarvan uiteindelijk 1 tot 2% overlijdt aan Lassakoorts. Mensen worden meestal geïnfecteerd door nauw contact met infectieuze knaagdieren of met hun excreties. Dit kan gebeuren door het eten van gecontamineerd voedsel of het inademen van infectieuze viruspartikels. Momenteel is er geen vaccin of goedwerkend geneesmiddel dat Lassakoorts kan voorkomen of genezen. De enige manier om infectie te voorkomen is door knaagdiercontrole, maar zelfs voor deze methode is niet geweten hoe effectief ze eigenlijk is of welke controletechnieken het meest efficiënt zijn.

Mastomys natalensis is niet alleen de primaire gastheer van LASV, maar ook van verschillende andere arenavirussen, waaronder Morogorovirus (MORV) dat voorkomt in knaagdierpopulaties in Tanzania. Het MORV-*M. natalensis* systeem wordt als een geschikt model beschouwd om algemene virus-gastheer interacties te bestuderen in natuurlijke omstandigheden. Voordelen zijn dat de ecologie van het knaagdier goed gekend is en het virus geen mensen infecteert, wat het bijvoorbeeld een veilig alternatief maakt om bepaalde aspecten van de ecologie van LASV te bestuderen.

Deze thesis heeft twee doelstellingen. De eerste is om beter te begrijpen hoe arenavirussen kunnen overleven in populaties van hun primaire gastheer. Hiervoor zullen we de transmissiedynamica van MORV bestuderen in knaagdierpopulaties in Tanzania. De tweede is om te onderzoeken welke knaagdiercontrolestrategieën het meest efficiënt zijn om het infectierisico van LASV voor mensen te verminderen. Hiervoor zullen we data gebruiken van veldexperimenten uitgevoerd in Guinee, alsook informatie die we verkregen hebben bij het bestuderen van MORV.

Hoe kunnen arenavirussen overleven in populaties van hun primaire gastheer?

Variatie in regenval zorgt voor seizoenale fluctuaties in de populatiedensiteiten van *M. natalensis* in Tanzania. Wanneer de densiteiten laag zijn vergroot de kans dat parasieten zich niet meer kunnen verspreiden omdat hun gastheer minder contact maakt met andere individuen in de populatie. In tegenstelling tot de verwachting overleeft MORV wel periodes van lage densiteiten. Om te onderzoeken hoe dit mogelijk is bestudeerden we eerst de virulentie van het virus (hoofdstuk 2 en 3). Mocht hieruit blijken dat MORV de levensduur van zijn gastheer

aanzienlijk vermindert, zou het zijn eigen overlevingskans verlagen doordat infectieuze gastheren minder lang leven. Aan de hand van morfologische gegevens (hoofdstuk 2) en overlevingsdata (hoofdstuk 3) toonden we aan dat dit niet het geval is. MORV lijkt zijn gastheer weinig tot geen schade toe te brengen. Mogelijk is dit een adaptie om te vermijden dat zijn gastheer uitsterft wanneer zijn dichtheid al door andere factoren wordt verlaagd.

Om te begrijpen hoe parasieten overleven in populaties van wilde dieren is het noodzakelijk om het infectieverloop in de gastheer te kennen. In laboratoriumomstandigheden is dit reeds goed gekend voor MORV infecties. *M. natalensis* werd onder deze omstandigheden intraperitoneaal geïnoculeerd en voor enkele weken opgevolgd. Maar omdat het verloop in natuurlijke omstandigheden kan veranderen ten gevolge van verschillen in genetisch materiaal, infectiedosis of algemene omgevingsverschillen, moest het gevalideerd worden aan velddata. Om het infectiepatroon te vergelijken gebruikten we bloed- en excretiestalen afkomstig van een vangst-hervangststudie die drie maanden duurde en waarbij muizen tweewekelijks werden gevangen (hoofdstuk 4). Uit deze studie bleek dat 52% van de natuurlijk geïnfecteerde dieren een infectieverloop had gelijkaardig aan wat we observeerden in het laboratorium. De verschillen konden we verklaren aan de hand van bepaalde situaties die we nog niet in het laboratorium hadden getest, zoals de aanwezigheid van maternale antilichamen of een antilichaam detectielimiet. Een interessant resultaat m.b.t. de overleving van het virus was dat sommige dieren (16%) tekenen vertoonden van chronische of latente infecties, hoewel we eerder veronderstelden dat MORV infecties acuut zijn bij *M. natalensis*.

De natuurlijke transmissiedynamica van MORV in populaties van *M. natalensis* werd uiteindelijk onderzocht door simulaties van een wiskundig model te vergelijken met serologische gegevens van een 10 jaar durende vangst-hervangst studie (hoofdstuk 5). We observeerden in deze dataset dat seropositieve dieren continue aanwezig waren (dus geen extinctie van het virus) en dat de seroprevalentie piekt juist na de densiteitspiek van de gastheerpopulatie. De wiskundige modellen waarin zowel verticale als densiteits-afhankelijke horizontale transmissie mogelijk was konden dit seizoenale patroon het meest accuraat verklaren. Bovendien suggereerden deze modellen dat MORV enkel kan overleven wanneer een bepaald percentage van muizen chronisch geïnfecteerd is. Dit gegeven overtuigde ons om verder onderzoek te verrichten naar dit infectiepatroon in natuurlijke omstandigheden, en gebeurde door wilde knaagdieren te vangen, de dieren in kooien te plaatsen en de infectie op te volgen voor acht weken. Overeenstemmend met de suggesties van het wiskundig model en de

vorige vangst-hervangststudie (hoofdstuk 4) vonden we dat sommige dieren (8%) inderdaad chronisch geïnfecteerd waren.

Welke knaagdiercontrolestrategieën zijn het meest efficiënt zijn om het infectierisico van LASV voor mensen te verminderen?

Er is nog maar weinig geweten over de ecologie van *M. natalensis* in West-Afrika en om beter te begrijpen hoe LASV zich verspreidt op lokaal niveau onderzochten we eerst hoe dit knaagdier zich voortbeweegt in dorpen in Guinee waarin Lassakoorts endemisch is. Aan de hand van verschillende veldexperimenten toonden we aan dat *M. natalensis* zich gemakkelijk verplaatst tussen de velden en de huizen van de dorpsbewoners (hoofdstuk 6). De experimenten ondersteunde de hypothese waarin wordt gesteld dat *M. natalensis* massaal migreert van de velden naar de huizen op zoek naar eten tijdens het droogseizoen, wat een verklaring kan zijn voor de hogere Lassakoorts incidentie bij dorpsbewoners tijdens deze periode. De experimenten verschaften bovendien nuttige informatie voor het parametriseren van wiskundige modellen die de transmissiedynamica van LASV konden simuleren (hoofdstuk 7).

In het laatste hoofdstuk (hoofdstuk 7) analyseerden we data van een knaagdiercontrole experiment dat was uitgevoerd in Guinee. We vonden dat een jaarlijkse controlestrategie effectief was om de verspreiding van LASV binnen de knaagdierpopulatie te verminderen. We vonden echter ook dat het effect van een dergelijke knaagdiercontrole klein was in vergelijking tot de arbeidsintensiteit (we vonden een reductie in seroprevalence van 5% per jaar). Omdat we tegelijkertijd vaststelden dat de knaagdierpopulatie na het verspreiden van gif snel terug aangroeit, besluiten we dat een jaarlijkse knaagdiercontrolestrategie niet geschikt is om het transmissierisico van LASV voor mensen te verlagen. De data die we verkregen van dit experiment (samen met de data van MORV en de vorige veldexperimenten in Guinee) waren wel zeer zinvol om een wiskundig model te parametriseren dat de verspreiding van LASV kan simuleren in West-Afrikaanse dorpen. Dit model suggereert dat het continu verlagen (i.t.t. één keer per jaar) of vaccineren van knaagdierpopulaties wel effectieve strategieën zijn om LASV onder controle te krijgen. De uitkomsten van dit model geven ook een eerste indicatie voor beleidsmakers hoe lang en frequent knaagdiercontrole zou moeten gebeuren om LASV transmissie te stoppen.

Introduction

Throughout history, infectious parasites have affected human societies by their disease-causing effects and rapid or insidious spread (Wolfe et al. 2007). While some of these parasites have thrived in a human population for more than thousands of years (e.g. tuberculosis bacteria were found in mummies from ancient Egypt), others seem to have emerged only very recently (Ksiazek et al. 2003; Ziskind and Halioua 2007). Especially during the past five decades, there is a significant rise in the number of such emerging infectious diseases (EIDs), which is linked to severe changes in socio-economic (e.g. globalisation), environmental (e.g. human encroachment of wild habitats) and ecological factors (e.g. climate change) (Jones et al. 2008).

Most EIDs originate from zoonotic parasites, defined here as those which have a non-human animal source, and most of them originate from wildlife (Daszak et al. 2000; Karesh et al. 2012). From these wildlife hosts, rodents encompass a disproportionate number of zoonotic reservoirs (i.e. their natural hosts) (Han et al. 2015). This may be because of their high contact rate with humans (rodents often roam around human dwellings) and fast-paced life history, which might favour offspring production over investment in the immune system (Karesh et al. 2012; Han et al. 2015). Among all zoonotic parasites, RNA viruses are the ones that emerge most frequently in humans (Woolhouse and Gaunt 2007; Morse et al. 2012). Given that these viruses use the error-prone RNA-polymerase for replication, important traits that can explain their zoonotic potential are the high mutation rate and host plasticity (Lauring and Andino 2010). These traits are also associated with increased human-to-human transmission and a wide host range distribution (Kreuder et al. 2015). For example, RNA viral strains related to the causative agent of the extremely infectious Spanish flu, influenza A virus H1N1, have been found all around the world in humans, pigs and birds (Girard et al. 2010; Medina 2018).

In order to devise strategies to mitigate and prevent zoonotic disease risk to humans, it is necessary to understand how these parasites are transmitted in populations of their reservoir hosts (Plowright et al. 2017; Forbes et al. 2018). However, for the vast majority of zoonotic parasites, such information remains little known (Han et al. 2016). This thesis aims to contribute to filling this research gap by describing the transmission dynamics of arenaviruses in the Natal multimammate mouse, *Mastomys natalensis* (Smith 1834). Before I will describe the main components of the studied system in this thesis (Morogoro virus, Lassa virus and the multimammate mouse), I will start with a brief overview of the most important concepts in disease ecology.

1. Parasite ecology: theory

1.1 The transmission term: -βSI

Transmission is the key component in all parasite-host interactions. Discussing transmission for all parasites would be far beyond the scope of this thesis, so here I will limit the description for directly transmitted microparasites. For these parasites, new infections depend on the per capita transmission rate (also called the force of infection: λ) and the number of susceptible hosts in the population (Begon et al. 2006; Keeling and Rohani, 2007). The transmission rate is the product of the underlying host contact rate (*c*), the probability (*v*) of successful transmission between infectious (*I*) and susceptible hosts (*S*), and the parasite prevalence (*I* or *I/N*, with *N* being the total population size). The overall transmission term is then given by $-cvSI/N$. Because it is practically impossible to differentiate between *c* and *v* when using empirical data, these parameters are often combined in one parameter, the transmission coefficient β.

The transmission rate has two general possibilities that differ in how host contact rate changes with population size: $\lambda = \beta I$ and $\lambda = \beta I/N$ (McCallum et al. 2001; Begon et al. 2002; Keeling and Rohani 2008). The first formulation reflects the situation where the contact rate increases with population size, which results in density-dependent transmission. This situation is assumed for many wildlife diseases, as the denser the population becomes the more likely it is that hosts come into contact with each other. The second formulation reflects the situation where the contact rate is independent of the population size, which results in frequency-dependent transmission. This situation is often assumed for sexually transmitted diseases. For parasites with a density-dependent contact rate the transmission term is given by $-\beta SI$, while it is given by $-\beta SI/N$ for parasites with a frequency-dependent contact rate. The difference between frequency- and density-dependent transmission becomes important if the population size fluctuates in time or space, which is often the case for wildlife populations. In reality, we are more likely to observe an intermediate relationship between these two extreme (theoretical) forms. For example, when a whole range of density- and frequency-dependent transmission shapes were modelled as descriptors of cowpox virus transmission in field voles (*Microtus agrestis*) an intermediate relationship came out as the best model fit (Smith et al. 2009).

1.2 The basic reproductive number: R_0

Another important concept in parasite ecology is the basic reproductive number, R_0, defined as 'the average number of secondary infections derived from a primary infection in an entirely susceptible population' (Kermack and McKendrick 1927; Heesterbeek and Dietz 1996). For

many directly transmitted parasites, R_0 is proportional to the transmission coefficient (β), the infectious period of the host (γ^{-1}) and the number of susceptible individuals (S) in the population. This gives the following equation: $R_0 = (\beta N)/\gamma$. A parasite will theoretically spread in a closed population when $R_0 > 1$, whereas it will not if $R_0 < 1$. The transmission threshold that must be crossed for a parasite to spread is then given by the condition $R_0 = 1$. In the real world, a population will rarely be totally susceptible to an infection. The effective reproductive rate (R_e) estimates the average number of secondary infections derived from an infectious case in a mixed population of both susceptible and non-susceptible hosts.

1.3 Population thresholds

Theory predicts the existence of population thresholds below which a parasite cannot invade or persist in the host population (Anderson and May 1978, 1979; Lloyd-Smith et al. 2005). An invasion threshold (N_T) occurs when R_e increases with density, as in density-dependent transmission, and corresponds to the population density for which $R_e = 1$. This threshold underlines disease control policies based on culling of animals and is often crossed in wildlife hosts that exhibit large density fluctuations in time or space, such as plague in great gerbils (*Rhombomys opimus*) and brucellosis in herds of bison (*Bison bison*) (Davis et al. 2004; Dobson & Meagher, 1996). In contrast, no invasion threshold exists for parasites with frequency-dependent transmission, and these parasites are (theoretically) predicted to invade even in very low-density populations. In this case, control measures aimed at reducing host densities would be useless and parasite extinction will only occur if the proportion of susceptibles is too low for transmission, $S_T < 1/R_e$, which can be achieved by vaccinating a sufficient number of animals (Morters et al. 2013; McCallum 2016). For example, management of rabies in wild foxes (*Vulpes vulpes)* revealed that vaccination is more effective than culling because the latter destabilises the social structure, resulting in an increased β caused by higher contact rates (Pastoret and Brochier 1996). Furthermore, stochastic models show that invasions can fail by chance even when $R_e > 1$ and that the effect increases at smaller population sizes (Lloyd-Smith et al. 2005). This phenomenon was first described in studies on measles, which was observed to persist in cities with more than 300.000 inhabitants only (Black 1966). The observation resulted in the definition of the critical population size (referred to as the critical community size for human populations), which corresponds to the population size at which a parasite will not fade out due to stochastic events over a predefined time period (e.g. 10 years for measles in human populations) (Bartlett 1957).

1.4 SIR models

Mathematical models are valuable tools for understanding epidemiological patterns and guiding policy decisions (Heesterbeek et al. 2015). Here, I will briefly discuss the standard SIR model, as it is a good starting point to understand the models used in chapter 5 and 7 of this thesis. The SIR model combines the previously described parameters into a dynamic system of coupled ordinary differential equations (ODE) that categorizes hosts within a population as **S**usceptible, **I**nfected and **R**ecovered (Kermack and McKendrick 1927; Keeling and Rohani, 2008). It assumes a closed population without demographics in which individuals are homogeneously mixed (all individuals have the same probability to make contact with each other) and immediately become infectious after infection (the latency period is zero) (Dietz 1967). Given that these assumptions are true, we get the following flow diagram and SIR equations (Fig 1):

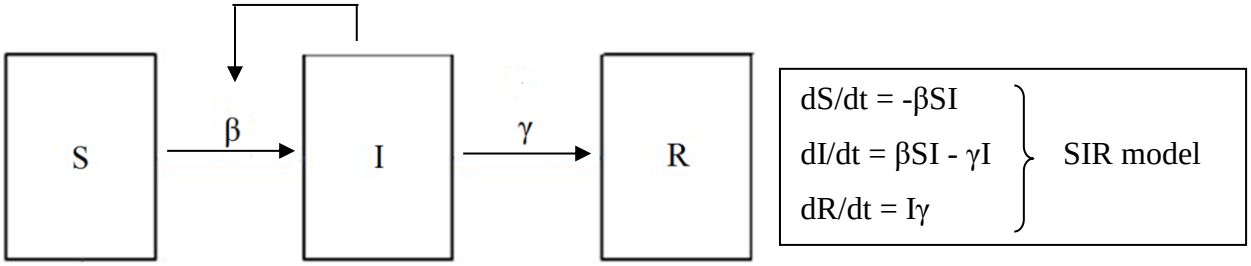

Fig 1: Flow diagram and ODE equations of the Susceptible-Infected-Recovered model.

In this model, individuals are assumed to be born in the susceptible compartment (S). They will transit to the infected compartment (I) depending on the transmission coefficient (β) and the number of infected individuals. Infected individuals will stay in this compartment until they clear the infection at the recovery rate γ (i.e. the reciprocal of the infectious period). After recovery, individuals transfer to the recovered compartment (R) where they stay permanently because they have acquired lifelong immunity against the parasite (Fig. 2). Despite its simplicity, the deterministic SIR model can already describe a wide range of infections by calculating the change in each category over small time intervals (Fig 2: *left*) (Hens et al. 2012). Examples of such models include those for influenza A (H1N1) virus in humans (Coburn et al. 2009), *Brucella abortus* in elk (Benavides et al. 2017) or Tularemia bacteria in house mice (Dobay et al. 2015). The SIR model also serves as a starting point for many other types of modelling formats, as demographic processes or compartments can easily be changed or added. Nevertheless, for other parasites the deterministic SIR model is an oversimplification of the real parasites' ecology. For example, even if the distribution of the infectious period could be the same for different populations, some individuals will recover slower than others just by chance

and could therefore produce more secondary cases. In this situation, stochastic models are more appropriate as they can incorporate these probabilistic or random effects (Fig 2: *right*) (Britton 2010).

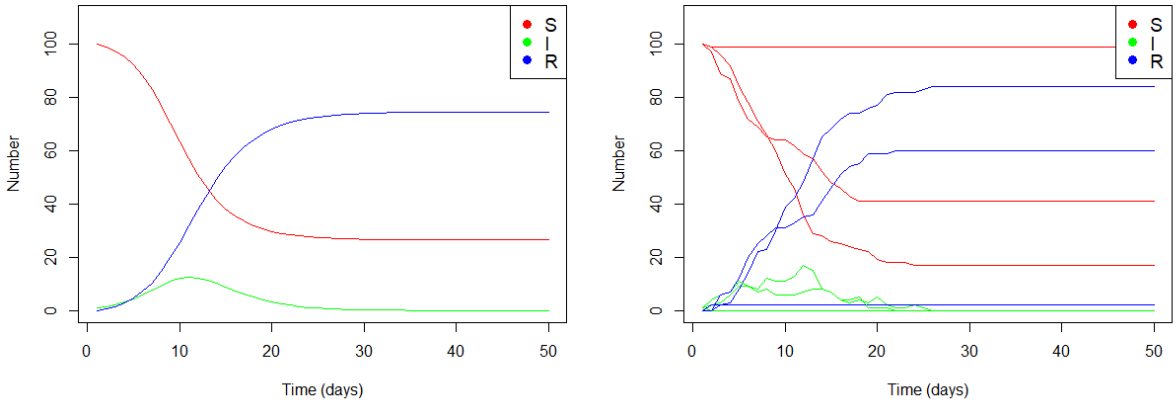

Fig 2: Typical SIR dynamics following the introduction of an infected individual in a completely susceptible population (n=100) with β=3.6 and γ=2. *Left*: Simulation of a deterministic model. *Right*: Three simulations of a discrete time stochastic model using the same parameters and starting values as the deterministic model.

2. Arenaviridae

The *Arenaviridae* are a family of segmented negative-strand RNA viruses of which several members can cause severe disease in humans. Currently, the family comprises 43 species that are divided into three genera: *Mammarenavirus*, *Reptarenavirus*, and *Hartmanivirus* (Radoshitzky et al. 2015; Maes et al. 2018). As indicated by the names, the genus *Mammarenavirus* encompasses those viruses that infect mammals, whereas the genera *Reptarenavirus* and *Hartmanivirus* encompass viruses that infect reptiles. Based on their antigenic properties, the genus *Mammarenavirus* is further sub-divided into the Old World and New World serocomplexes. All arenaviruses belonging to the Old World complex have a member of the rodent family *Muridae* as their reservoir host, whereas the New World arenaviruses circulate in rodents of the family *Cricetidae*, except for Tacaribe virus that has been isolated from bats (Günther and Lenz 2004). The fact that most mammarenaviruses have been found in only one rodent species or even sub-species, together with phylogenetic studies in both viruses and hosts, suggests apparent co-divergence between them (Zapata and Salvato 2013; Gryseels et al. 2017). However, as some arenaviruses (e.g. Lassa virus) have been found in multiple unrelated host species, the degree of host specificity may vary between arenaviruses (Gryseels et al. 2017; Olayemi et al. 2018).

All arenavirus share some common characteristics. They have a pleomorphic body with a diameter between 60 and 350nm. The presence of ribosomes (20-25nm) within this body resembles sand-like particles in electron microscopy slides, hence the family's name ('arena' means sand in Latin) (Carstens 2012). The genome of arenaviruses consists of two independent RNA-segments: a short (S) and long (L) fragment (Fig 3; Günther and Lenz 2004). The S-RNA fragment (~3400 nucleotides) codes for the viral glycoprotein precursor (recognition and binding to host membrane) and the nucleoprotein (RNA replication and transcription). The L-RNA fragment (~7300 nucleotides) codes for the RNA-dependent RNA-polymerase (RNA replication) and a small zinc-binding Z-protein (RNA synthesis and viral budding) (Rossi and Jenik 1996). A last important common feature is the presence of a viral envelope, which protects the genome against the extracellular environment and interacts with the host receptor cells (Vennemal et al. 1996).

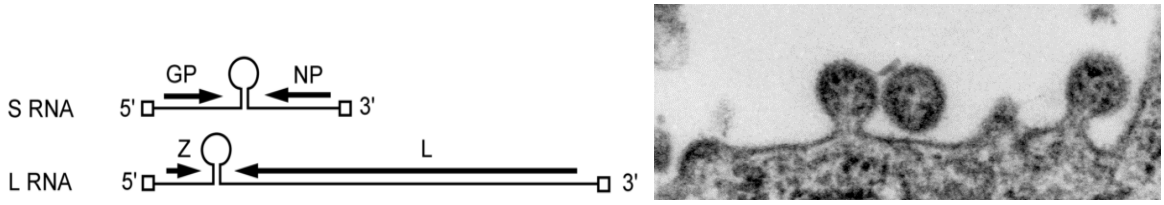

Fig 3: *Left*: genome organization of an arenavirus (GP=glycoprotein precursor, NP= nucleoprotein, Z= zinc-binding Z-protein, L= RNA-dependent RNA polymerase). Right: electron microscope image of arenaviruses budding from a host cell membrane. Both figures were derived from Günther and Lenz 2004.

2.1 Lymphocytic Choriomeningitis Virus: the lab model

Lymphocytic Choriomeningitis Virus (LCMV) is the family prototype of the *Arenaviridae* (Carstens 2012). Although I do not directly investigate this virus in my thesis, I will briefly discuss it as it is the best-studied arenavirus (probably one of the best-studied viruses in general) and I will often refer to it in the following chapters. LCMV was discovered serendipitously in 1933 from isolation of human material obtained during an epidemic of encephalitis in St. Louis, USA (Bredeck 1933; Amstroing and Lillie 1934). Not long after, LCMV was detected in a colony of laboratory mice and a series of inoculation experiments was set up to unravel the virus' biology and pathogenesis (Traub 1936a, b, c). The first interesting result was that inoculation of neonatal mice caused persistent infections, whereas inoculation of adults caused acute infections. The second was that transmission of LCMV between lab mice occurred via both direct (horizontal and vertical) and indirect transmission routes. During the following eighty years, the study of LCMV in laboratory mice and rats led to several key discoveries in

the field of viral immunology such as: Major Histocompatibility Complex (MHC) restriction, T-cell memory, T-cell exhaustion or the role of immune pathology in diseases (Oldstone 2002; Zhou et al. 2012). LCMV is also known for its ability to cause meningitis in humans and spontaneous abortions in pregnant woman, although numbers of case reports are generally low and infections often remain asymptomatic (Barton et al. 2002; Bonthius 2012). Humans mostly become infected by direct or indirect contact with the reservoir rodent host (*Mus musculus*) in wild or laboratory environments, or by organ transplantation (Fischer et al. 2006; Jamieson et al. 2006).

2.2 Lassa virus: the agent of Lassa fever

Lassa virus (LASV) is a West African arenavirus that causes severe hemorrhagic Lassa fever in humans (Buckley et al. 1970; McCormick JB 1999). Both virus and disease are named after a town in Nigeria where the first human case was recorded in 1969 (Carey et al. 1972). Currently, it is assumed that LASV is endemic in Nigeria and the countries of the Mano River Union (Sierra Leone, Guinea and Liberia), although some isolated cases have also been observed in the neighbouring countries (Ivory Coast, Ghana, Togo and Benin) (Fichet-Calvet and Rogers 2009; Patassi et al. 2017; Whitmer et al. 2018). An often-cited report of 1987 concluded that between 200.000 and 300.000 infections occur each year, with a fatality rate of 1-2% (Mccormick et al. 1987). However, the current situation might be completely different and more recent data suggest a substantial increase in the incidence rate, exemplified by the unprecedented outbreak in Nigeria in 2017-2018 (Safronetz et al. 2010; Kouadio et al. 2015; Roberts 2018; Fichet-Calvet et al. unpublished results). The increased incidence can be partly explained by the availability of better diagnostic tools, especially after the 2014-2016 Ebola epidemic in this region, but also by the extreme changes in land use, climate and human mobility during the past three decades (Redding et al. 2016; Gibb et al. 2017; Awosanya 2018). Because there is no human vaccine or efficient drug yet, rodent control and adjusting human behaviour are currently considered to be the only options for LASV prevention (Fichet-Calvet et al. 2007). For this reason, LASV was recently added to the World Health Organization's list of priority pathogens of epidemic potential for which there are no, or insufficient, countermeasures (World Health Organisation, 2018).

For a long time *Mastomys natalensis* was assumed to be the sole reservoir host of LASV, but recent field studies suggest that other rodents may serve as reservoirs too (e.g. LASV RNA was found in *Mastomys erythroleucus* and *Hylomyscus pamfi* in Nigeria) (Olayemi et al. 2016, 2018). Nevertheless, because *M. natalensis* is most often found in proximity with humans (the

rodent typically comprises more than 90% of all species trapped indoor in rural villages in West Africa), we can assume that *M. natalensis* is the main reservoir leading to zoonotic infections (Fichet-Calvet et al. 2007, 2009). One possible reason why *M. natalensis* is so abundant inside houses in West Africa is that villagers store their harvest inside (often under the bed), which might attract *M. natalensis* and increase rodent-to-human contact (Fig 4) (Fichet-Calvet et al. 2007). Humans can become infected through ingestion of contaminated food or water, inhalation of aerosolized virus particles, or direct consumption of infected animals (Stephenson et al. 1984; McCormick JB 1999; Bonwitt et al. 2017). Human-to-human transmission is thought to be rare, although certain activities may increase the likelihood of infection, such as nosocomial exposure or close contact in the same household (Carey et al. 1972; Kernéis et al. 2009; Lo Iacono et al. 2015). Similar as for LCMV, LASV transmission between rodents is assumed to occur via both direct (vertical and horizontal) and indirect transmission (Demby et al. 2001; Fichet-Calvet et al. 2014). In addition, LASV is suggested to be a reversed zoonosis (Fichet-Calvet E. 2014). This hypothesis states that humans become vectors by excreting the virus in urine and saliva on the ground, infecting rodents through contact with the contaminated soil. Although this hypothesis may sound negligible in explaining the epidemiology of the disease, I will show in chapter 7 that this transmission route may have important implications when developing LASV control strategies.

Fig 4: *Left*: Typical rural house in West Africa (Tambaya, Guinea). Villagers dry their harvest in front of the house before storing it inside, which attracts *M. natalensis* (picture made by Mariën J.). *Right*: QR-code with a link to three camera trap movies that show some typical places where *M. natalensis* occurs in rural houses (Faranah province, Guinea) and might infect humans with LASV: on the floor, between kitchen material or on the roof. You can also follow this link:

https://www.youtube.com/watch?v=XkxcjGRToyI&list=UUJIVHc8L8x_ixHzU7RHDpSw&index=1

2.3 Morogoro virus: The field model

Morogoro virus (MORV) is an arenavirus that has been discovered in Tanzania and can be regarded as a strain of Mopeia virus (Günther et al. 2009; Maes et al. 2018). The virus is genetically closely related to LASV and has the same reservoir host, but while LASV is limited to the West African A-I and A-II lineages of *M. natalensis*, MORV only occurs in the B-V lineage (S. Gryseels 2015, PhD thesis; Olayemi et al. 2016b). Because transmission of MORV to other sympatric rodent species, or even subspecific clades of *M. natalensis*, has never been observed, *M. natalensis* B-V is currently assumed to be the only suitable host for this virus (de Bellocq et al. 2010; Gryseels et al. 2017). MORV seems to be endemic in highly fluctuating populations of this host and can be detected even at very low densities (Borremans et al. 2011). Depending on the study, prevalence ranges between 0 and 25% in Tanzania, whereas seroprevalence peaks were observed up to 70% (Günther et al. 2009; de Bellocq et al. 2010; Marien et al. unpublished data). Given that the virus is not pathogenic for humans and the population ecology of the host is well known, the MORV-*M. natalensis* system is considered a suitable model for examining parasite-host interactions in natural conditions. The system is especially considered a safe substitute for studying closely related but pathogenic arenaviruses such as LASV or LCMV. For these reasons, the transmission ecology of MORV has been extensively investigated the last decade, although several important aspects remain to be elucidated for a thorough understanding (Borremans et al. 2011, 2015; Gryseels et al. 2017).

3. *Mastomys natalensis*: the reservoir host

The Natal multimammate mouse *Mastomys natalensis* is a widely distributed rodent (family *Muridae, subfamily Murinae*) in sub-Saharan Africa (Kingdon et al. 2013). The animal thanks its name to the two rows of 8-12 mammae that females possess, which correspond to the litter size (sometimes > 20 pups) when giving birth (Leirs 1994). Its natural habitat consists of savannah and grassland areas, but the animal currently thrives in agricultural fields and human dwellings (Coetzee 1975). In agriculture, *M. natalensis* is considered to be the most important pest species from Africa, as outbreaks can cause crop losses up to 80% at both the household and regional level (Mwanjabe et al. 2002; Mulungu 2017). The rodent is also notoriously known for hosting several human diseases, including *Yersinia pestis* (bubonic plague), *Leptospira interrogans* (leptospirosis) and LASV (Lassa fever) (Monath 1987; Holt et al. 2006; Neerinckx et al. 2008; Meerburg et al. 2009). Besides MORV and LASV, *M. natalensis* is the reservoir

host of four other known arenaviruses: a Mobala-like virus in West Africa and Mopeia, Gairo and Luna viruses in Southern East Africa (S. Gryseels, 2015, PhD thesis).

3.1 Ecology in Tanzania

Because of its relevance in agriculture and public health, the ecology of *M. natalensis* has been extensively studied in Tanzania over the past 30 years (Telford 1989, Leirs 1994; Sluydts et al. 2009; Singleton et al. 2010; Mulungu 2017). The population dynamics of the rodent are heavily depending on rainfall, which is bimodal in this region with long (March-May) and short (November-December) rains. The variation in rainfall results in strong density fluctuations between seasons, generally ranging from 20-300 individuals per hectare (Leirs H. 1994; Sluydts et al. 2009). Breeding of *M. natalensis* is triggered at the end of the long rains by sprouting young grass and lasts until November, when the population size peaks (Leirs et al. 1990; Mlyashimbi et al. 2018). Shortly hereafter, the population decreases due to competition, food deprivation and other environmental factors, to reach its lowest point around May (Leirs et al. 1990). Occasional outbreak densities can be reached after heavy rainfall at the start of the new year, as this stimulates growth and fecundity of new recruits. The result is that mice from two generations can breed during the same season, leading to outbreak densities up to 1000 individuals per hectare at some years (Leirs et al. 1990). Other interesting aspects of the rodent are its promiscuous behavior and complete lack of territoriality which is rarely seen in small mammals (Veenstra 1958; Kennis et al. 2008; Borremans et al. 2013). Because home range overlap is generally high and increases significantly with density, contact rates are assumed to be density-dependent, probably nonlinearly (Borremans et al. 2013; 2016; 2017). The home range size is generally assumed to be small, although estimations of its absolute size depend on the used measuring technique (e.g. $\pm 650 m^2$ by capture-mark-recapture and $\pm 1200 m^2$ by radio-tracking methods) (Leirs et al. 1997; Borremans et al. 2013).

3.2 Ecology in Guinea

In contrast to the rodent populations of East Africa, populations of West Africa are poorly studied (Swanepoel et al. 2017). Some information can be extrapolated, but the few studies that have been conducted (mostly in Guinea) revealed important differences. For example, while *Rattus* species comprise the largest part of the indoor rodent population in Tanzania, *M. natalensis* is the main rodent found in rural houses in Guinea. This may be because *Rattus* scares away *M. natalensis* from houses and is simply less common in Guinea, leaving space for more opportunistic species (Demby et al. 2001; Monadjem et al. 2011). Another difference is that population densities of *M. natalensis* are generally lower in Guinea than in Tanzania, which

can be explained by the absence of large agricultural fields in West Africa, as smaller fields lack the food supply to sustain high rodent densities (Fichet-Calvet 2014). Nevertheless, as in Tanzania, *M. natalensis* populations show clear seasonal density fluctuations. While the rodent is as numerous inside as outside houses during the rainy season, its density increases significantly inside but decreases outside houses during the dry season (Fichet-Calvet et al. 2007). This pattern can be explained by a change in habitat preference driven by fluctuations in food availability. During the dry season, fields are left fallow and rodents might be attracted by the harvest that is stored inside the houses. This supposed change in habitat preference might explain the increased incidence in human Lassa fever cases during the dry season, which is noted by doctors working in local hospitals (Mccormick et al. 1987; Bausch et al. 2001; Asogun et al. 2012).

4. Thesis objectives and outline

Although the transmission mechanisms of arenaviruses have been extensively investigated in laboratory conditions (especially for LCMV), only a few studies tested fundamental ecological questions related to the transmission dynamics in natural conditions. Using a combination of long-term field data, field/lab experiments and mathematical modelling, my thesis aims to provide an answer to some of these questions.

Before embarking on my PhD project in 2014, it was assumed that MORV could not locally persist in highly fluctuating populations of *M. natalensis*, because transmission was thought to be density-dependent, the infection predominantly acute and the immune response lifelong (Borremans et al. 2011; Goyens et al. 2013). However, as MORV-RNA and antibodies were detected in a long-time capture-mark-recapture time series on a continuous basis (10 years), it seemed that MORV can nevertheless persist in these fluctuating populations. The result triggered me to investigate how this was possible and led to the first objective of this thesis: **exploring the mechanisms that allow an arenavirus to persist in fluctuating populations of its reservoir host** (Fig 5). We developed nine not mutually exclusive hypotheses (H1 to H9) that can explain how MORV can persist in fluctuating populations of *M. natalensis*. Although I was not able to test all the proposed hypotheses during my PhD, I will provide them all here. The hypotheses highlighted in bold are the ones that I specifically tested during my PhD.

MORV can persist through the yearly population bottlenecks of *M. natalensis* because

H1: the virus causes no or only limited adverse effects on the reservoir host.

H2: a subset of hosts become chronically infectious. These animals can continue the transmission chain when host densities become too low for viral transmission.

H3: the virus can survive for a longtime outside of the rodent host, which can become infected indirectly.

H4: the virus might manipulate the host's behaviour in order to increase its contact rate when host densities are low.

H5: sexual contacts during the low-density season (coinciding with the breeding season) increase the overall contact rate between hosts and may facilitate viral transmission between hosts.

H6: the virus can not only be transmitted horizontally but also vertically. Vertical transmission is known to contribute to the intergenerational maintenance of a parasite and might prevent extinction during the low-density (breeding) season.

H7: maternal antibodies may temporally reduce the transmission rate during high host density periods. Proportionally fewer individuals will be immune during the subsequent low density periods, which facilitates transmission because more individuals can become infected.

H8: contact rates of *M. natalensis* scale non-linearly with density. Contact rates between hosts remain higher than expected at low densities. Therefore, the transmission chain will not be broken due to insufficient contacts between hosts at low densities.

H9: viral reinvasion occurs through metapopulation dynamics.

The second objective of this PhD was to develop an ecological disease model that could predict which **rodent control strategies are effective to control LASV in Guinea**. The model was developed using information derived from the MORV-system (the previous objective) and additional field experiments performed in Lassa fever endemic villages in Guinea (Fig 5). MORV is considered a safe and appropriate alternative to study certain aspects of LASV ecology because the two viruses are genetically very similar (e.g. 62% nucleotide similarity for L-gene) and both have *M. natalensis* as their reservoir host. Three hypothesis (H10-H12) were tested to meet the second objective.

H10: *M. natalensis* moves easily between houses and proximate cultivations in rural villages in Guinea. Rodent control should therefore be performed inside and outside houses to be effective.

H11: Rodent control can reduce LASV transmission in the rodent population.

H12: Rodent control is a sustainable approach to eliminate LASV from the rodent population and to reduce spillover risk to humans in rural villages in Guinea.

The outline of this thesis is as follows (Fig 5). Chapters 2 and 3 focus on the hypothesis that arenaviruses have no or only limited adverse effects on their reservoir hosts. Chapter 4 compares arenavirus infection patterns between experimentally and naturally infected rodents. Here, we explore if laboratory data are useful for interpreting field samples or that important differences can occur due to conditions that were not yet tested in the lab. Chapter 5 analyses the transmission dynamics of MORV in highly fluctuating populations of *M. natalensis* based on long-term field data and a mathematical model. In this chapter, we also investigate why chronic infections are so important for arenavirus persistence in rodent populations. Chapter 6 provides basic ecological data on movement behaviour of *M. natalensis* in Lassa fever-endemic villages in Guinea. The data obtained in this field study were used to parameterise the mathematical models explained in the next chapter (e.g. information on action radius size and rodent density). Chapter 7 describes the mathematical model developed to simulate LASV transmission in rodent populations in Guinea. The model is mostly based on information obtained from the previous chapters and a rodent control experiment performed in Guinea (by Fichet-Calvet et al). Finally, chapter 8 provides an overall discussion and conclusions about the main results of this thesis.

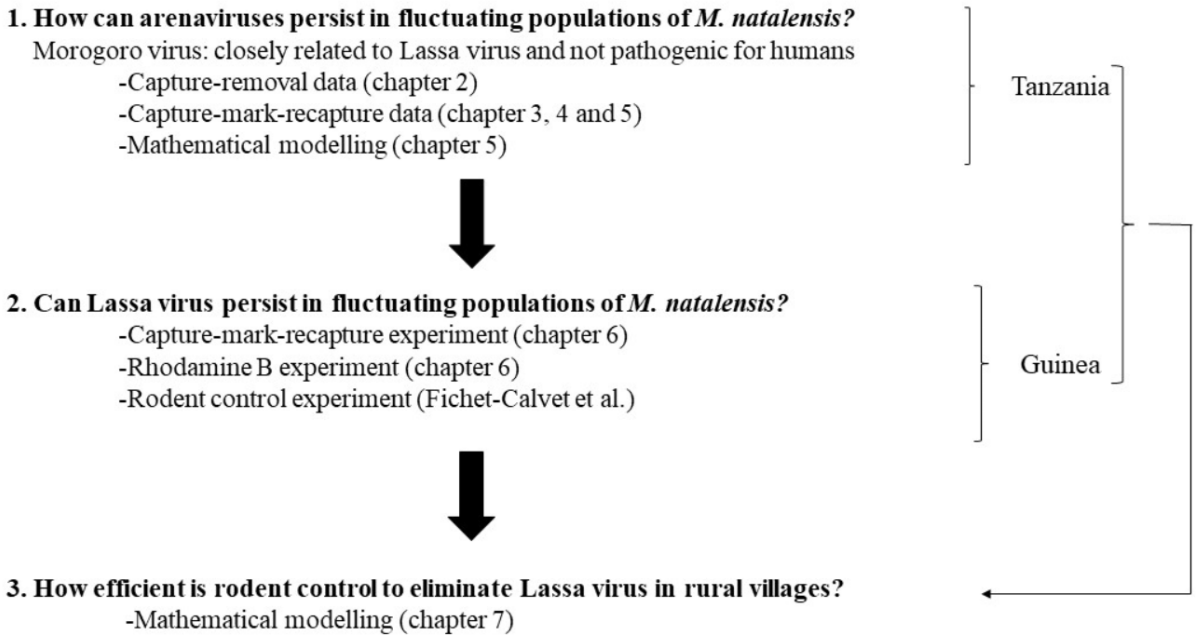

Fig 5: a schematic representation of the thesis outline.

No measurable adverse effects of Lassa, Morogoro and Gairo arenaviruses on their rodent reservoir host in natural conditions

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Abstract

***Background*:** In order to optimize net transmission success, parasites are hypothesized to evolve towards causing minimal damage to their reservoir host while obtaining high shedding rates. For many parasite species however this paradigm has not been tested, and conflicting results have been found regarding the effect of arenaviruses on their rodent host species. The rodent *Mastomys natalensis* is the natural reservoir host of several arenaviruses, including Lassa virus that is known to cause Lassa haemorrhagic fever in humans. Here, we examined the effect of three arenaviruses (Gairo Morogoro and Lassa virus) on four parameters of wild-caught *Mastomys natalensis*: body mass, head-body length, sexual maturity and fertility. After correcting for the effect of age, we compared these parameters between arenavirus-positive (arenavirus RNA or antibody) and negative animals using data from different field studies in Guinea (Lassa virus) and Tanzania (Morogoro and Gairo viruses).

***Results*:** Although the sample sizes of our studies (1297, 749 and 259 animals respectively) were large enough to statistically detect small differences in body conditions, we did not observe any adverse effects of these viruses on *Mastomys natalensis*. We did find that sexual maturity was significantly positively related with Lassa virus antibody presence until a certain age, and with Gairo virus antibody presence in general. Gairo virus antibody-positive animals were also significantly heavier and larger than antibody-free animals.

Conclusion: Together, these results suggest that the pathogenicity of arenaviruses is not severe in *M. natalensis*. This is a plausible result given that adaptations of viruses towards low virulence might optimize their transmission success. They also suggest that sexual behaviour might increase the probability of *M. natalensis* to become infected with arenaviruses.

Introduction

The Natal multimammate mouse, *Mastomys natalensis* (Smith, 1834), is the natural host of six known Old World arenaviruses (Arenaviridae, *Mammarenavirus*): Lassa (LASV) and a Mobala-like virus in West Africa, and Morogoro (MORV), Mopeia, Luna and Gairo viruses (GAIV) in Southern East Africa (Monath et al.1974; Wulff et al. 1977; Günther et al. 2009; Ishii et al. 2011; Gryseels et al. 2015; Gryseels et al. 2016; Olayemi et al. 2016). Of these six viruses, only LASV is known to cause Lassa haemorrhagic fever in humans, who can become infected by close contact with infectious rodents or their excretions (Monath et al. 1974).

Annually around 200,000 people are affected, with a fatality rate of 1-2% (Monath 1987; McCormick JB 1999; World Health Organization 2016).

While the pathogenic effects of LASV on humans are well-documented (Walker et al. 1987; Günther and Lenz 2004), little attention has been paid to the effects on its reservoir host. Nevertheless, knowledge of how parasites affect their reservoir hosts is relevant in the study of wildlife diseases, as this helps to understand how host population dynamics or behaviour affect the spread of the parasite through the population (Anderson and May 1979). In general, in order to optimize net transmission success, parasites face a trade-off between within-host reproduction, which requires the utilisation of host resources, and between-host transmission probability, which requires the hosts to be sufficiently healthy and long-lived to encounter new susceptible individuals (Anderson and May 1982; Frank 1996; Alizon et al. 2009). Usually, a long evolutionary history in a particular host results in low virulence towards the natural reservoir (Knell 2004; Dwyer et al. 2007).

Most information about arenavirus pathogenicity is derived from inoculation studies on laboratory house mice (*Mus musculus*) with lymphocytic choriomeningitis virus (LCMV), an Old World arenavirus that shares 60% amino acid similarity with LASV (Kerber et al. 2011). House mice are the reservoir hosts of LCMV and most LCMV strains result in symptom-free infection, despite high levels of viral growth (Childs et al. 1993; Oldstone 2002). Nevertheless, severe disease (including meningitis, reduced growth and death) did develop for some mouse breed - LCMV strain combinations (Hotchin and Cinits 1958; Oldstone and Dixon 1968; Mims 1970; Oldstone et al. 1982). Variation in pathogenicity was also noted in vesper mice (*Calomys callosus* and *Calomys musculinus*.) which are the natural hosts of respectively New World Machupo and Junin arenaviruses. Inoculation of *C. callosus* with Machupo virus resulted in a dual response in which about half of the infected animals became immunocompetent with transient viremia and no disease symptoms, whereas the other half became immunotolerant and exhibited persistent viremia and reduced body size, fertility and lifespan (Webb et al. 1975). Inoculation with Junin virus caused a similar response in *C. musculinus,* where adults remained asymptomatic while juveniles experienced reduced body size, fertility and lifespan (Vitullo et al. 1987; Vitullo and Merani 1990).

Few studies have examined the effects of arenaviruses in *M. natalensis*. Two inoculation studies, on LASV and MORV, showed no or only mild overt disease symptoms in their rodent host (Walker et al. 1975; Borremans et al. 2015). MORV inoculations in adult wild type *M. natalensis* caused significant temporary 7% weight loss 10 days post infection in about half of

the animals. Although these animals recovered completely in the laboratory, such weight loss in natural circumstances might have significant consequences for survival probability. In contrast to MORV inoculation studies, two field studies found that LASV reduces growth and fecundity of *M. natalensis*, and suggest that LASV severely affects its rodent host (Demartini et al. 1975; Lalis et al. 2015). These studies should however be interpreted with caution, as the first provided limited statistical proof, while the latter used body mass category as an estimate to correct for an age effect on prevalence, which is a potentially unreliable proxy (Leirs et al. 1990b).

Knowledge of the exact age is essential to estimate pathogenicity effects, since age correlates with many morphometric and sexual parameters. More importantly, in case of acute infections with long-term presence of antibodies (observed for LASV-related arenaviruses in *M. natalensis* (Demby et al. 2001; Borremans et al. 2011; Gryseels et al. 2015), older animals are more likely to have already encountered the infection, and developed antibodies, during their lifetime simply due to the longer time window they have been alive. In this situation, the probability of detecting active, recent infection decreases with age, as more susceptible (antibody-negative) animals are available in the younger age categories compared to the older categories (Borremans et al. 2011). Therefore, if one wants to investigate the effect of infection on an age-dependent host variable (e.g. body mass, head-body length or sexual maturity), the effect of age should be disentangled from that of the variable (Kallio et al. 2015).

In this paper, we want to systematically analyse data from three different *M. natalensis*-borne arenaviruses in the same way, in order to confidently test whether infection affects the host in natural conditions. We will test the relationships between arenavirus (LASV, MORV and GAIV) infection and body mass, body length, sexual maturity and fertility. We correct for the effects of age by using eye lens weight (ELW), which is known to be a good proxy for age because the eye lens is continuously growing, independent of environmental factors (Lord 1959; Morris 1972; Leirs et al. 1990a; 1994; Augusteyn 2014). We previously investigated the relationships between LASV infection and several parameters such as village, habitat, season, age and abundance in Guinea (Fichet-Calvet et al. 2007; 2008; 2014). Using more recent data, we here expand this analysis to body condition and increase the dataset from 553 to 1297 individuals. For MORV, a previous investigation on its effect on *M. natalensis* body condition was performed on a small dataset (n = 171) during one season and locality only, where no significant relationship between MORV infection and body mass index was found, although a model including body mass index did result in the best prediction of active MORV infection

(Borremans et al. 2011). Here we supplement this dataset with new data in order to arrive at a sample size of 749 individuals. Our recent work on GAIV tested the relationship between infection and host condition (using infection status as dependent variable) (Gryseels et al. 2015), but here we re-analyse these data in exactly the same way as the other two viruses (using body condition as dependent variable), as this allows a direct comparison between the three viruses.

Methods

Guinean study sites, rodent sampling, and LASV screening of rodents

For the examination of the relationships between LASV and *M. natalensis* body condition, we re-analysed data from previous publications (Fichet-Calvet et al. 2007; Fichet-Calvet et al. 2008; Fichet-Calvet et al. 2014b; Fichet-Calvet et al. 2016) and also present new data. Here, we provide a summary of the data collection procedures, but refer to the mentioned publications for more details. Rodents were captured in thirteen villages with reported human LASV cases in Upper Guinea during an intermittent time span between 2003 and 2015. Traps were placed both indoors and outdoors during the rainy and dry season. Captured rodents were humanely killed and measured morphometrically. Sexual maturity of females was determined by pregnancy, lactation status and by signs of scars in a large uterus (width >4 mm). Body mass of pregnant females was adjusted for foetus + uterus mass. Fertility of pregnant females was determined by counting the number of foetuses inside the uterus. Males were considered sexually mature if the vesiculae seminales were swollen and their surface exceeded 100 mm^2. Eyes were preserved in 10% formalin, the lenses were extracted, cleaned and dried for 2 hours at 100° C, and then weighed to the nearest 0.1 mg. Whole blood, spleen, kidney, and liver were collected during the autopsies and stored in liquid nitrogen or in -20°C. Detection of LASV viral RNA (vRNA) was performed and examined by RT-PCR on blood and spleen of 1298 *M. natalensis* (Vieth et al. 2007; Olschläger et al. 2010). Detection of anti-LASV antibodies was performed on 1139 *M. natalensis* and examined by indirect immunofluorescence assay (Wulff and Lange 1975; Hufert et al. 1989). For this assay, Vero cells infected with LASV strain Bantou were spread on immunofluorescence slides, air dried, and acetone-fixed. Antibodies in positive samples would then bind to antigens presented by the Vero cells and be visualized with anti-mouse IgG-FITC secondary antibodies. In total, 168 (13%) LASV vRNA and 318 (28%) antibody positive individuals were detected.

Tanzanian study sites, rodent sampling, and MORV or GAIV screening of rodents

For the examination of the relationships between MORV or GAIV infection and *M. natalensis* body condition, we (re-)analysed data from previous publications (Borremans et al. 2011; Gryseels et al. 2015; Gryseels et al. 2016) and here present new antibody data from the nine localities where MORV circulates as described in (Gryseels et al. 2016). Rodents were trapped outdoors in the Morogoro region in Tanzania intermittently between 2008 and 2012. Captured rodents were humanely killed and identified morphometrically. Reproductive status was measured by investigating external characteristics of the reproductive organs, as described in (Leirs 1994). Eye lens weight (ELW) was measured using the method described for the LASV studies, except that they were dried for >2 hours at 80° C. Organs were preserved in RNAlater for viral detection, and blood on serobuvard filter paper (+/-15 µl/punch; Serobuvard, LDA 22, Zoopole, France) for antibody detection. In total, 743 and 259 *M. natalensis* were tested for respectively MORV and GAIV vRNA using RT-PCR reaction (Vieth et al. 2007; Günther et al. 2009), and 683 and 259 *M. natalensis* were tested for the presence of anti-MORV and anti-GAIV antibodies using indirect immunofluorescence assay. Vero cells infected with MORV strain 3017/2004 were spread on immunofluorescence slides, air dried, acetone-fixed, and visualised as described for LASV. For MORV and GAIV respectively, 48 (6%) and 29 (11%) animals tested vRNA positive, and 73 (11%) and 25 (9%) animals tested antibody positive (Borremans et al. 2011; Gryseels et al. 2015; 2016).

Statistical analysis

We tested the relationships between arenavirus infection and four different measures of *M. natalensis'* body condition: body mass, head-body (HB) length and sexual maturity for all viruses, and fertility for LASV only with linear mixed models and generalised linear mixed models. For all the investigated parameters it was important to disentangle the effect of age from that of the measured variable. For this reason, we developed models with one of the parameters of interest as dependent variable and ELW (proxy for age), infection status (RNA/antibody positive or negative) and their interaction as independent fixed variables. We also included sex, season (rainy/ dry) and their interaction with ELW and infection status as independent fixed variables, and year and village as independent random variables to correct for effects of year and location. We log-transformed body mass, HB length and ELW since we observed a linear relation between log(body mass) and log(HB length) on the one hand and log(ELW) on the other hand. The relationships to sexual maturity (i.e. the probability of being

sexually active, 1 = active, 0 = inactive) were assessed assuming a binomial distribution (logit-link function). The relationships to fertility (i.e. number of fetuses inside the uterus, only for sexually mature females) were assessed assuming a Poisson distribution (log-link function). We performed separate analyses for three different infection statuses: (1) only arenavirus RNA, (2) only antibody, and (3) either arenavirus RNA or antibody presence, or both.

In one of the 10 villages investigated in 2013 in Upper Guinea, no LASV was detected. We therefore compared the effect of infection in *M. natalensis* living in this LASV negative village (Tambaya, n = 50) to those living in the nearest LASV positive village (Brissa, n = 62). Season and year were removed from this model as these animals were collected in the same period, and LASV village (positive/negative) was implemented as an independent fixed variable.

We used the lmer and glmer functions of the lme4 package (version 1.1-7) of the R statistical software version 3.3.0 (R Core Team 2014) to run the mixed models. When fitting the models, we started with the fully parameterised models (all two-way interactions between the independent fixed variables) and sequentially dropped variables that had the highest insignificant p-values. Significance was tested with the likelihood ratio test.

Results

Because the effects of the parameters ELW, infection status and their interactions on the body condition variables (body mass, head-body length, sexual maturity and fertility) were of main interest in this study, we summarized these in table 1. The effects of other parameters (sex, season and their interaction) on the body condition variables, were presented in supplementary tables (S3 and S4).

The three body condition predictor variables (body mass, head-body length and sexual maturity) are all highly correlated with age in the three investigated populations of *M. natalensis* ($\chi_1^2 > 25.90$, $p < 0.01$) (Figures 1, 2 and 3). As reported previously, presence of either vRNA or antibody is also correlated significantly with age for all three viruses ($\chi_1^2 > 4.75$, $p < 0.03$) (Supplementary Table 1).

Correcting for this general age effect, we observed almost no effect of either active or past arenavirus infection on body condition (Table 1).

For none of the investigated viruses, presence of vRNA was significantly related to any of the predictor variables, nor were any of the interactions between vRNA presence and ELW significantly related to any of the predictor variables (Table 1).

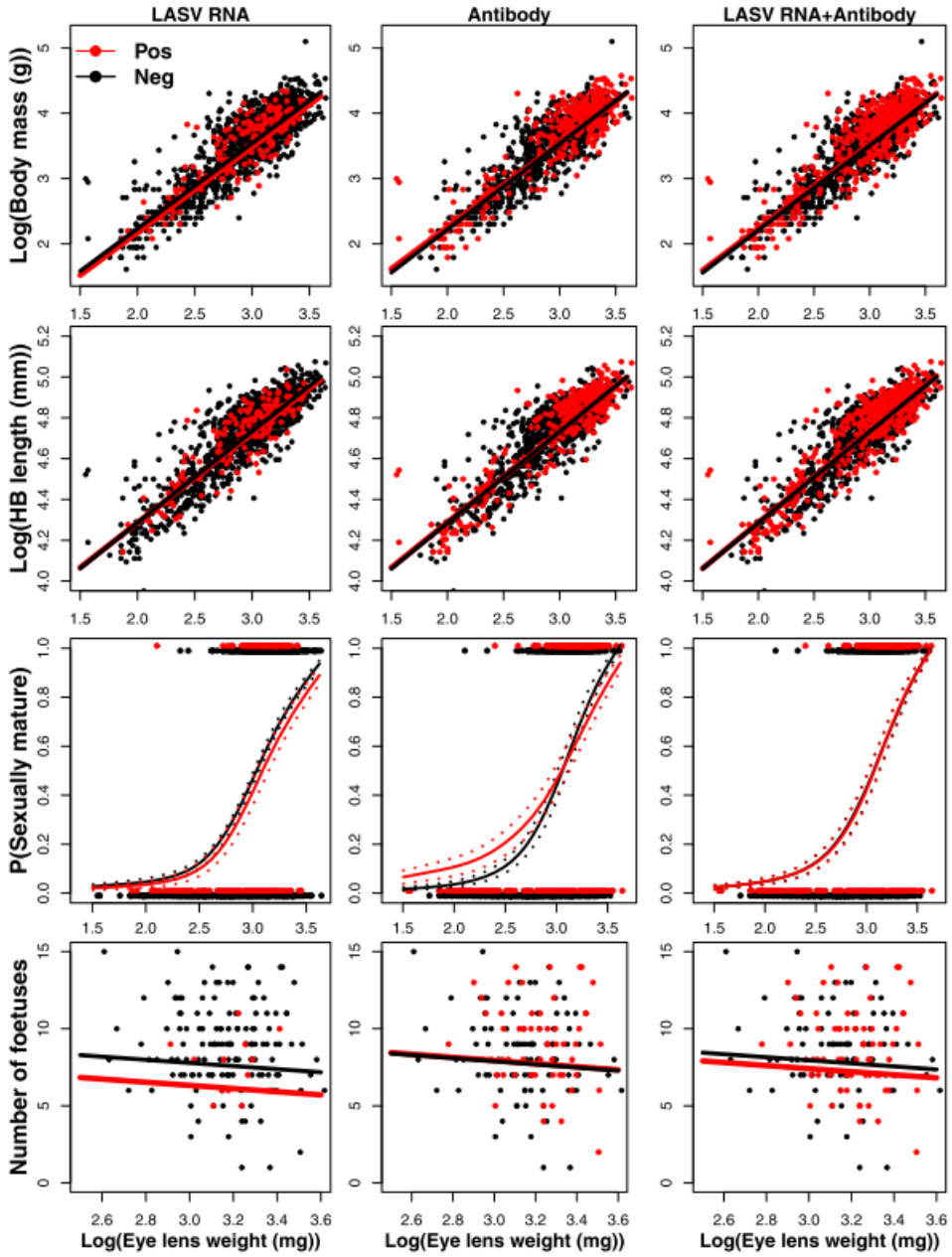

Figure 1: Correlations between body mass, head-body length, sexual maturity (1 = active and 0 = inactive) and fertility (# foetuses) and Eye lens weight (as a proxy for age) in *M. natalensis* infected by Lassa virus. Red = positive, black = negative. Dashed lines represent standard errors on the predicted probabilities.

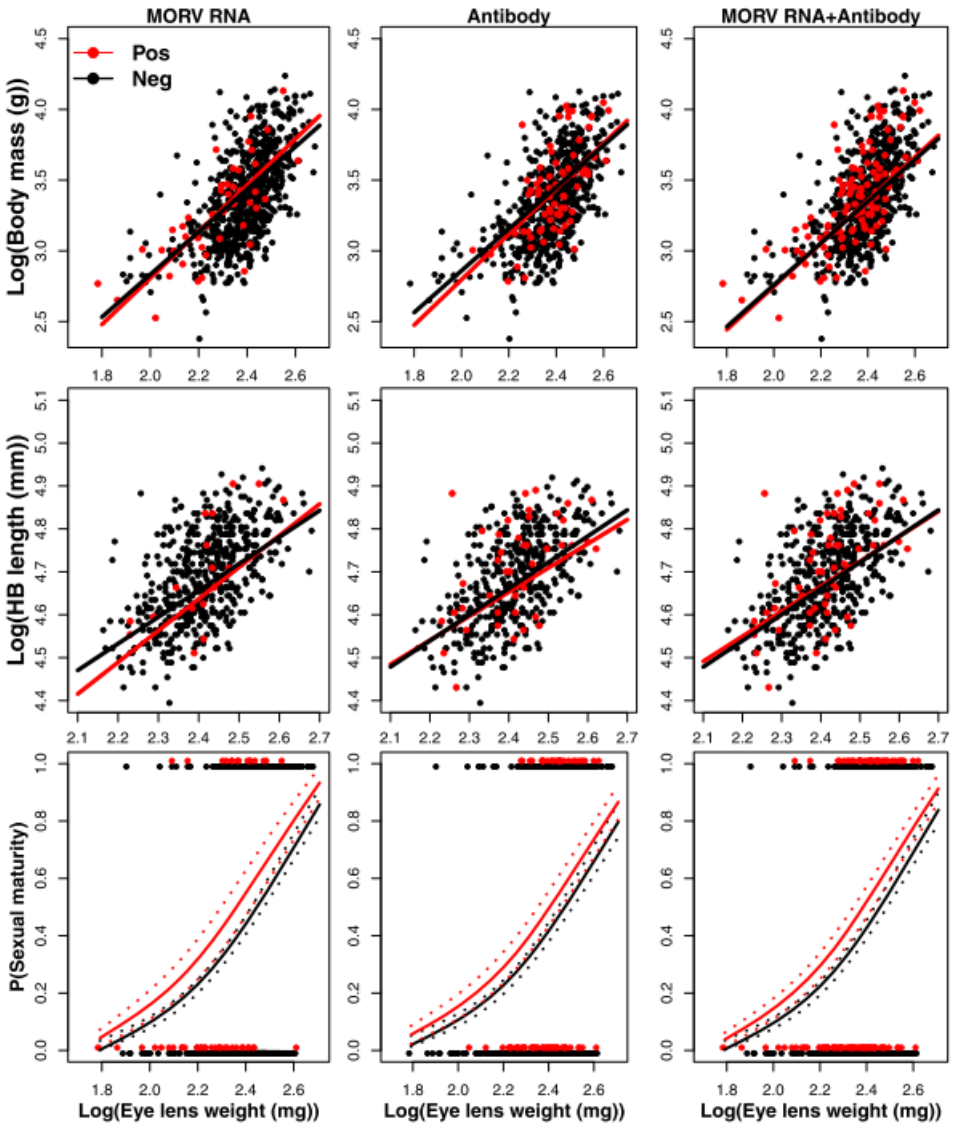

Figure 2: Correlations between body mass, head-body length, sexual maturity (1 = active and 0 = inactive) and Eye lens weight (as a proxy for age) in *M. natalensis* infected by Morogoro virus. Red = positive, black = negative. Dashed lines represent standard errors on the predicted probabilities.

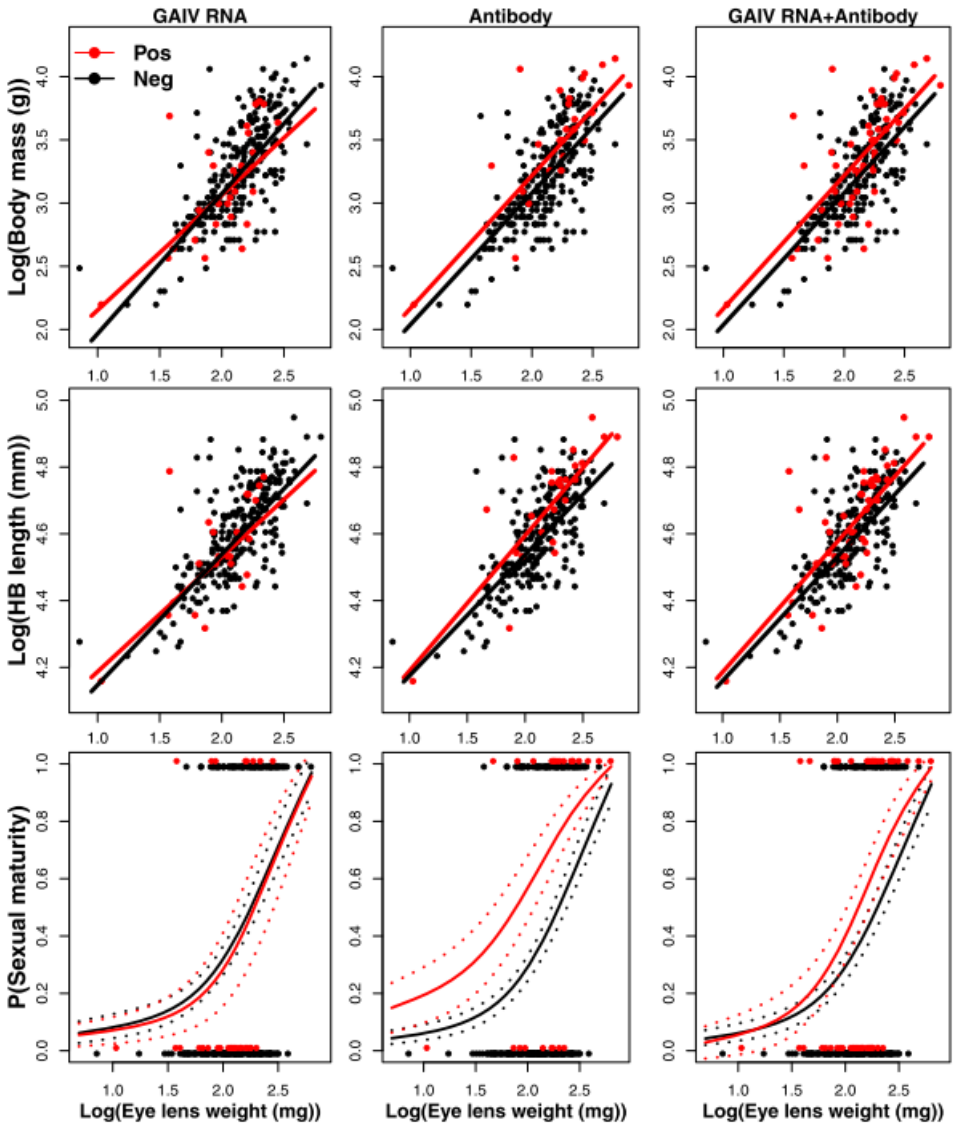

Figure 3: Correlations between body mass, head-body length, sexual maturity (1 = active and 0 = inactive) and Eye lens weight (as a proxy for age) in *M. natalensis* infected by Gairo virus. Red = positive, black = negative. Dashed lines represent standard errors on the predicted probabilities.

Antibody presence was not significantly related to any of the predictor variables for MORV. For GAIV, we found significant relationships between antibody presence and all three body condition predictor variables (Table 1, Fig. 3): antibody-positive *M. natalensis* were significantly heavier (χ_1^2 = 13.28, p<0.01) and larger (χ_1^2 = 8.45, p<0.01) than antibody negative ones, and had a significantly higher probability of being sexually mature (χ_1^2 = 8.57, p<0.01). For LASV, antibody presence alone was not significantly related to body mass and HB length. The relationship between LASV antibodies and sexual maturity could not be assessed due to a significant interaction between antibody and ELW (χ_1^2 = 5.42, p = 0.02), in which young antibody-positive *M. natalensis* had a higher probability of being sexually mature than

antibody-negative animals (Fig. 1). This difference disappeared for older animals where more animals are sexually mature (χ_1^2 = 1.86, p = 0.06). Interactions between antibody presence and ELW had no significant effect on any of the body condition variables in the case of MORV and GAIV.

The presence of either vRNA, antibody or both (indicative of successful infection at any point in the past) was not significantly related to any of the predictor variables for MORV and LASV, nor was the interaction between this definition of infection status and ELW significantly related to any of the predictor variables. For GAIV, there were significant relationships between the infection status and all three body condition predictor variables: past or recent infected animals where significantly heavier (χ_1^2 = 8.60, p<0.01) and larger (χ_1^2 = 6.23, p = 0.01), and had a higher probability of being sexually mature (χ_1^2 = 6.14, p = 0.01) than animals that were never infected (Table 1, Fig. 3).

We could analyse the relationship between infection and fertility for the LASV dataset only. Fertility in females as measured by the number of foetuses was independent of age (χ_1^2 = 1.84, p = 0.17), and no significant difference in number of foetuses was observed between LASV RNA/antibody-positive and negative pregnant females (χ_1^2 < 2.14, p > 0.14) (Table 1). For LASV, we could also compare a LASV-negative village (Tambaya) with its nearest LASV-positive village (Brissa). We did not find any significant differences in body mass, head-body length or sexual maturity between *M. natalensis* in the two villages (χ_1^2 < 2.62, p > 0.09) (Supplementary data: Table 2 and Figure 1).

Discussion

We did not observe any significant negative associations between arenavirus infection and *M. natalensis* morphometric or sexual parameters, suggesting that these viruses are harmless for the reservoir hosts in natural conditions. Our results are in contrast with the studies of Demartini *et al.* (1975) and Lalis *et al.*(2015). Demartini *et al.* (1975) found that LASV infections resulted in less heavy animals and inflammatory lesions. As these authors noted, important limitations of their study were the small sample size (N=28) and lack of information about the animals' age. While Lalis *et al.* (2015) also found no association between LASV presence and host body mass, they did find that LASV positive animals had a significantly smaller body size, skull and size of the reproductive organs in the adult category. Their adult category based on the body mass (>40 g) was highly variable and can cover a range of 40-97 g (ELW = 15-38 mg in fig 5 in EFC et al. 2008). Among this body weight class, 73% (47/64) of the LASV positive animals were belonging to the youngest ELW class (15-25 mg). We can suggest that their 30 LASV

positive adult samples were possibly younger, smaller and more inactive sexually than their 38 LASV negative ones.

Our results are consistent with the laboratory inoculation studies of Walker *et al.* (1975) and Borremans *et al.* (2015), who observed no or only mild disease symptoms of arenaviruses in *M. natalensis*. Walker *et al.* (1975) showed that neonatal animals inoculated with LASV exhibited a persistent tolerant infection with no histopathological signs of disease. Nevertheless, some animals infected as adults developed moderate (not deadly) meningoencephalitis. Borremans *et al.* (2015) found that inoculation of *M. natalensis* with MORV could decrease the weight of animals between days 7 and 15 post-infection (±7% of normal weight in 40 % of animals), but this decrease was only temporary and did not further affect the animals' growth rates.

It should be noted that we did not provide proof that these viruses never affect their reservoir host. For example, our data does not exclude that some animals become sick, die quickly from disease and thus are not trapped during field studies. Also, we only checked four host parameters, while viruses might affect their host in many different ways (e.g depending on the life history traits, differences in hormone levels, brain size or temperature may be found) (Oldstone 2002). Such complex relationships between parasite infection and host fitness were indeed observed in other rodent-borne diseases. For example, infection of Puumala hantavirus in bank voles (*Myodes glareolus*) was initially assumed to be asymptomatic (Verhagen et al. 1986; Bernshtein et al. 1999; Kallio et al. 2006), but recent long-term field studies observed negative effects on the survival probability and an equivocal impact on the fecundity of females (Kallio et al. 2011; Tersago et al. 2012a; Kallio et al. 2015). An important difference between Old World arenaviruses and hantaviruses, however, is that the latter causes persistent infections in their reservoir hosts (Voutilainen et al. 2015), while the former infects their hosts mainly acutely (Fichet-Calvet et al. 2014; Borremans et al. 2015). The lower survival probability caused by hantaviruses could therefore perhaps be explained by an accumulation of small deleterious effects over time, which might not be the case during the relatively short arenavirus infections. Nevertheless, performing survival analyses would be a logical next step to further explore possible effects of arenaviruses in *M. natalensis*, and could be done by investigating long-term capture-mark-recapture data.

In general, our study supports the hypothesis that the pathogenicity of arenaviruses is low in their natural hosts. This is likely an adaptation of these viruses to increase the probability of transmitting the infection to other individuals. In contrast, highly pathogenic viruses might fail

in transmission because they reduce the host's infectious period, or reduce its contact rate as a trade-off for energy to eradicate the virus (Anderson and May 1979; Barber and Dingemanse 2010). A non-pathogenic lifestyle would be especially beneficial for MORV, since MORV is endemic in populations of *M. natalensis* that fluctuate heavily between seasons, generally ranging from 20-300 individuals per hectare (Leirs 1994; Sluydts et al. 2007; Günther et al. 2009). Mathematical models suggest that these fluctuations impose a severe impediment for MORV persistence, because during the low density periods, the transmission chain is heavily dependent on a few susceptible and infectious individuals only (Goyens et al. 2013). Nevertheless, MORV can persist in these highly fluctuating populations, and can be detected even at very low densities (Borremans et al. 2011). In order to survive these bottlenecks, we assume that adaptation has driven MORV to a low-pathogenic lifestyle. A similar situation is suggested for the persistence of Junin arenavirus in populations of *Calomys musculinus*. Vitullo and Merani (1990) found that Junin virus did not affect the fitness of adult *C. musculinus* and, based on a mathematical model, they assumed that these unaffected animals are a prerequisite for viral persistence.

After correcting for the effect of age, we found that GAIV antibody-positive *M. natalensis* were significantly heavier, larger and reached sexual maturity earlier than antibody-free rodents. We also found that LASV antibody-positive rodents reached sexual maturity earlier until a certain age. These results indicate that arenavirus infections do not effect sexual maturity of their host. In fact, they are compatible with the hypothesis that sexual behaviour increases the probability of becoming infected with arenaviruses, and that sexual transmission could therefore be an important mechanism for the spread of arenaviruses in populations of *M. natalensis*. Although this relationship was not found for LASV in older animals or for MORV at all, sexual transmission would indeed be a highly beneficial transmission route for arenaviruses. Arenaviruses are assumed to be transmitted horizontally (at least partly) by as yet unconfirmed pathways (Fichet-Calvet et al. 2007; 2014; Borremans et al. 2011; Gryseels et al. 2015), and *M. natalensis* has a promiscuous mating system in which both males and females endeavour to copulate with numerous mates (Veenstra 1958; Leirs 1994; Kennis et al. 2008). Sexual transmission is also assumed to be important in other virus-rodent host systems. Cage experiments showed that the transmission rate of Machupo arenavirus in *C. callosus* was considerably higher in opposite sex pairs than in same sex pairs, and high concentrations of virus and antibody were found in the reproductive organs (Webb et al. 1975). In natural populations of bank voles infected with Puumala hantavirus, seroconversion was observed to

occur more frequently during the reproductive season and in sexually active individuals (Bernshtein et al. 1999). However, a recent long-term field study showed that seroconversion rates of bank voles are significantly higher outside the reproductive season, which occurs in winter (Voutilainen et al. 2016). This last result supports the hypothesis of an active indirect transmission through contaminated soil during wet and cold conditions (Sauvage et al. 2003). The two conflicting results highlight that patterns within the same parasite-host system can differ considerably due to different environmental conditions.

Otherwise, the observed relation between antibodies and sexual maturity in our study might be explained by the hypothesis that arenavirus replication enhances sexual maturity or behaviour of its host. This might be likely, as it has been shown that LCMV can severely disrupt hormonal regulation in laboratory mice (Oldstone et al. 1982; 1984; Carlos et al. 1992). Similarly, Kallio et al.(2015) suggest that infection with Puumala hantavirus enhances breeding in young female bank voles, but not in old ones.

Conclusion

We do not find any evidence that *M. natalensis*-borne arenaviruses negatively affect their natural host in natural circumstances. While adverse effects that lead to low capture probability in a subset of the infected population cannot be ruled out, our results suggest that the pathogenicity of arenaviruses is low in *M. natalensis* in general. This implies that these viruses do not significantly affect the population dynamics of *M. natalensis*.

Furthermore, as our study indicates that sexual behaviour can be important for arenavirus transmission in natural populations of *M. natalensis*, it would be interesting to determine the extent to which sexual transmission occurs. If an important mode of transmission, and considering mating frequency could be expected to be density-dependent in *M. natalensis*, control measures aimed at reducing the abundance of rodents during the breeding season may be highly effective for virus eradication.

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Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request. Voucher specimens of all individuals analysed for Morogoro and Gairo virus are maintained at the Evolutionary Ecology group at the University of Antwerp.

Author Contributions

Conceived the study: J.M. and E.F.-C. Wrote the paper: J.M. B.B. and S.Gr, Performed the experiments: B.B. S.Gr, B.S. G.N.B. B.B-Z. J.G-B. and E.F.-C. Performed the analyses: J.M. and L.D.B. Supervised field and laboratory work: J.G-B, S.Gu, H.L. N.M. and E.F-C. All authors read and approved the final manuscript.

Ethics approval

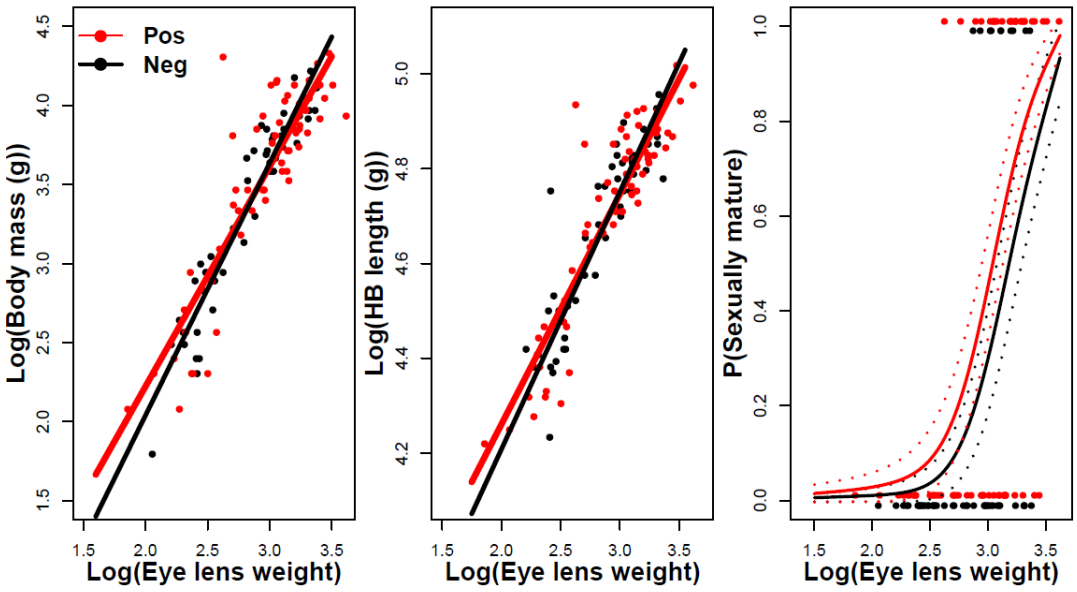
For Guinea, approval for the investigations were obtained from the National Ethics Committee of Guinea (permit n° 2003/PFHG/05/GUI and 12/CNERS/12). For Tanzania, all the procedures followed the Animal Ethics guidelines as laid out by the Sokoine university of Agriculture (Morogoro, Tanzania).

Tables

Table1: Effects of age (Eye lens weight - ELW) and viral infection (viral RNA - vRNA and antibody - AB) on body mass, head-body length, sexual maturity and fertility in *M. natalensis* infected by Lassa (LASV), Morogoro (MORV) and Gairo (GAIV) viruses. Effect estimates were shown on a log-scale to allow linear comparison. The last two columns represent analyses grouping animals that were either vRNA or antibody positive. n= number of sampled *M. natalensis*; * = statistically significant, NP= not possible to calculate because of significant interaction.

			ELW		**vRNA**		**Interaction vRNA x ELW**		**AB**		**Interaction AB x ELW**		**vRNA or AB**		**Interaction vRNA or AB x ELW**	
Virus	*Predicted*	*n*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*
LASV	Body mass	1297	1670.30	<0.01*	0.53	0.46	0.11	0.74	1.78	0.18	0.49	0.49	2.08	0.14	0.13	0.71
	Head-body length	1296	1751.80	<0.01*	0.16	0.68	0.09	0.75	0.05	0.81	0.15	0.70	0.29	0.65	<0.01	0.97
	Sexual maturity	1297	345.21	<0.01*	1.72	0.54	0.32	0.57	NP	NP	5.42	0.02*	0.01	0.91	2.78	0.10
	Fertility	151	1.84	0.17	2.14	0.14	0.21	0.65	2.14	0.14	0.03	0.85	0.10	0.75	0.08	0.78
MORV	Body mass	747	331.22	<0.01*	0.01	0.94	0.85	0.36	0.02	0.90	0.85	0.35	<0.01	0.95	1.26	0.26
	Head-body length	514	127.46	<0.01*	0.04	0.83	1.50	0.22	0.01	0.94	0.03	0.87	<0.01	0.97	0.13	0.72
	Sexual maturity	749	37.99	<0.01*	0.16	0.69	0.27	0.60	0.08	0.77	0.01	0.93	<0.01	0.96	0.03	0.84
GAIV	Body mass	258	176.09	<0.01*	0.03	0.86	0.52	0.46	13.28	<0.01*	0.01	0.93	8.60	<0.01*	<0.01	0.99
	Head-body length	254	175.00	<0.01*	0.14	0.71	0.29	0.59	8.45	<0.01*	0.95	0.33	6.23	0.01	0.23	0.63
	Sexual maturity	256	25.90	<0.01*	0.09	0.76	0.12	0.72	8.57	<0.01*	0.49	0.48	6.14	0.01	0.53	0.46

Supplementary data



S. Figure 1: Relationships between body mass, head-body length, sexual maturity (1 = active and 0 = inactive) and Eye lens weight (as a proxy for age) in *M. natalensis* infected by Lassa virus (LASV). Red = LASV positive village (Brissa), black = LASV negative village (Tambaya). Dashed lines represent standard errors on the predicted probabilities.

S. Table1: Relation between viral infection (viral RNA - vRNA and antibody - AB) and age (eye lens weight) in *M. natalensis* infected by Lassa (LASV), Morogoro (MORV) and Gairo (GAIV) viruses.

		vRNA		**AB**		**Interaction AB x vRNA**	
	n	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*
LASV	1293	1.09	0.29	56.48	<0.01*	36.20	<0.01*
MORV	739	20.15	<0.01*	0.55	0.45	4.76	0.03*
Gairo	255	1.93	0.16	13.55	<0.01*	7.64	<0.01*

S. Table2: Effects on body mass, head-body length and sexual maturity of *M. natalensis* between a Lassa virus (LASV) positive and negative village (Brissa versus Tambaya). n= number of sampled *M. natalensis*

			Village		**Interaction ELW x Village**	
Virus	*Predicted*	*n*	χ^2_1	*p-value*	χ^2_1	*p-value*
LASV	Weight	102	0.47	0.50	2.61	0.10
	Head-body length	102	1.65	0.19	1.07	0.30
	Sexual maturity	102	2.58	0.11	0.32	0.57

S. Table 3: Effects of sex and viral infection (viral RNA - vRNA and antibody - AB) on body mass, head-body length, sexual maturity and fertility in *M. natalensis* infected by Lassa (LASV), Morogoro (MORV) and Gairo (GAIV) viruses. Estimate effects were shown on a log-scale to allow linear comparison. The last two columns represent animals that were vRNA or antibody positive. n= number of sampled *M. natalensis*; * = statistically significant, NP= not possible to calculate because of significant interaction with ELW.

			sex		**Interaction sex x ELW**		**Interaction Sex x vRNA**		**Interaction sex x AB**		**Interaction sex x vRNA or AB**	
Virus	*Predicted*	*n*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*
LASV	Body mass	1297	NP	NP	12.37	<0.01*	1.46	0.23	0.33	0.56	2.01	0.15
	Head-body length	1296	NP	NP	7.96	<0.01*	2.56	0.11	0.05	0.87	1.07	0.30
	Sexual maturity	1297	NP	NP	30.90	<0.01*	1.62	0.20	0.14	0.71	0.34	0.56
MORV	Body mass	747	75.50	<0.01*	2.02	0.15	1.42	0.23	0.33	0.56	0.59	0.44
	Head-body length	514	NP	NP	12.89	<0.01*	1.78	0.18	0.68	0.41	1.10	0.29
	Sexual maturity	749	125.06	<0.01*	0.68	0.41	0.93	0.33	0.74	0.38	0.03	0.87
GAIV	Body mass	258	0.84	0.36	2.97	0.09	0.02	0.90	0.88	0.34	0.35	0.56
	Head-body length	254	0.59	0.44	2.22	0.13	0.42	0.51	0.43	0.51	0.96	0.33
	Sexual maturity	259	12.15	<0.01*	1.67	0.19	0.05	0.94	0.02	0.87	0.03	0.86

S. Table 4: Effects of season and viral infection (viral RNA - vRNA and antibody - AB) on body mass, head-body length, sexual maturity and fertility in *M. natalensis* infected by Lassa (LASV), Morogoro (MORV) and Gairo (GAIV) viruses. Estimate effects were shown on a log-scale to allow linear comparison. The last two columns represent animals that were vRNA or antibody positive. n= number of sampled *M. natalensis*; * = statistically significant, NP[a]= not possible to calculate because of significant interaction with ELW, NP[b]= not possible to calculate because there were not enough data sampled in one of the two seasons.

			season		**Interaction season x Lens**		**Interaction season x vRNA**		**Interaction season x AB**		**Interaction season x vRNA or AB**	
Virus	*Predicted*	*n*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*	χ^2_1	*p-value*
LASV	Body mass	1297	0.97	0.32	0.05	0.82	3.88	0.05	1.34	0.25	7.23	0.01*
	Head-body length	1296	0.27	0.59	0.28	0.59	2.56	0.11	1.52	0.22	6.42	0.01*
	Sexual maturity	1297	0.32	0.57	0.51	0.47	0.32	0.57	0.99	0.32	0.89	0.36
	fertility	151	2.17	0.14	0.96	0.32	0.21	0.64	1.14	0.28	0.41	0.51
MORV	Body mass	747	3.49	0.06	0.10	0.75	1.24	0.27	0.01	0.94	0.13	0.72
	Head-body length	514	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]
	Sexual maturity	749	NP[a]	NP[a]	7.64	0.01	0.13	0.71	0.26	0.61	0.78	0.38
GAIV	Body mass	258	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]
	Head-body length	254	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]
	Sexual maturity	256	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]	NP[b]

Arenavirus infection correlates with lower survival of its natural rodent host in a long-term capture-mark-recapture study

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Abstract

Background: Parasite evolution is predicted to select for levels of parasite virulence that maximise transmission success. When host population densities fluctuate heavily, low levels of virulence with limited impact on the host are expected, as this should increase the likelihood of surviving periods of low host density. We examined the effects of Morogoro arenavirus on the survival and recapture probability of the Natal multimammate mouse (*Mastomys natalensis*) using a seven-year capture-mark-recapture time series. *M. natalensis* is the natural host of Morogoro virus and is known for its strong seasonal density fluctuations.

Results: We found a small but significant negative correlation between Morogoro virus antibody presence and survival probability of *M. natalensis* (effect size: 5-8% per month depending on the season). We also observed a significant positive correlation between antibody presence and recapture probability (effect size: 8%).

Conclusion: The small negative correlation between host survival probability and Morogoro virus antibody presence could imply that either the virus has a detrimental effect on host wellbeing, or that hosts with lower survival probability are more likely to obtain Morogoro virus infection (e.g. due to particular behaviour or lower body/immune condition). Because recapture probability was also higher for antibody-positive animals, risky behaviour might be correlated with infection by Morogoro virus.

Introduction

The classical theory that explains how parasites evolve predicts a trade-off between virulence and transmission (Anderson and May 1982a; Ewald 1983; Alizon et al. 2009). This trade-off balances virulence and within-host reproduction so that transmission is maximized over the lifetime of infection. Parasites expressing intermediate levels of virulence should be favoured in this scenario, which is supported by sev

eral empirical examples of viruses with high transmission success (e.g. infections of myxoma virus in rabbits, HIV in humans, and Cauliflower Mosaic Virus in *Brassica rapa*) (Fraser et al. 2007; Doumayrou et al. 2013; Dwyer et al. 2014). However, not all studies found evidence for evolution towards intermediate virulence, but instead suggested evolution towards high or low virulence (Ebert and Bull 2003; Bull and Lauring 2014).

Host population density is a key factor to determine whether either low or high virulence will be favourable (King et al. 2009; Messinger and Ostling 2009; Cressler et al. 2016). The effect can be understood in the framework of a trade-off between a parasite's competitive ability and its persistence. Transmission rates are lower at low host densities, therefore a strain that can maintain a long infectious period (and thus low virulence) will have a persistence advantage over high virulent strains that kill the host or reduces its contact rate before transmission can take place. In contrast, a strain with a short infectious period but high reproductive rate will have an advantage at high host density, as it will outcompete strains with lower transmission rate and has little selective pressure to reduce its virulence. These host density effects could be especially important during virulence evolution of wildlife parasites, as their hosts often display strong density fluctuations (Boots and Mealor 2007).

For many wildlife parasites, however, virulence levels have not been assessed, and conflicting results have been found regarding the virulence of arenaviruses (Arenaviridae, *Mammarenavirus*) in their natural rodent host (Mariën et al. 2017). Most arenaviruses seem to be restricted to a single rodent species or even sub-species (Childs et al. 1993; Gryseels et al. 2017) suggesting special adaptation to those specific hosts. Some arenaviruses can also infect humans, potentially causing severe disease or death. Lassa arenavirus (LASV) can for example cause Lassa fever which annually affects around 200,000 people in West Africa (McCormick JB 1999; World Health Organization 2016). Other examples include Junín, Machupo, Guanarito and Sabia arenaviruses that cause sporadic outbreaks of haemorrhagic fevers in South-America. Although the pathogenic effects of arenaviruses on humans are relatively well-documented, little is known about their effect in their natural rodent hosts (Günther and Lenz 2004; Charrel et al. 2011).

Most information about arenavirus virulence in rodents is derived from laboratory inoculation studies, e.g. lymphocytic choriomeningitis virus (LCMV) in *Mus musculus*, LASV and Morogoro virus (MORV) in *Mastomys natalensis*, Machupo virus in *Callomys callosus* and Junin virus in *Callomys musculinus* (Walker et al. 1975; Webb et al. 1975; Oldstone 2002; Borremans et al. 2015). The inoculated rodents from these studies typically remained symptom-free despite temporary high viral loads, although severe disease symptoms have also been observed for LCMV (Weigand et al. 1963; Oldstone and Dixon 1968; Mims 1970). Nevertheless, several factors that influence virulence can differ between laboratory and natural settings, including variation in infection route or dose, viral strain, stress levels or individual life histories (Mariën et al. 2017). In order to examine the effects of arenavirus infection in

natural conditions, we recently analysed capture-removal studies in which we related body condition variables (head-body length, body weight, fecundity and maturation rate) of wild *M. natalensis* to their infection status (Mariën et al. 2017). Although we found no adverse relationship between MORV infection and body condition, we were not able to exclude the hypothesis that animals become lethargic or die quicker due to infection and thus have a lower probability to be captured. For this reason we now investigate whether MORV reduces the survival and recapture probability of *M. natalensis* (its reservoir host) using a seven-year capture-mark-recapture (CMR) dataset.

MORV infection in *M. natalensis* provides an interesting model system for examining parasite-host interactions, as the ecology and evolution of both the virus and the rodent host have been studied intensively (Leirs 1994; Sluydts et al. 2007; Günther et al. 2009; Borremans et al. 2011; Borremans et al. 2015; Gryseels et al. 2017). In particular, it provides a safe alternative to studying closely related but pathogenic arenaviruses such as LASV. MORV is endemic to East Africa where seroprevalence in *M. natalensis* has been found to range between 5 and 50% (Goüy de Bellocq et al. 2010; Borremans et al. 2011). In this region, *M. natalensis* populations experience seasonal density fluctuations, generally ranging from 20-250 individuals per hectare (Sluydts et al. 2009). These fluctuations are the result of seasonal breeding, driven by a bimodal rain pattern with short (November-December) and long (March-May) rainy periods (Leirs et al. 1990). Reproduction starts shortly after the long rains and continues until the end of the dry season in October. Population density peaks around November after which it decreases rapidly, probably due to a combination of high competition between individuals and changing environmental conditions such as decreased food availability and rainfall (Leirs et al. 1990). Despite the seasonal periods of low densities, MORV manages to persist and can be detected even at very low host densities (Borremans et al. 2011). This may be surprising for a (mainly) directly transmitted parasite of which the host's contact rate is assumed to be density-dependent and infection predominantly acute, and is probably only possible if a proportion of animals becomes infected chronically (Borremans et al. 2011; 2015; 2016; Mariën et al. 2017). We showed indeed that animals infected as neonates in the laboratory establish a chronic infection, whereas the infection in adults is cleared within 45 days (Borremans et al. 2015).

We hypothesize that MORV displays low virulence in its reservoir host, as longer host survival combined with chronic infection in some animals would allow MORV to persist during the seasonal periods of low host population density (Goyens et al. 2013). For this reason, we predict

that no adverse relationship exists between MORV infection status and *M. natalensis* survival probability.

Methods

Study area and trapping

A capture-mark-recapture study was performed between May 2010 and April 2017 on a mosaic field (maize and fallow land) on the campus of the Sokoine University of Agriculture in Morogoro, Tanzania (6°51'S; 37°38'E). A robust trapping design was used with trapping sessions conducted every month (primary capture occasion) for three consecutive nights (secondary capture occasions). Sherman live traps (Sherman Live Trap Co. Tallahassee, FL, USA) were placed in a rectangular 300x100m grid and spaced evenly at 10m intervals. The traps were baited in the evening with a mixture of peanut butter and corn flour and checked in the morning. Trapped animals were transported to the lab, where species, sex, weight and reproductive status were recorded (Leirs 1994; Sluydts et al. 2007). Mice were considered to be adults if signs of sexual activity were observed (scrotal testes in males; perforated vagina, lactating nipples or pregnancy in females). Blood samples were taken from the retro-orbital sinus and preserved on prepunched filter paper (±15 µL/punch; Serobuvard, LDA 22, Zoopole, France). Blood was only sampled once per monthly session, so if an animal was recaptured in the same three-day session, blood was not taken again. Each rodent was individually marked by toe clipping (Borremans, Sluydts, R.H. Makundi, et al. 2015), and released at its previous trap location.

Serology

Filter papers were dried and stored in the dark at ambient temperature in a locked plastic bag with dehydrating silica gel. Since the year 2014, blood samples were preserved at -20ºC after drying. Dried blood spots on filter paper were punched out and eluted in a 100µl solution of phosphate buffer saline and 0.25% NH_3 (Borremans 2014). Blood samples were analysed for the presence of antibodies (Ab) by indirect immunofluorescence assay using MORV-infected Vero cells as antigens and polyclonal rabbit anti-mouse IgG (Dako, Denmark) as secondary antibodies (Günther et al. 2009).

CMR data

The CMR dataset consisted of 8274 separate captures of 3884 unique *M. natalensis*, of which 855 individuals were seropositive at least once, and 168 seroconversion events were detected

where the infection status of animals changed from Ab-negative to positive in between trapping sessions. Antibody status was used as an indication of recent or past MORV infection, except for very young individuals that may have maternally-derived Ab (Demby et al. 2001). We therefore removed the youngest animals (body weight at first capture < 15g) from the dataset. A small proportion of animals showed an apparent loss of Ab. These negative samples were considered to be false negatives due to Ab titers falling below the detection threshold of the Ab-assay, as *M. natalensis* normally exhibits long-term Ab production after MORV infection (Borremans et al. 2015; Mariën et al. 2017).

The CMR data were analysed using R (R Core Team 2016) package 'marked' (Laake et al. 2016), which provides functions that allow efficient interfacing with CMR analysis software MARK (White and Burnham 1999). For survival analysis, we assumed a multivariate multistate Cormack-Jolly-Seber model that allows for parameter estimations in systems where different states (e.g. Ab-positive/negative) can be assigned to surviving individuals (Johnson et al. 2016). All parameter estimates were based on the primary capture occasions (i.e. the monthly trapping occasions), which were standardized to a time span of 30 days. Because time intervals between primary occasions varied between 22 and 55 days in reality, we included these differences into the model's design matrix.

Goodness of fit test

A goodness-of-fit (GOF) test was carried out with the program U-CARE to evaluate possible effects of confounding factors (Pradel et al. 2003; Choquet et al. 2009). Major deviations against assumptions on 'transience' and 'trap-dependence' were found (see results). The null hypothesis on 'transience' states that there is no difference in the re-encounter probability of newly trapped and recaptured individuals. Because we were interested in survival of resident animals only (not in migration), we decided to remove all transient animals from the CMR data set (Sluydts et al. 2007). Transient animals were defined as individuals that were captured only once. These individuals were (most likely) not re-encountered because they moved outside of the trapping grid, and not because they died shortly after release. Removing transient individuals obviously solved the problem against the assumption on 'transience', but was only possible on the condition that Ab-prevalence did not differ between transient and resident individuals. This assumption was tested using a generalized linear model with binomial distribution and logit-link function (see results).

The null hypothesis on 'trap-dependence' states that when individuals are caught, they become aware of the trap and will seek it or avoid it at the next trapping occasion (e.g. *M. natalensis*

becomes trap-happy in our dataset). This effect is likely to be strongest just after a capture occasion. In order to correct for trap-awareness, we implemented an immediate trap effect in the model using trappability states, in which individuals were able to move in a Markovian way between a 'trap-aware' state (after occasions when they are captured) and a 'trap-unaware' state (after occasions when they are not captured) (Pradel and Sanz-Aguilar 2012).

Modelling

The multistate model estimates three probabilistic events: the monthly probability that animals survive (Φ); the monthly probability that animals are recaptured (P) given that they were still alive; and the monthly probability that animals move between states (transition, ψ) given that they were alive in that state (Johnson et al. 2016). Trapped individuals were assigned an infection and a trappability state on each capture occasion: (1) Ab-negative and trap-aware, (2) Ab-positive and trap-aware, (3) Ab-negative and trap-unaware, (4) Ab-positive and trap-unaware, and (5) not captured. Because *M. natalensis* is assumed to stay MORV Ab-positive during its entire life, transitions from Ab-positive to Ab-negative states were not possible (Borremans et al. 2015).

Each of the parameters (Φ, P, ψ) was fitted by the following fixed factors: time, age, and infection status. It was not possible to fit fully time-dependent models, because our CMR study contained too many capture occasions (84) which would overparameterise the models. We therefore simplified the fully time-dependent model into a seasonal one (breeding season: may-Oct; non-breeding season: Nov-Apr), as seasonal effects have been shown to account for the largest variation in survival of *M. natalensis* in Morogoro (Julliard et al. 1999; Sluydts et al. 2007). We did not include a year effect in the models for several reasons that are further explained in the supplementary information. An age factor was included into the models to correct for the positive relation between *M. natalensis'* age and Ab-prevalence (Borremans et al. 2011). Older animals are more likely to be Ab-positive because Abs remain present after infection throughout an animal's lifetime, and older animals have had more opportunities to have encountered the infection than younger animals. Without this correction, we might have found that infected individuals have lower survival only because they are old. We used the logarithm of body weight on first capture as proxy for age, as we recently found that body weight was not affected by MORV infection and related linearly to log(eye lens weight), which is an unbiased indicator of age in rodents (Leirs 1994; Mariën et al. 2017). However, as it is known that variation in body weight increases significantly in adult animals, we removed all individuals of which the body weight was higher than 35g on first capture (Leirs 1994).

The most complex model contained all possible main effects and their interactions. A trappability factor was added to the recapture and transition models, but not to the survival models. The modelling itself occurred in subsequent steps: first we modelled transition, then recapture and finally survival. The models were ranked according to Akaike information criterion (AICc) and the one with the lowest AICc was selected as starting point for the next modelling step. During the first steps, survival and/or recapture were fixed and modelled by an interaction between season and weight and a trappability effect (for recapture only).

All models were implemented in R using the R packages marked, mvtnorm, dplyr and ggplot2 (Mi et al. 2009; Laake et al. 2016; Gómez-Rubio 2017). The R code can be found in the supplementary material. After removal of the transient individuals and individuals with a body weight <15g and >35g, the remaining data set contained 1219 individuals of which 325 were at least once seropositive and 118 seroconverted.

Results

Goodness of fit test

The GOF test showed major deviations against assumptions on transients (TEST 3.SR one sided test for transience, $\chi^2 = 83$, df = 63, p = 0.005) and trap-dependence (TEST M.ITEC, $\chi^2 = 132$ df = 44, $p < 0.001$, animals became trap-happy). Because Ab-prevalence was not significantly different between transient (individuals that were captured only once during one secondary trap interval) and resident animals [GLM, $\chi^2 = 1.6$, df=1, p = 0.201, Ab prevalences were 20% (CI 18-21%) and 18% (CI 16-20%) respectively], we could safely remove the transient animals from our data set. More than half of the animals (56%) were captured only once in the three hectare open grid. This pattern matched previous findings and suggests that the recapture probability at the primary trapping session is fairly low for *M. natalensis* in this experimental setup (Sluydts et al. 2007). While the deviation on transients hereafter disappeared (TEST 3.SR one sided test for transience, $\chi^2 < 1$, df = 59, $p > 0.999$), the deviation on trap-dependence remained in the reduced data set (TEST M.ITEC: $\chi^2 = 170$, df = 57, $p < 0.001$). We corrected for this trap-dependence by implementing two possible trappability states in the models (see methods).

Model selection

The transition model with the lowest AICc value included two interactions: one between infection (Ab-presence) and season and one between season and weight ($\psi_{I*S+S*W}$, AICc = 8660,

-2lnL = 8628, par = 16) (supplementary material: table 1). This model was 1 AICc units removed from a transitions model that fitted second best and which also contained interactions between infection, weight and season ($\psi_{I*W*S,}$ AICc = 8661, -2lnL = 8625, par = 18). After modelling transition we modelled recapture. The recapture model with the lowest AICc included an infection effect only (P_I, AICc = 8655, -2lnL = 8627, par = 14). Two other recapture models were only 1 AICc unit removed from the best fitting recapture model. These models contained an additive effect between infection and season (P_{I+S}, AICc = 8656, -2lnL = 8626, par = 15) or an interaction between infection and season (P_{I*S}, AICc = 8656, -2lnL = 8624, par = 16). During the modelling of survival, we found two models that had the same lowest AICc value. The first model included an additive effect between infection and season (Φ_{I+S}, AICc = 8645, -2lnL = 8619, par = 13). The second model included an interaction between infection and season (Φ_{I*S}, AICc = 8645, -2lnL = 8617, par = 14). One survival model was 1 AICc unit removed from the two best fitting models. It contained an additive effect between infection, season and weight (Φ_{I+S+W}, AICc = 8646, -2lnL = 8618, par = 14). We eventually choose the survival model with the lowest AICc value. This final model contained the following factors: $\psi_{I*S+S*W}$, P_I, Φ_{I+S}.

Survival estimates

The best fitting survival model included differences between season and infection status (Fig 1). During the breeding season, Ab-positive individuals had a monthly survival probability of 0.77 (95% CI 0.72 to 0.80) compared to 0.82 (95% CI 0.80 to 0.84) for Ab-negative animals. During the non-breeding season, Ab-positive individuals had a monthly survival probability of 0.47 (95% CI 0.43 to 0.52) compared to a survival probability of 0.55 (95% CI 0.52 to 0.58) for Ab-negative animals.

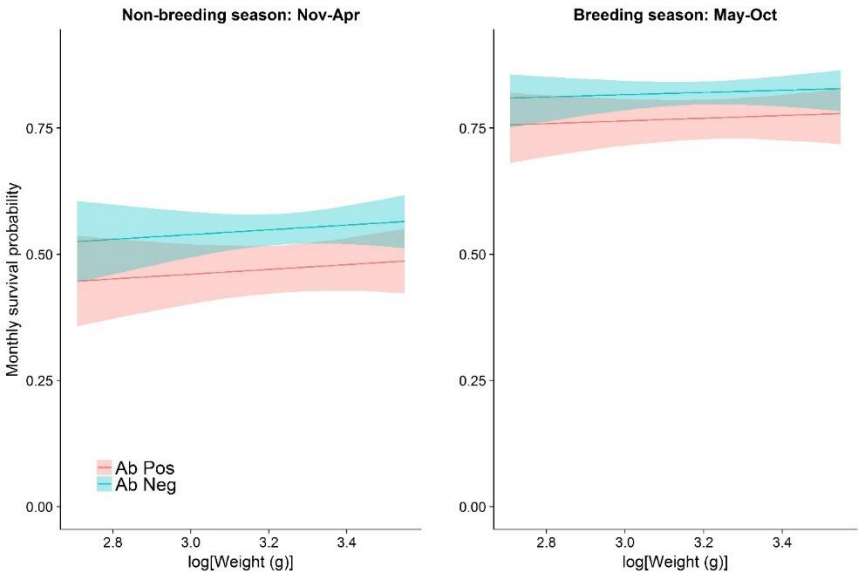

Fig 1: Monthly survival probability of MORV Ab-negative (blue) and Ab-positive (red) *M. natalensis* in function of log(body weight), a proxy for rodent age. The survival probability is given for the non-breeding (left) and breeding season (right). Solid lines and envelopes represent probabilities that an animal survived and 95% confidence interval (CI).

Recapture estimates

The best-fitting recapture model included an infection effect only (P_I). The recapture probability of Ab-positive animals was higher than the recapture probability of Ab-negative animals irrespective of age or season (estimate: 0.08 [95% CI 0.11, 0.03]). After correction for trap-dependency effects, the recapture probability of Ab-positive animals was 0.31 (CI 0.25, 0.37) compared to 0.23 (CI 0.20,0.25) for Ab-negative animals.

Discussion

The survival models indicate that the presence of anti-MORV Ab correlates with a 5-8% lower survival probability of *M. natalensis* in natural conditions. In our previous study we did not observe any adverse effects of MORV on the hosts' body condition (Mariën et al. 2017), but these two results are not necessarily contradictory. Parasites can impair host health through a variety of mechanisms that may affect survival probability but not the body condition parameters that we evaluated (body weight, body length, and reproductive maturity), such as behavioural changes or an increased susceptibility to secondary infection. Furthermore, the effect on survival probability observed here was small, so the power to detect very small effects on body condition parameters in our previous study, with more limited sample size (n=743, 73 were Ab positive), was perhaps not high enough.

Both field studies are actually in line with an inoculation experiment that showed how body weight of inoculated *M. natalensis* can decrease between days 7 and 15 post-infection (approx. 7% of normal body weight in 40% of animals) but recovers quickly without affecting further growth rates (Borremans et al. 2015). While such rapid recovery might explain why we did not observe any significant effects during the previous study, the temporary decrease in body weight does suggest that MORV can provoke adverse effects in some individuals. It is possible that a proportion of wild animals indeed become sick, which die quickly, and are therefore not included in our previous study. Such pathogenic effects would be observable during a CMR study, which might explain the small effect on survival that we found here.

Negative associations between infection and host fitness have been reported for other rodent-borne parasites. Hantavirus infections were initially assumed to be asymptomatic in rodents as no obvious pathology (such as reduction in body weight or fecundity) was observed (Verhagen et al. 1986; Bernshtein et al. 1999; Compton et al. 2004). However, recent CMR studies showed that hantaviruses can affect the survival probability of rodents depending on sex and reproductive status. For example, Puumala hantavirus decreased survival of reproductively inactive bank voles (*Myodes glareous*) by 14%, while Sin Nombre hantavirus decreased survival of male deer mice (*Peromyscus maniculatus*) by 13% (Kallio et al. 2011; Luis et al. 2012; Tersago et al. 2012a). Cowpox virus infections in voles and mice were also initially assumed to be asymptomatic (Bennett et al. 1997; Chantrey et al. 1999). Nevertheless, CMR studies showed that infections can correlate both positively and negatively with survival probability depending on the season. A positive relation between cowpox infection and survival probability was for example observed in bank voles and wood mice (*Apodemus sylvaticus*) during summer when reproduction is occurring, while negative effects were observed during winter (Telfer et al. 2002). Infected field voles (*Microtus agrestis*) had on the other hand an overall lower survival probability of 10-22% compared to uninfected field voles (Telfer et al. 2002; Burthe et al. 2008).

Because it is difficult to determine causality in a correlative model, the observed negative relationship between survival and MORV Abs may in fact be explained by several not mutually exclusive hypotheses (Fig 2). One possibility is that MORV has a direct negative effect on survival of *M. natalensis* because of the costs to eradicate the virus (Fig 2, H1). Another possibility is that Ab-positive mice have a lower survival probability because of prior poor conditions (e.g. secondary infections) that might increase susceptibility to MORV (Fig 2, H2). This situation has been observed for cowpox virus infections in *M. agrestis* (Beldomenico et al.

2009). Yet, this seems unlikely for MORV as no negative relationship seems to exist between MORV infection and *M. natalensis'* body condition (Mariën et al. 2017). Alternatively, the negative association between MORV infection and host survival may be explained by confounding host behavioural traits that affect both the probability of survival and infection (Fig 2, H3). For example, individuals with a risky lifestyle could be more susceptible to both predation and parasitism (Barber and Dingemanse 2010). Vanden Broecke et al. (2017) showed that such consistent behavioural differences (i.e. personality types) exist in *M. natalensis*, as some individuals were consistently more explorative than others. Although they found no significant relation between explorative behaviour and MORV infection (possibly due to the low number of infected animals in their study), other personality types may still influence MORV infection, such as boldness. Since bolder individuals might be more likely to get trapped (Boyer et al. 2010) and our results show a higher recapture probability (~8%) for Ab-positive individuals, it could indeed be possible that animals with a bolder personality are more likely to get infected by MORV.

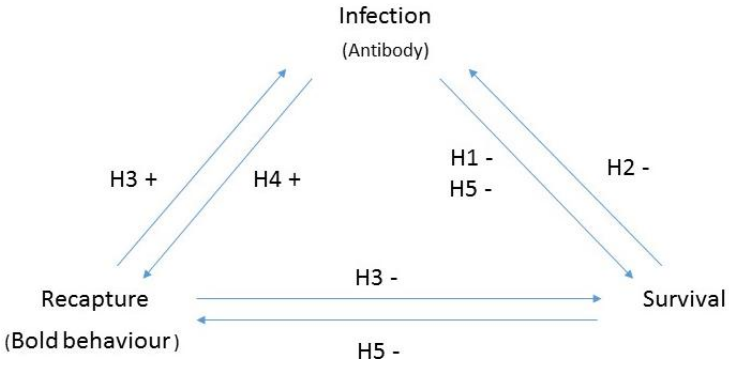

Fig 2: Schematic view of the possible relationship between infection, survival and recapture as described in the discussion. A '+' sign indicates that the two variables are proportionally related, '-' sign indicates that two variables are inversely proportionally related, H = hypothesis.

The positive relation between Ab-status and recapture probability can also be explained by two other hypotheses (Fig 2). Because it has been shown that arenaviruses can affect host behaviour directly (e.g. persistent LCMV infections impair the learning capacity of laboratory mice), MORV might increase the recapture probability of *M. natalensis* by decreasing neophobic behaviour in the rodent (Fig 2, H4) (Gold et al. 1994; De La Torre et al. 1996; Kunz et al. 2006). Similarly, *Toxoplasma gondii* decreases neophobic behaviour in brown rats (*Rattus norvegicus*) which is suggested to increase the rat's recapture probability and susceptibility to predators (Webster et al. 1994). Otherwise, the positive relation between infection and recapture probability might be explained indirectly by unavoidable side effects of infection on host health

(i.e. sickness effects) (Fig 2, H5) (Barber and Dingemanse 2010). Infected *M. natalensis* might need to recover from infection and therefore increase risk-taking behaviour to search for extra food, which might result in a higher recapture probability.

Conclusion

We found a significant negative relation between *M. natalensis* survival probability and MORV Ab-status. However, the effect of infection was small (5-8%) and probably negligible compared to the effects of environmental factors such as rainfall (25-30%), which is known to be an important driver of survival and reproduction in *M. natalensis* (Leirs et al. 1990; Sluydts et al. 2007). Since we previously observed no relationship between MORV and the body condition or reproductive maturity of its host and now only a small effect on its survival (Mariën et al. 2017), it seems that MORV does not significantly affect the population dynamics of *M. natalensis*. Combined, our two field studies suggest that MORV displays low virulence in its natural rodent host, which could represent an adaptation of the arenavirus to persist in the seasonally fluctuating host populations.

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Availability of data

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Conceived the study: JM and VS. Wrote the paper: JM, VS, BB, BvdB and SGr. Performed the experiments: JM, BB, SGr, BvdB and JG-B. Performed the analyses: JM and VS. Supervised field and laboratory work: LSM, JG-B, CS, SGu, LM, AK, M and HL. All authors read and approved the final manuscript.

Ethics approval

All the procedures followed the Animal Ethics guidelines of the Research Policy of Sokoine University of Agriculture as stipulated in the ''Code of Conduct for Research Ethics'' (Revised

version of 2012) and the guidelines in (Sikes and Gannon 2007). The used protocol was approved by the University of Antwerp Ethical Committee for Animal Experimentation (2015–69) and adhered to the EEC Council Directive 2010/63/EU.

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Tables

Table 1: Modelling of transition, recapture and survival. Highlighted (bold) models were selected in each step and used as starting point for the subsequent step. I = infection (antibody positive or negative), S = season (breeding and non-breeding season), W = weight (proxy for age of *Mastomys natalensis*), AICc (sample size corrected version of Aikaike information criterion), -2Lnl (-2*log likelihood) and Par (number of identifiable parameters).

Transition	Recapture	Survival	AICc	Lnl	Par
I*S+S*W	**S*W**	**S*W**	**8660**	**8628**	**16**
I*W*S	S*W	S*W	8661	8625	18
I+W*S	S*W	S*W	8662	8632	15
I*S+W	S*W	S*W	8662	8632	15
I*W+S*W	S*W	S*W	8662	8630	16
I*W+I*S+S*W	S*W	S*W	8663	8629	17
I+W+S	S*W	S*W	8664	8636	14
I*W	S*W	S*W	8664	8636	14
I+W	S*W	S*W	8665	8639	13
I*W+S	S*W	S*W	8668	8638	15
I*S+I*W	S*W	S*W	8668	8636	16
I*S+I*W	**I**	**S*W**	**8655**	**8627**	**14**
I*S+I*W	I+S	S*W	8656	8626	15
I*S+I*W	I*S	S*W	8656	8624	16
I*S+I*W	I*S+W	S*W	8657	8621	17
I*S+I*W	~	S*W	8657	8631	13
I*S+I*W	I+W	S*W	8657	8627	15
I*S+I*W	I+S+W	S*W	8658	8626	16
I*S+I*W	S	S*W	8659	8631	14
I*S+I*W	W	S*W	8659	8631	14
I*S+I*W	I*W	S*W	8659	8627	16
I*S+I*W	I*S+W*S	S*W	8659	8623	18
I*S+I*W	I*S+W*I	S*W	8659	8623	18
I*S+I*W	S+W	S*W	8660	8630	15

I*S+I*W	I*W+S	S*W	8660	8626	17
I*S+I*W	S*W+I	S*W	8660	8626	17
I*S+I*W	I*S+S*W+I*W	S*W	8661	8623	19
I*S+I*W	S*W	S*W	8662	8630	16
I*S+I*W	I*W+W*S	S*W	8662	8626	18
I*S+I*W	I*S*W	S*W	8662	8622	20
I*S+I*W	**I**	**I+S**	**8645**	**8619**	**13**
I*S+I*W	I	I*S	8645	8617	14
I*S+I*W	I	I+S+W	8646	8618	14
I*S+I*W	I	I*S+W	8647	8617	15
I*S+I*W	I	I*W+S	8647	8617	15
I*S+I*W	I	I*S+W*I	8647	8615	16
I*S+I*W	I	S*W+I	8648	8618	15
I*S+I*W	I	I*S+W*S	8649	8617	16
I*S+I*W	I	I*W+W*S	8649	8617	16
I*S+I*W	I	I*S+S*W+I*W	8649	8615	17
I*S+I*W	I	I*S*W	8650	8614	18
I*S+I*W	I	S	8652	8628	12
I*S+I*W	I	S+W	8653	8627	13
I*S+I*W	I	S*W	8655	8627	14
I*S+I*W	I	I	8846	8822	12
I*S+I*W	I	I+W	8846	8820	13
I*S+I*W	I	I*W	8848	8820	14
I*S+I*W	I	~	8863	8841	11
I*S+I*W	I	W	8863	8839	12

Supplementary information

Explanation for not using 'year' as variable in the models

We did not include 'year' as variable in the models for four reasons:

- We were not primarily interested in the effect of year on survival but only in the effect of MORV infection. We assumed that it was only necessary to include season and weight as fixed factors in the models, as we observed significant differences in prevalence according to season (Mariën et al, unpublished data) and because it was essential to correct for an age effect (as explained in the main text). In an ideal scenario we would have implemented year as a random variable in the models, but there were only seven years, which is not enough for the implementation of a random variable.
- Because we also observed differences in prevalence between years, we tested if there was a correlation between yearly prevalence and yearly survival: we found a positive correlation (Fig 1). This positive correlation is most likely caused by the fact that

animals which survive longer become older, and are therefore more likely to developed antibodies due to the longer time window they have been alive. Because we already corrected for age effects, we assumed that it was not necessary to correct for this positive correlation between prevalence and survival. If we would have found a negative correlation between yearly prevalence and yearly survival probability, it would have been necessary to include year as factor in the model. In that case, year would be a confounding factor which might have explained the negative correlation between MORV Ab presence and survival probability. For example, if survival would have been low during certain years (e.g. because of climate conditions) but Ab prevalence high, it would be logic to find a negative correlations between MORV infection and survival. This was however not the case.

- If we included year as fixed effect in the models, we would have increased the number of parameters substantially (number of parameters x7). This would have made the models too complex for the amount of data that we had. It would have been especially problematic in our situation, as we used the AIC approach for model selection which is often claimed to over-fit models which are overly parametrized (relative to the true general model).
- Even if we added year as fixed effect to the models, we still selected a model that included an infection effect as the best model. Estimates of the infection effect were almost similar between models with and without year, as year was not significant. The errors on the estimates were however considerably larger in models with year. This suggests that adding or leaving out year would not have severely affected our results.

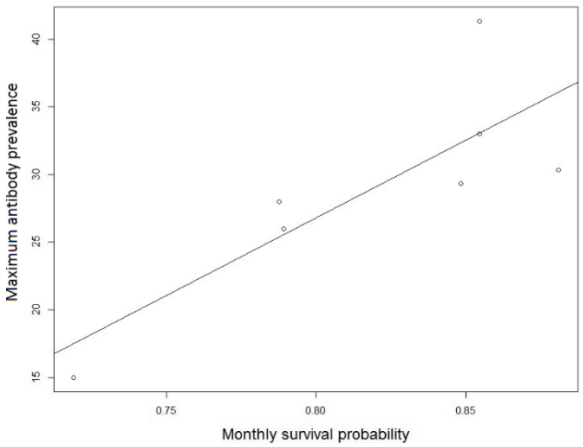

Figure 1: Correlation between monthly survival probability and maximum antibody prevalence measured per year (2010 until 2017).

Arenavirus dynamics in experimentally and naturally infected rodent hosts

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Abstract

Infectious diseases of wildlife are typically studied using data on antibody and pathogen levels. In order to interpret these data, it is necessary to know the course of antibodies and pathogen levels after infection. Such data are typically collected using experimental infection studies in which host individuals are inoculated in the laboratory and sampled over an extended period, but because laboratory conditions are controlled and much less variable than natural conditions, the immune response and pathogen dynamics may differ. Here, we compared Morogoro arenavirus infection patterns between naturally and experimentally infected multimammate mice (*Mastomys natalensis*). Longitudinal samples were collected during three months of bi-weekly trapping in Morogoro, Tanzania, and antibody titer and viral RNA presence were determined. The time of infection was estimated from these data using a recently developed Bayesian approach, which allowed us to assess whether the natural temporal patterns match the previously observed patterns in the laboratory. A good match was found for 52% of naturally infected individuals, while most of the mismatches can be explained by the presence of chronically infected individuals (35%), maternal antibodies (10%) and an antibody detection limit (25%). These results suggest that while laboratory data are useful for interpreting field samples, there can still be differences due to conditions that were not tested in the laboratory.

Introduction

Understanding disease transmission in populations relies on a correct interpretation of infection data. When data are difficult to measure in natural conditions, e.g. length of the infectious period, immune response duration and dynamics, or transmission mechanisms (vertical, horizontal, sexual, etc.), challenge experiments are typically used, where healthy individuals are inoculated under controlled laboratory conditions (Gallagher and Clifton-Hadley 2000; Carrat et al. 2008; Hardestam et al. 2008; Kerr 2012). The validity of such experimental data is conditional on the assumption that infection patterns in natural conditions are the same as those in a controlled laboratory setting. Dissimilarities between natural and laboratory infections can result from variance in many factors, including presence of different genetic strains, inoculum or infection volumes, transmission routes, stress levels, individual life histories (e.g. reproductive status, past infection) and trade-offs between immune defence and other fitness related traits (Hill & Beran 1992; Schmid-Hempel & Ebert 2003; Martin et al. 2008; Franca et al. 2012;Voutilainen et al. 2015; Jones et al. 2015). While all these factors can vary in both

laboratory and natural conditions, the first three might be more important for differences observed in laboratories and the last three for differences observed in nature.

Comparing data from animals sampled in the wild to data from laboratory animals can however be difficult. An important reason for this is the need to know exactly when a sampled individual became infected in the field in order to interpret the consequent antibody or parasite kinetics, or to determine the infectious and immune period (Begon et al. 2009; Voutilainen et al. 2015). Determining the exact time of infection (TOI) in natural populations is most often unfeasible because usually not all individuals can be captured repeatedly, as a large effort is required to collect sufficient long-term data from wild animals, and because the sampling frequency (typically 4 weeks or more) is often too low for quantifying infection patterns (Pollock et al. 1990; Samiel et al. 1999; Telfer et al. 2002; Lachish et al. 2007; Tersago et al. 2012; Cooch et al. 2012). While it is therefore not surprising that few studies have performed a cross-validation of infection data from laboratory and natural conditions, there is nevertheless a great need for such comparisons.

The aim of this study is to compare temporal variations in Morogoro virus (MORV) shedding and the immune response between experimentally and naturally infected multimammate mice (*Mastomys natalensis*). MORV is an East African arenavirus closely related to Mopeia and Lassa virus, the latter of which causes severe haemorrhagic Lassa fever in humans in West Africa (Wulff et al. 1977; Günther, et al. 2009). Due to their similarities and the fact that they are not pathogenic, MORV and Mopeia virus are considered to be safe alternatives for research on Lassa virus ecology and vaccine development (Lukashevich et al. 1999; Borremans et al. 2011; Rieger et al. 2013).

A recent experimental infection study in controlled laboratory conditions revealed that IgG levels as well as the presence of viral RNA (vRNA) exhibit a predictable temporal pattern post-inoculation, with relatively little variation between individual *M. natalensis* (Borremans et al. 2015). One day after inoculation, viraemia starts and continues for a period of 15 days after which the virus disappears in blood, while MORV RNA remains detectable in urine, saliva and faeces until about 40 days after inoculation. Antibodies (IgG) against MORV are detectable from day 7 post infection (pi), and follow a clear, predictable pattern characterized by a high initial increase phase peaking around day 20 pi, followed by a decrease phase that reaches a minimum approximately 70 days pi, after which Ab titers again start to increase until reaching a final equilibrium concentration from day 160 pi onwards (last sampled at 210–211 days p.i.) (Borremans et al. 2015). The mice used in this challenge study were part of a breeding colony

initiated in 1999 using mice from Morogoro (Tanzania), which is the same location where we collected field samples for the current comparative study. By focusing our efforts on a brief but high-intensity sampling effort in the field, we were able to obtain a dataset of sufficiently high temporal resolution to allow us to quantify individual infection dynamics of naturally infected rodents, and compare these qualitatively with the temporal patterns of the experimental data (Borremans et al. 2016).

Methods

Field and molecular work

Between 30 July 2013 and 18 October 2013, a rodent capture-mark-recapture (CMR) study was performed in five grids of 100m by 100m in Morogoro, Tanzania. The timing of the study corresponds with the second half of *M. natalensis'* breeding season, during which period we expect the highest MORV incidence rates. In each grid, 100 Sherman live traps were placed at 10m intervals and baited with a mixture of peanut butter and ground maize. Trapping sessions of three consecutive nights each were repeated every other week. Blood and saliva samples were taken from each animal and preserved on prepunched filter paper (±15 µL/punch) (Borremans 2014). Animals were individually marked using toe clipping (Borremans et al. 2015), and weight, sex, and reproductive state were recorded (Leirs, 1994). All individuals that were captured during at least two different trapping sessions were analysed for the presence of anti-MORV antibodies (Abs) by indirect immunofluorescence assay and vRNA by RT-PCR (Günther et al. 2009). Antibody titers were estimated using two-fold dilution series, starting with a minimum dilution of 1:10 (Borremans et al. 2015). A more detailed explanation of the field and laboratory work can be found in the supplementary information (supplementary text 1 and 2).

Comparing field and laboratory data

In order to compare the Ab and vRNA patterns of naturally infected mice with those obtained through experimental infection (Borremans et al. 2016), we needed to simultaneously plot both datasets on a figure showing Ab titer and vRNA presence against time after infection, and in order to do this it was necessary to know the exact time of infection (TOI). While the exact TOI is known for the experimentally infected mice, this is obviously not the case for those that were infected naturally. As the best possible alternative, we used the TOI that resulted in the best possible match between the measured data of a wild mouse and the laboratory data. If a good

match between laboratory and natural infection patterns can be found, this would be a strong indication that experimentally acquired data are representative for natural infections; it is impossible to statistically test the match unless the exact TOI in natural conditions is known.

To estimate the TOI of naturally infected mice, we used the method described in Borremans et al. 2016. Briefly, this method integrates existing data on Ab presence and titer, vRNA presence in blood and excretions (saliva or urine), and body weight into one semiparametric, Bayesian model that can be used to estimate the most likely TOI given the available information. The method takes into account all available data about a captured individual, which generally means that the error on the estimated TOI will be smaller for individuals that were re-captured more often. Here, the infection data used to inform the TOI estimation model originates from the experimental data described above, which means that the use of this method will result in the best possible match between the experimental and natural patterns. Therefore, it is important to bear in mind that this method is potentially positively biased towards finding a good match between temporal patterns, does not allow obtaining statistical proof for the matches and will only allow the identification of obvious discrepancies between experimental and natural temporal infection patterns.

Because it may be possible that discrepancies between temporal patterns of laboratory and field data only occur in either Ab or vRNA data and not necessarily in both simultaneously, the TOI was estimated using two scenarios. In the first (method 1), only Ab titer data were used, in order to test whether Ab titer patterns are similar regardless of the vRNA patterns. In the second scenario (method 2), both vRNA data from blood and excretions and Ab data were incorporated. Additionally, to slightly improve estimation of the TOI, all scenarios take into account body weight by limiting the maximum age of animals. Individuals with a body weight below 20g (= juveniles) were assumed to be younger than 120 days following (Leirs 1994), which means that they could not have been infected before this day. We also considered a third scenario where only vRNA data were used to estimate the TOI, but because this method lacks validity (see below) the results of this method were not further discussed in this article.

Only mice that were recaptured during multiple trapping sessions and were Ab-positive during at least one of these sessions were retained for TOI estimation. Thus, mice that were recaptured and were vRNA-positive at least once but never became Ab-positive were not included. It is possible that they could have been infected at a young age after which they developed Abs at titers under the detection threshold of the used immunofluorescence assay (see discussion and Begon et al. 2009), which is a condition that was not investigated in the laboratory before. After

the TOI estimations, field and experimental data were plotted together to allow a qualitative assessment of the match between infection patterns. R statistical software (R Core Team 2014) was used for data manipulation, TOI estimation and plotting.

We defined a good match for a tested individual if all its Ab titers or vRNA presence data fell within the credible band (CB) of the laboratory data. More detailed information about this calculation can be found in the supplementary information (supplementary information text 3).

Testing the validity of the different TOI methods

Because the TOI method is biased towards finding a good match between field and laboratory data, we also tested whether the obtained results are statistically different from those resulting from random data, where observation data (Ab, vRNA) are randomised for each individual, i.e. when the presumed temporal pattern (which contains information on the TOI) was broken. If this was not the case, this would be an indication that: (1) there is insufficient information to estimate TOI sufficiently accurate to make a meaningful comparison between field and laboratory data (e.g. because animals were not recaptured frequently enough in this study); or (2) the observed temporal dynamics in the field data differ considerably from those measured in the laboratory. We assessed this using a permutation test. For each permutation, we randomly permuted for each individual the Ab titers or vRNA presence in blood and excretions and then calculated the percentage of individuals that matched the laboratory data. We considered 10,000 permutations of the field data.

Results

During 10,800 trap nights (the number of traps times the number of trapping nights), we captured 1,133 *M. natalensis* individuals of which 220 were recaptured at least once during a different trapping session. All samples of recaptured individuals (590 samples) were analysed for both Abs and MORV vRNA (Table 1).

We found that 10% (21/220) of rodents were vRNA-positive at least once and never Ab-positive, 9% (19/220) were at least Ab-positive once and never vRNA-positive, 10% (23/220) were vRNA- and Ab-positive at least once at the same or a later capture of which 78% (18/23) were simultaneously vRNA- and Ab-positive at least once (Table 1). From the 21 vRNA-positive mice that were never found to be Ab-positive, 13 mice were vRNA-positive during one capture session but not during later recaptures. This occurred in eight mice two weeks later, in four mice four weeks later, and in one mouse eight weeks later. Two Ab-negative individuals

were vRNA-positive during two consecutive recaptures (one x two weeks and one x four weeks later); and six individuals were positive during their last recapture. This means that 26% (15/57) of infected mice showed no signs of an active Ab response two weeks or more after after vRNA detection. The six mice that were vRNA-positive at their last recapture were not included in this sum because we cannot determine whether they were sampled before they could produce Abs (day six after inoculation in laboratory conditions).

For the TOI analyses, we used only the 42 individuals that were Ab-positive at least once. When TOI was estimated based on Ab levels only (method 1), the temporal patterns of natural and experimental Ab dynamics were remarkably similar (i.e. there were very few instances of a bad match). We found that 88% (37/42) of individuals matched the laboratory immune response, of which 91% (82/90) of all the collected field data observations fell within the 95% credible band (CB) of the laboratory data (Table 2; Fig 1). Of the remaining ten data points, seven were Ab-negative while based on the laboratory data they would have been expected to be Ab-positive (Fig 1 and e.g. supplementary Fig S21).

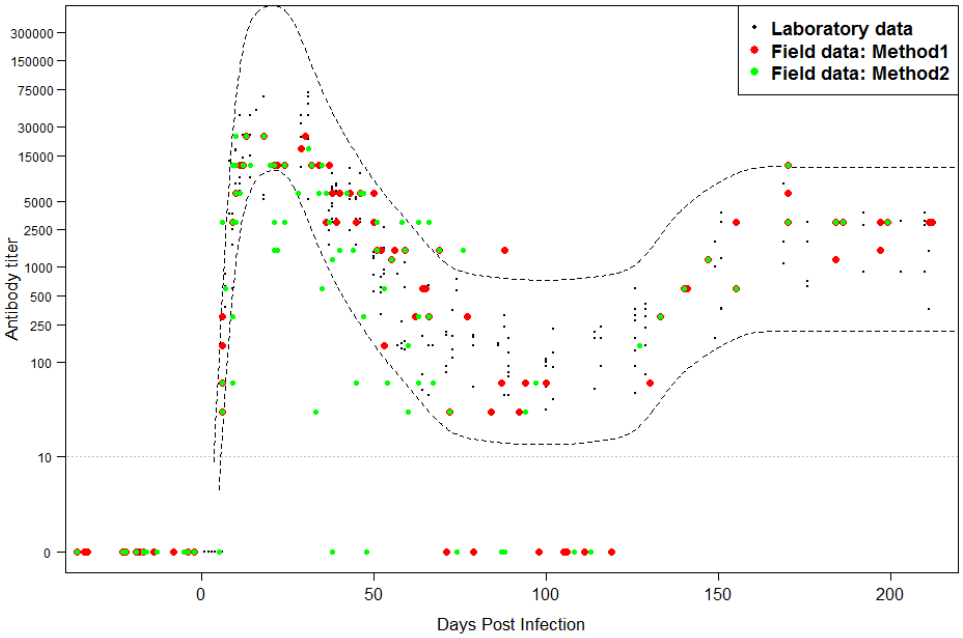

Fig 1. Temporal Ab dynamics of naturally and experimentally MORV-infected *M. natalensis*. Small black dots represent Ab levels from experimentally infected rodents in the laboratory (data derived from (Borremans et al. 2015)), red and green dots represent Ab titers from naturally infected rodents observed in this study. The TOI of the red field data (dots) was estimated using the TOI estimation method based on Ab titer only (method 1). The TOI of the green field data (dots) was estimated using the TOI estimation method based on Ab titer and vRNA (method 2). Ab patterns of 42 naturally infected individuals were plotted together in this graph.

When both Ab levels and presence of vRNA were taken into account (method 2), vRNA and Ab level patterns were still roughly in agreement with laboratory results. For the Ab response, we found that 67% (28/42) of individuals matched the laboratory results, of which 78% (73/94) of all the collected field data fell within the 95% CB of the laboratory data (Table 2; Fig 1). We found that 71% (27/38) of individuals matched the vRNA dynamics of the laboratory data in blood and 87% (34/39) in excretions, of which 85% (69/81) and 93% (76/82) of all the collected field data fitted the predicted probabilities respectively (Fig 2). For the combined results of temporal Ab and vRNA patterns, we found a good match with the laboratory patterns in 52% (22/42) of naturally infected mice (supplementary Table 2). The mismatches were due to the prolonged presence of vRNA in blood or excretions (e.g. supplementary Fig S6), the absence of Abs at times that they would be expected to be positive (Fig S21), the potential presence of maternal Abs (Fig S29), and overall lower Ab titers in naturally infected animals (Fig S1).

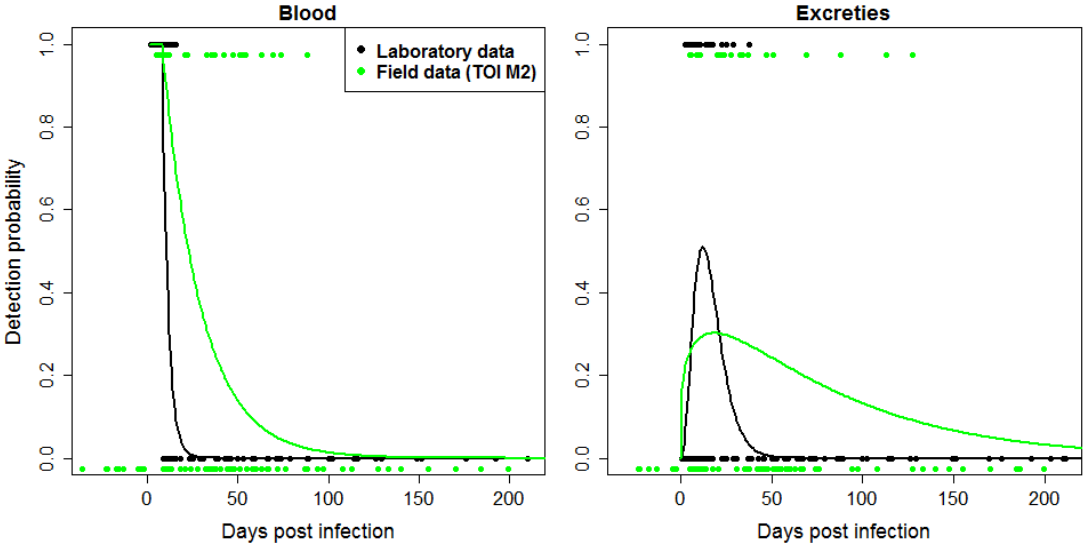

Fig 2. Temporal detection probability of MORV vRNA presence in blood (left) and excretions (right). The black points/curve represent raw data (points) and the proportion of samples (curve) that were vRNA-positive in laboratory conditions (i.e. vRNA presence probability; data derived from Borremans et al. 2015). The green points/curve represent raw data (points) and the proportion of samples (curve) that were vRNA-positive in field conditions, and were estimated using the TOI estimation method based on all available data (method 2, Ab titer, vRNA presence in blood and excretions, weight cut-off). vRNA patterns of 42 naturally infected individuals were plotted together in this graph.

Testing validity of TOI methods

When TOI was estimated based on Ab titers alone (method 1) or on both Ab titers and vRNA in blood and excretions (method 2), real field data matched laboratory data significantly better than random permutations (p-value < 0.0005 for both methods) (Fig 3). In contrast, if TOI was

estimated based on vRNA observations alone (method 3), real field data did not match laboratory data better than permutations (p-value = 0.9291) (Fig 3). We conclude that methods 1 and 2 are appropriate to make a meaningful comparison between field and laboratory data, but that it is not possible to make such a comparison based on vRNA data alone (method 3) because good matches between laboratory and randomised data were much too likely. Results of method 3 were therefore not further discussed in this paper.

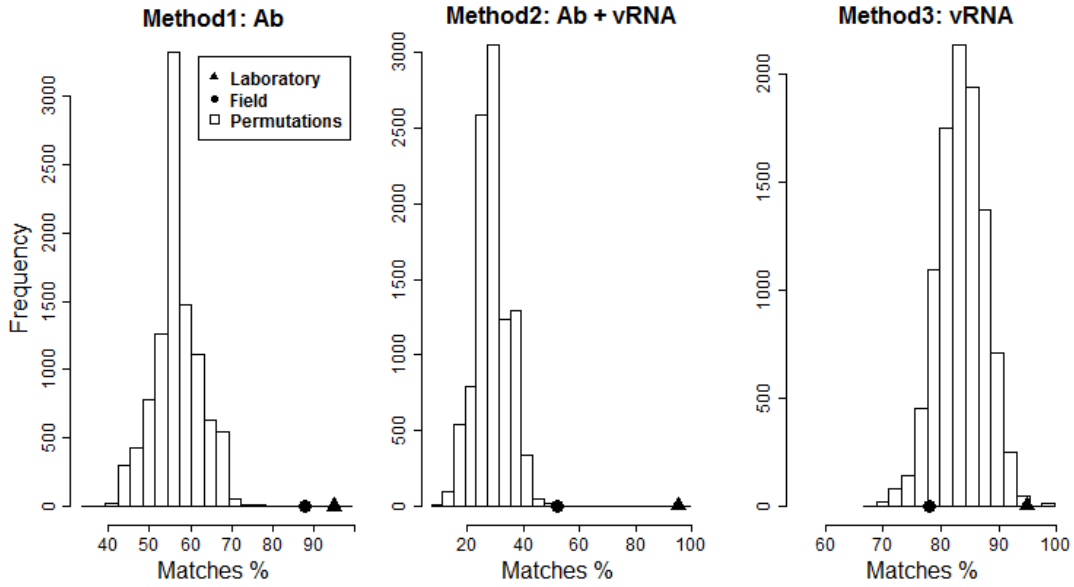

Fig 3: Percentages of individuals for which the infection patterns in viral shedding and/or host immune response matched the laboratory data. Random permutations of field data (bars) were compared with real field data (dots) and laboratory data (triangles, by default 95%) for the three different TOI methods: Ab titers (method 1), Ab titers and vRNA in blood and excretions (method 2), and vRNA in blood and excretions (method 3).

Discussion

We found that 74% of infected individuals in the field developed an Ab response within at least two weeks after the infection was detected. When both Ab and vRNA data of these Ab-positive individuals were taken into account, we found a good match between field and laboratory infection patterns for 52% of individuals. This percentage is significantly higher than in case the Ab or vRNA observations are randomised for every individual, but is clearly lower than the 95% matching percentage that is to be expected in the laboratory. This lower percentage could be due to several mechanisms, which are discussed below.

MORV vRNA-positive without antibody response

First, we found that 26% of mice developed no Ab response after MORV infection in the field (with animals tested at least two weeks after MORV infection was detected), in contrast to

laboratory conditions where all inoculated mice seroconverted within 6 days. One possibility to explain this result is that these mice developed Abs at titers that fell below the detection threshold of the used immunofluorescence assay. Such lower Ab titers could be due to infection at a young age. Studies based on early serological methods thought that neonatal *M. musculus* infected with lymphocytic choriomeningitis arenavirus (LCMV) did not develop Abs and were assumed to be immuno-tolerant (Burnet & Fenner 1949; Weigand & Hotchin 1961). However, when analysed later using more sensitive immune assays, Abs did appear to be present, but at low titers (Oldstone & Dixon 1967; Oldstone 2002). This may also be the case for neonatally infected *M. natalensis* in nature, although we have previously observed that laboratory-infected neonatals develop a chronic MORV infection with the presence of Abs at normal titres (Borremans et al. 2015).

Another hypothesis would be that these mice indeed never produced Abs. Wild animals need to allocate their limited resources between immune defence and other fitness related traits (Schmid-Hempel and Ebert 2003). As a result, they could have cleared the virus by the cell-mediated immune response alone. Although this response is not yet investigated for MORV infections in *M. natalensis*, it is known that cytotoxic T-cells can indeed clear LCMV infections without the help of Abs in B cell-deficient mice (Matloubian et al. 1994; Asano & Ahmed 1996).

Lower antibody titers

The majority of mice (74%) did develop a clear immune response in the field, although Ab titers were systematically lower than in the experimental data (Fig 1, Fig 3). This was most distinct in the supplementary Fig S1 (individual 354060F1), Fig S2 (364060F1) and Fig S33 (760F6) where the Ab titers just fell outside the CB of the laboratory data. It may also have been the case for individuals shown in Fig S12 (390F3), Fig S21 (260F5), Fig S22 (370F5), Fig S29 (210F6) and Fig S37 (3100F6), where Ab titers of positive samples were generally low and where the laboratory results would be matched perfectly if the negative samples were in fact low, undetectable titers. Such an apparent loss of detectable Abs was also observed for a small percentage (<5%) of *Microtus agrestis* naturally infected with cowpox virus when analysed by indirect immunofluorescence assay (Chantrey 1999; Begon et al. 2009). Because *M. agrestis* normally shows a long-term Ab production, these samples were considered to be false negatives. Combined, our results imply that Ab titer values should be increased slightly when they are used for detailed analyses such as TOI estimation (Borremans et al. 2016).

Chronic infections

Evidence for chronic infection was found for a number of individuals (13%), and was most convincingly seen in Fig S26 (individual 38F6), where an individual is depicted for which all samples were positive for Ab and vRNA in blood and almost all samples in excretions. There is also some evidence for chronic infection during which virus presence in blood or excretions is not constant but intermittent, suggesting temporary flare-ups of excretion; examples are shown in Fig S6 (15F3a), Fig S10 (330F3), Fig S22 (370F5), Fig S27 (48F6), Fig S36 (2680F6) and Fig S37 (3100F6). A chronic infection could also be an explanation for two individuals (20100F4 and 355060F1) that remained Ab-negative but were vRNA-positive in blood or excretions during two consecutive capture sessions (respectively 2 and 4 weeks interval).

If these patterns are indeed the result of chronic infection, this means that viraemia is not always transient in field conditions, as opposed to laboratory conditions where vRNA is only detectable for a short period in infected adults (until day 15 in blood and day 40 in excretions). For hantaviruses, chronic infection usually seems to result in temporary viraemia, after which the virus retreats into certain organs and is shed at lower concentrations (Yanagihara et al. 1985; Fulhorst et al. 2002), although chronic infection can also result in persistent viraemia, which has been observed for Black Creek Canal hantavirus, and for natural infections with Sin Nombre and Puumala hantaviruses (Billings et al. 2010; Bagamian et al. 2012; 2013; Voutilainen et al. 2015).

For arenaviruses, both transient and persistent viraemia have been observed in rodent hosts. Most arenaviruses establish a chronic infection when experimentally inoculated in newborn hosts, but are cleared from the rodent's body quickly when inoculated in adults: less than two weeks in blood and less than one month in excreta. This age-at-infection effect for natural hosts has been experimentally observed for the Old World arenaviruses (LCMV in *M. musculus*, Lassa and Morogoro viruses in *M. natalensis*) (Buchmeier et al. 1978; Borremans et al. 2015; Walker et al. 1975), and the New World arenaviruses (Tamiami virus in *Sigmodon hispidus*, Catarina virus in *Neotoma micropus* and Whitewater Arroyo virus in *Neotoma albigula* (Murphy et al. 1976; Fulhorst et al. 2001; Milazzo & Fulhorst 2012;). Other New World arenaviruses (Machupo virus in *Calomys callosus*, Junin virus in *Calomys musculinus* and Guanarito virus in *Zygodontomys brevicauda*) establish a chronic infection in all inoculated newborns and in about half of the adults (Webb et al. 1975; Vitullo et al. 1987; Fulhorst et al. 1999). In contrast, Latino arenavirus causes acute or chronic infections in newborn and acute infections in adult *C. callosus* (Webb et al. 1975). For the MORV system, it would be interesting

to perform additional CMR studies during the beginning of *M. natalensis'* breeding season (i.e. may-June). During this low host density period, the transmission chain is heavily dependent on a few susceptible and infectious individuals only (Goyens et al. 2013). A better estimation of the percentage of chronically infected animals might help to understand how MORV can persist through these host population bottlenecks.

Maternal antibodies

The presence of maternal Abs could explain one, and perhaps two of the 20 animals with different infection patterns than expected from the laboratory. Fig S29 (individual 210F6) shows a young individual (body weight at first capture was 19g) of which the first sample was Ab-positive and the second negative, while all samples were vRNA-negative. It could therefore be the case that this individual still had some maternal Abs at very low titers when it was first sampled, but then lost the Abs. Another aberrant pattern was observed for a young individual shown in Fig S10 (330F3) (body weight at first capture were 20g). Here, maternal Abs might explain the presence and rapid decrease of Ab titers, followed by an vRNA-positive blood and excretion sample. For the latter individual, initial Ab titers were as high as those observed after inoculation in the laboratory, which is unusual for maternal Abs although high maternal Ab titers have also been observed against Sin Nombre hantavirus in naturally infected *Peromyscus maniculatus* (Borucki et al. 2000) and for a number of different pathogens in *Microtus pennsylvanicus* (Glass et al. 1990). Because it is suggested that maternal Abs can play a substantial role in the transmission dynamics of parasites [e.g. Puumala hantavirus in *Myodes glareolus* (Kallio et al. 2006; 2010; 2013; Voutilainen et al. 2016)], it would be interesting to further investigate to which extent maternal Abs might influence the dynamics of MORV.

Recent infection without positive MORV vRNA sample

Finally, there is some indication that blood is not always vRNA-positive (or concentrations lie below the detection threshold of the PCR assay) during the first week after infection. Five individuals [depicted in Fig S5 (3710100F1), Fig S9 (210F3), Fig S11(340F3), Fig S23 (630F5) and Fig S34 (880F6)] show an Ab-titer pattern that strongly suggests recent infection, albeit without an vRNA-positive blood sample shortly after infection. This pattern can also be explained by Ab titers that temporarily (e.g. somewhere between the 100-120 day time interval) lie below the detection threshold and increase again two weeks later, as such mimicking the situation in the laboratory where Ab-titres show the highest-slope increase between day 6 and 20 after infection.

Conclusion

The majority of MORV infection patterns observed in the field seems to fit the laboratory data, which means that more often than not it is possible to use laboratory patterns of MORV as a basis for the interpretation of field samples. Note that although we found a relatively good match between laboratory and field data, we do not provide evidence that natural infection patterns are generally the same as those in the laboratory (it is possible to reject a statistical null hypothesis but not to prove it). What we did find in this study is that based on the observed similarities there is no evidence to reject the assumption that natural and laboratory infection patterns are similar. For the remaining cases where we did observe a mismatch between field and laboratory patterns, simple hypotheses (Ab detection threshold, chronic infection, and maternal Abs) exist that could explain the patterns, and should thus be considered when interpreting field samples. Overall, our results are encouraging, as they support the use of experimental infection studies for analysing infection patterns in natural as well as laboratory studies, although they do show that extrapolation to field data should be done with caution. Results of controlled infection experiments can then be used to estimate TOI of animals in natural populations, which in turn enables estimating epidemiological parameters (e.g. incidence or basic reproductive number) more accurately than when based on momentary absence/presence information only.

Ethics Approval

All the procedures followed the Animal Ethics guidelines of the Research Policy of Sokoine University of Agriculture as stipulated in the "Code of Conduct for Research Ethics" (Revised version of 2012), and the guidelines in Sikes and Gannon (Gannon 2007). The used protocol was approved by the University of Antwerp Ethical Committee for Animal Experimentation (2015–69) and adhered to the EEC Council Directive 2010/63/EU.

Authors' contributions

Conceived the study: JM and BB. Wrote the paper: JM and BB. Performed the experiments: JM, BB, SGr, BBZ and BvdB. Performed the analyses: JM, BB and JR. Supervised field and laboratory work: SGu, RM, AM and HL. All authors read and approved the final manuscript.

Tables

Table 1: Number of *M. natalensis* recaptured during multiple trapping sessions.

Times captured	Total	Number of individuals			
		Antibody & MORV RNA negative	Antibody positive[a]	MORV RNA positive[b]	Antibody & MORV RNA positive[c]
2	149	104	15	17	14
3	39	30	3	2	4
4	27	20	1	1	5
5	3	2	0	1	0
6	2	0	0	0	0
7	1	1	0	0	0
Total	221	157	19	21	23

[a] Ab-positive during at least one trapping session, but always vRNA-negative

[b] vRNA-positive during at least one trapping session, but always Ab-negative

[c] Ab and vRNA-positive during at least one trapping session

Table 2: Matches between field and laboratory data of *M. natalensis* infected with MORV for the two different TOI methods.

	Time of infection method			
	1. Antibody	**2. Antibody & MORV vRNA**		
	Ab titers	Ab titers	Blood	Excretions
Individuals	88% (37/42)	67% (28/42)	71% (27/38)	87% (34/39)
Observations	91% (82/90)	78% (73/94)	85% (69/81)	93% (76/82)

For both TOI estimation methods the observed percentages of matches between field and laboratory data are given on the individual level and total number of observations: method 1 is based on Ab titers only and method 2 on both Ab titres and vRNA presence in blood and excretions.

Supplementary texts

Supplementary text 1: Field work

Between 30 July 2013 and 18 October 2013, a rodent capture-mark-recapture (CMR) study was performed in five grids of 100m by 100m in Morogoro, Tanzania. In each grid, 100 Sherman live traps (Sherman Live Trap Co. Tallahassee, FL, USA) were placed at 10m intervals and baited with a mixture of peanut butter and ground maize. Distances between the grids were at least 1.5km (coordinates of the fields are given in supplementary table 1). Trapping sessions of

three consecutive nights each were repeated every other week. Captured *M. natalensis* were transported to the nearby laboratory (Pest Management Centre - Sokoine University of Agriculture, approx. 2km from the field sites), where weight, sex and reproductive status were determined, and mice were individually marked using toe clipping (Borremans et al. 2015). Blood samples were taken from the retro-orbital sinus and preserved on prepunched filter paper (±15 µL/punch; Serobuvard, LDA 22, Zoopole, France). Saliva was collected by putting a small filter paper slip into the mouth of the animal for a few seconds. If the animal urinated, a urine sample was taken directly from the animal using filter paper. After sampling, animals were again released on the exact capture location. Filter papers were dried and stored in the dark, at ambient temperature (maximum temperature was 28°C) for two months, after which they were preserved at -20ºC in a locked plastic bag with dehydrating silica gel, as described by (Borremans 2014b).

Supplementary text 2: Detection and quantification of antibody and MORV RNA

All individuals that were captured during at least two different trapping sessions were analysed for the presence of anti-MORV antibodies (Abs) and viral RNA (vRNA). Blood samples were analysed for the presence of Abs by indirect immunofluorescence assay using MORV-infected Vero cells *as antigens and* polyclonal rabbit anti-mouse IgG (Dako, Denmark) as secondary antibodies. Dried blood spots on filter paper were punched out and eluted in phosphate buffer saline. Antibody titers were estimated using two-fold dilution series, starting with a minimum dilution of 1:10. Viral RNA extraction was performed on both blood and excretion (saliva and urine) samples as described by (Borremans et al. 2015), using the QIAmp vRNA Mini Kit (Qiagen, Hilden, Germany). RT-PCR was done following the protocol described in Günther et al. 2009. MoroL3359-forward and MoroL3753-reverse primers were used to target a 340 nucleotide portion of the RNA-dependent RNA polymerase gene of MORV (Günther, et al. 2009). All amplicons were confirmed by Sanger-sequencing at the Vlaams Instituut voor Biotechnologie (Antwerp, Belgium) and comparing the sequences to known MORV sequences in the software Geneious 7.0.6 (Kearse et al. 2012).

Supplementary text 3:Definition of a good match between field and laboratory data

We defined a good match for a field tested individual if all the Ab titers and/or vRNA presence/absence data fell within a particular credible band (CB). This CB was calculated

following (Borremans et al. 2016), and adjusted such that, given the number of recaptures for this particular individual, there is 95% chance that all observations fall within the CB. Consequently, the width of the CB depends on the number of recaptures of the individual. For each TOI method we calculated the percentage of *individuals* that matched the laboratory data. Note that the use of a 95% CB implies that in the theoretical situation where the Ab and/or vRNA temporal dynamics are identical to those measured in the laboratory, 95% of the mice would match the laboratory data. If the dynamics are different to those measured in the lab, one expects that the percentage of matches would be lower. We also calculated the percentage of *observations* that matched (fell inside) the laboratory data's 95% CB for each TOI method. Again, in case of dynamics similar to those in the laboratory, 95% of the data would match the laboratory data, and this percentage will be lower if the dynamics are different.

Supplementary Figures

The supplemenary figures described in the discussion can be found at the following link: https://link.springer.com/article/10.1007/s10393-017-1256-7

Supplementary Information Tables

Table1: Coordinates of fields on which rodents were trapped

Field	Corner	Coordinates
F1	1a	S06° 50.695' E037° 39.240'
F1	10a	S06° 50.675' E037° 39.202'
F1	1j	S06° 50.633' E037° 39.227'
F1	10j	S06° 50.657' E037° 39.262'
F3	1a	S06° 50.640' E037° 39.630'
F3	10a	S06° 50.600' E037° 39.617'
F3	1j	S06° 50.624' E037° 39.578'
F3	10j	S06° 50.664' E037° 39.599'
F4	1a	S06° 51.604' E037° 37.771'
F4	10a	S06° 51.573' E037° 37.791'
F4	1j	S06° 51.595' E037° 37.832'
F4	10j	S06° 51.595' E037° 37.832'
F5	1a	S06° 51.279' E037° 37.704’
F5	10a	S06° 51.237' E037° 37.726’
F5	1j	S06° 51.302' E037° 37.747’
F5	10j	S06° 51.301' E037° 37.758’
F6	1a	S06° 51.044' E037° 37.969’
F6	10a	S06° 51.002' E037° 37.995’
F6	1j	S06° 51.604' E037° 37.771’
F6	10j	S06° 51.064' E037° 38.015’

Table 2: Comparison of the Ab and vRNA patterns between field and laboratory mice for each recaptured Ab-positive mouse and both TOI methods

Fig	Individual	Method1: Ab	Method2: Ab+vRNA	Weight[a]	#Captured[b]	Field
1	354060F1	match	mismatch	40	2	F1
2	364060F1	match	match	44	2	F1
3	371090F1	match	match	42	2	F1
4	3510100F1	match	mismatch	40	3	F1
5	3710100F1	match	match	45	2	F1
6	15F3a	match	mismatch	36	4	F3
7	15F3b	match	mismatch	15	2	F3
8	38F3	match	match	17	3	F3
9	210F3	match	match	25	4	F3
10	330F3	match	mismatch	20	2	F3
11	340F3	match	match	48	2	F3
12	390F3	match	mismatch	54	2	F3
13	550F3	match	match	35	2	F3
14	1670F3	match	mismatch	33	2	F3
15	20100F3	match	mismatch	24	2	F3
16	140F4	match	match	46	2	F4
17	360F4	match	match	60	4	F4
18	890F4	match	match	25	3	F4
19	1100F4	match	match	83	2	F4
20	4080F4	match	match	38	2	F4
21	260F5	mismatch	mismatch	52	3	F5
22	370F5	mismatch	mismatch	28	4	F5
23	630F5	match	match	25	2	F5
24	3090F5	match	mismatch	28	2	F5
25	4090F5	match	match	11	2	F5
26	38F6	match	mismatch	38	4	F6
27	48F6	match	mismatch	45	4	F6
28	180F6	match	match	46	2	F6
29	210F6	mismatch	mismatch	19	3	F6
30	340F6	mismatch	match	47	4	F6
31	420F6	match	match	44	2	F6
32	670F6	match	mismatch	19	2	F6
33	760F6	match	mismatch	46	4	F6
34	880F6	match	match	43	2	F6
35	1770F6	match	match	50	2	F6
36	2680F6	match	mismatch	37	2	F6
37	3100F6	mismatch	mismatch	41	4	F6
38	4530F6	match	match	62	2	F6
39	8100F6	match	match	22	2	F6
40	11090F6	match	mismatch	27	2	F6
41	30100F6	match	mismatch	21	3	F6

42	110100F6	match	match	22	2	F6

[a]Weight= weight of the animal on its first capture

[b]#captured= number of times that the animal was recaptured in the field

Table 3: Comparison of Morogoro virus (MORV) antibody (Ab) titers between field and laboratory mice. For each field data point the difference with the credible band (CB) of the laboratory data was given. 'CB' indicates that the field data point falls within the CB. Time of infection (TOI) was based on method 1 (only Ab titer).

Individual	TOI	Ab titers	Day capture	Difference with CB
354060F1	-34	0	0	Not possible
354060F1	-34	60	40	CB
364060F1	92	30	0	CB
364060F1	92	300	41	CB
371090F1	9	3000	0	CB
371090F1	9	12000	12	CB
3510100F1	141	600	0	CB
3510100F1	141	3000	14	CB
3510100F1	141	6000	29	CB
3710100F1	-2	0	0	Not possible
3710100F1	-2	12000	14	CB
15F3a	170	6000	0	CB
15F3a	170	3000	27	CB
15F3a	170	3000	41	CB
15F3a	170	3000	53	CB
15F3b	11	12000	0	CB
15F3b	11	6000	32	CB
38F3	-50	0	0	Not possible
38F3	-50	0	27	Not possible
38F3	-50	60	56	CB
210F3	-47	0	0	Not possible
210F3	-47	0	29	Not possible
210F3	-47	0	33	Not possible
210F3	-47	6000	57	CB
330F3	13	24000	0	CB
330F3	13	1500	56	CB
340F3	-19	0	0	Not possible
340F3	-19	6000	29	CB
390F3	-33	0	0	Not possible
390F3	-33	150	39	CB
550F3	12	12000	0	CB
550F3	12	18000	17	CB
1670F3	39	3000	0	CB
1670F3	39	1500	13	CB
20100F3	-22	0	0	Not possible
20100F3	-22	30	28	CB
140F4	-36	0	0	Not possible
140F4	-36	30	42	CB
360F4	170	12000	0	CB
360F4	170	3000	16	CB
360F4	170	3000	29	CB
360F4	170	3000	59	CB
890F4	24	12000	0	CB
890F4	24	6000	14	CB
890F4	24	3000	26	CB
1100F4	133	300	0	CB

1100F4	133	1200	14	CB
4080F4	170	3000	0	CB
4080F4	170	3000	14	CB
260F5	84	30	0	CB
260F5	84	0	14	2.23
260F5	84	0	27	2.26
370F5	71	1.1	0	2.72
370F5	71	60	16	CB
370F5	71	60	29	CB
370F5	71	60	59	CB
630F5	-22	0	0	Not possible
630F5	-22	60	28	CB
3090F5	-8	0	0	Not possible
3090F5	-8	150	14	CB
4090F5	-8	0	0	Not possible
4090F5	-8	300	14	CB
38F6	36	3000	0	CB
38F6	36	1500	15	CB
38F6	36	600	29	CB
38F6	36	300	41	CB
48F6	18	24000	0	CB
48F6	18	12000	14	CB
48F6	18	3000	27	CB
48F6	18	1500	41	CB
180F6	-4	0	0	Not possible
180F6	-4	6000	14	CB
210F6	92	30	0	CB
210F6	92	0	14	2.37
340F6	30	24000	0	CB
340F6	30	6000	16	CB
340F6	30	1500	26	CB
340F6	30	1500	58	0.16
420F6	59	1500	0	CB
420F6	59	30	13	CB
670F6	-22	0	0	Not possible
670F6	-22	60	28	CB
760F6	170	3000	0	CB
760F6	170	1200	14	CB
760F6	170	1500	27	CB
760F6	170	3000	42	CB
880F6	-17	0	0	Not possible
880F6	-17	3000	26	CB
1770F6	40	6000	0	CB
1770F6	40	150	13	CB
2680F6	51	1500	0	CB
2680F6	51	600	13	CB
3100F6	79	1.1	0	2.23
3100F6	79	60	15	CB
3100F6	79	0	26	2.13
3100F6	79	0	40	2.26
4530F6	34	12000	0	CB
4530F6	34	6000	16	CB
8100F6	55	1200	0	CB
8100F6	55	300	11	CB
11090F6	10	6000	0	CB
11090F6	10	300	52	CB
30100F6	11	12000	0	CB
30100F6	11	12000	11	CB

30100F6	11	12000	26	CB
110100F6	140	600	0	CB
110100F6	140	600	15	CB

Table 4: Comparison of Morogoro virus (MORV) antibody (Ab) titer and vRNA presence/absence between field and laboratory mice. For each field data point the difference with the credible band (CB) of the laboratory was given. 'CB' indicates that the field data point falls within the CB. Time of infection (TOI) was based on method 2 (both Ab and vRNA titer in blood and urine).

Individual	TOI	Ab titers	MORV RNA blood	MORV RNA excretions	Day capture	Difference with CB Ab	Difference with CB blood	Difference with CB excretions
354060F1	5	0	yes	yes	0	CB	CB	CB
354060F1	5	60	No	NA	40	1.77	CB	NA
364060F1	6	30	yes	No	0	CB	CB	CB
364060F1	6	300	No	No	41	CB	CB	CB
371090F1	9	3000	yes	No	0	CB	CB	CB
371090F1	9	12000	No	No	12	CB	CB	CB
3510100F1	7	600	yes	No	0	CB	CB	CB
3510100F1	7	3000	No	No	14	0.77	CB	CB
3510100F1	7	6000	No	No	29	CB	CB	CB
3710100F1	-2	0	No	No	0	Not possible	Not possible	Not possible
3710100F1	-2	12000	NA	NA	14	CB	NA	NA
15F3a	10	6000	No	No	0	CB	CB	CB
15F3a	10	3000	yes	yes	27	CB	0.01	CB
15F3a	10	3000	No	No	41	CB	CB	CB
15F3a	10	3000	No	No	53	CB	CB	CB
15F3b	10	12000	NA	No	0	CB	NA	CB
15F3b	10	6000	yes	No	32	CB	0.01	CB
38F3	-50	0	No	No	0	Not possible	Not possible	Not possible
38F3	-50	0	No	No	27	Not possible	Not possible	Not possible
38F3	-50	60	yes	No	56	CB	CB	CB
210F3	-46	0	No	No	0	Not possible	Not possible	Not possible
210F3	-46	0	No	No	29	Not possible	Not possible	Not possible
210F3	-46	0	No	No	33	Not possible	Not possible	Not possible
210F3	-46	6000	No	No	57	CB	CB	CB
330F3	13	24000	No	No	0	CB	CB	CB
330F3	13	1500	yes	yes	56	CB	0.03	0.03
340F3	-19	0	NA	NA	0	Not possible	NA	NA
340F3	-19	6000	NA	No	29	CB	NA	CB
390F3	88	0	No	yes	0	2.43	CB	0.03
390F3	88	150	No	yes	39	CB	CB	0.03
550F3	14	12000	NA	No	0	CB	NA	CB
550F3	14	18000	NA	No	17	CB	NA	CB
1670F3	9	3000	yes	No	0	CB	CB	CB
1670F3	9	1500	yes	yes	13	1.63	CB	CB
20100F3	5	0	yes	No	0	CB	CB	CB
20100F3	5	30	yes	yes	28	4.04	0.02	CB

140F4	-36	0	No	NA	0	Not possible	Not possible	NA
140F4	-36	30	yes	No	42	CB	CB	CB
360F4	170	12000	NA	No	0	CB	NA	CB
360F4	170	3000	NA	No	16	CB	NA	CB
360F4	170	3000	No	No	29	CB	CB	CB
360F4	170	3000	NA	NA	59	CB	NA	NA
890F4	32	12000	No	NA	0	CB	CB	NA
890F4	32	6000	No	No	14	CB	CB	CB
890F4	32	3000	No	No	26	CB	CB	CB
1100F4	133	300	No	No	0	CB	CB	CB
1100F4	133	1200	NA	No	14	CB	NA	CB
4080F4	170	3000	No	No	0	CB	CB	CB
4080F4	170	3000	No	No	14	CB	CB	CB
260F5	60	30	NA	No	0	Not possible	NA	CB
260F5	60	0	yes	No	14	Not possible	0.02	CB
260F5	60	0	No	NA	27	Not possible	CB	NA
370F5	38	1.1	No	No	0	6.28	CB	CB
370F5	38	60	yes	No	16	0.52	0.01	CB
370F5	38	60	No	No	29	CB	CB	CB
370F5	38	60	No	No	59	CB	CB	CB
630F5	-22	0	No	No	0	Not possible	Not possible	Not possible
630F5	-22	60	NA	No	28	CB	NA	CB
3090F5	113	0	No	yes	0	2.42	CB	0.03
3090F5	113	150	No	No	14	CB	CB	CB
4090F5	-5	0	No	NA	0	Not possible	Not possible	NA
4090F5	-5	300	No	yes	14	CB	CB	CB
38F6	6	3000	yes	yes	0	0.93	CB	CB
38F6	6	1500	yes	yes	15	1.36	CB	CB
38F6	6	600	yes	No	29	0.52	0.01	CB
38F6	6	300	yes	yes	41	CB	0.01	0.01
48F6	10	24000	No	No	0	CB	CB	CB
48F6	10	12000	No	NA	14	CB	CB	NA
48F6	10	3000	No	No	27	CB	CB	CB
48F6	10	1500	yes	yes	41	CB	0.01	0.01
180F6	-4	0	No	No	0	Not possible	Not possible	Not possible
180F6	-4	6000	yes	NA	14	CB	CB	NA
210F6	94	30	No	No	0	CB	CB	CB
210F6	94	0	No	No	14	2.38	CB	CB
340F6	18	24000	No	No	0	CB	CB	CB
340F6	18	6000	No	yes	16	CB	CB	CB
340F6	18	1500	No	No	26	CB	CB	CB
340F6	18	1500	No	No	58	CB	CB	CB
420F6	59	1500	No	No	0	CB	CB	CB
420F6	59	30	No	NA	13	CB	CB	NA
670F6	-19	0	NA	NA	0	Not possible	NA	NA
670F6	-19	60	No	yes	28	0.47	CB	CB
760F6	24	3000	No	yes	0	0.75	CB	CB
760F6	24	1200	No	No	14	CB	CB	CB

760F6	24	1500	No	No	27	CB	CB	CB
760F6	24	3000	No	No	42	CB	CB	CB
880F6	-16	0	No	NA	0	Not possible	Not possible	NA
880F6	-16	3000	No	NA	26	CB	CB	NA
1770F6	47	6000	No	No	0	CB	CB	CB
1770F6	47	150	No	No	13	CB	CB	CB
2680F6	40	1500	No	NA	0	CB	CB	NA
2680F6	40	600	yes	No	13	CB	0.03	CB
3100F6	48	1.1	No	No	0	5.25	CB	CB
3100F6	48	60	No	No	15	CB	CB	CB
3100F6	48	0	NA	NA	26	2.53	NA	NA
3100F6	48	0	yes	NA	40	2.19	0.01	NA
4530F6	12	12000	yes	No	0	CB	CB	CB
4530F6	12	6000	No	yes	16	CB	CB	CB
8100F6	55	1200	No	No	0	CB	CB	CB
8100F6	55	300	No	No	11	CB	CB	CB
11090F6	11	6000	yes	yes	0	CB	CB	CB
11090F6	11	300	yes	No	52	CB	0.03	CB
30100F6	9	12000	yes	No	0	0.21	CB	CB
30100F6	9	12000	NA	yes	11	CB	NA	CB
30100F6	9	12000	No	No	26	CB	CB	CB
110100F6	140	600	No	No	0	CB	CB	CB
110100F6	140	600	No	No	15	CB	CB	CB

Seasonal host dynamics translate into density-dependent arenavirus cycles

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(Manuscript in preparation)

Abstract

A key aim in wildlife disease ecology is to understand how host and parasite characteristics influence parasite transmission and persistence. Host population density is one of the factors that can severely affect transmission, and theory predicts certain transmission-density patterns depending on how parasites are transmitted between individuals. Here, we present the results of a study investigating the dynamics of Morogoro arenavirus in a population of multimammate mice (*Mastomys natalensis*) using a ten-year capture-mark-recapture time series from Tanzania. *Mastomys natalensis* is the natural host of Morogoro virus and known for its strong seasonal density fluctuations. We observed that Morogoro virus seroprevalence correlates positively with host density with a lag of one to four months. By fitting the field data to a mathematical model, we find that the seasonal seroprevalence pattern can be best explained by a combination of vertical and density-dependent horizontal transmission, and that a small number of animals needs to be infected chronically to ensure viral persistence. Because of these persistent infections, we predict rodent control to be impractical to eliminate arenaviruses from *Mastomys* populations, which is highly relevant given the similarities between Morogoro and Lassa arenavirus.

Introduction

Ecological factors can exert strong pressures on wildlife populations, often resulting in substantial seasonal or multi-annual density fluctuations (Stenseth et al. 2002, Krebs 2013; Myers 2018). These fluctuations can influence the transmission dynamics of many parasites, and have been linked to disease outbreaks in both wildlife and humans (Davis et al. 2004; Mills 2005; Altizer et al. 2006; Hartley et al. 2012). A common expectation for directly transmitted parasites is that prevalence and persistence should increase with host density (Anderson & May, 1979). Although numerous field studies have focused on this relationship, linear positive correlations are only rarely observed (Davis et al. 2005; Lloyd-Smith et al. 2005a). This could be partly explained by the short duration of most field studies (mostly < than five years), but also by the fact that density will not always affect prevalence in a simple linear way. After all, transmission dynamics result from the complex interplay between a range of host and parasite characteristics including demography, transmission modes, shedding pattern, environmental survival and immune response (Hudson et al 2002; Begon et al. 2009).

Host density can affect parasite prevalence in different ways (Davis et al. 2005). For directly transmitted parasites, these differences are mainly the result of the way in which host contact rate changes with density, which can be linear (density-dependent transmission), constant (frequency-dependent transmission) or nonlinear (e.g. power or sigmoidal relation; Begon et al. 2002; Ryder et al. 2007; Smith et al. 2009). In addition, it is known that a lack of correlation between prevalence and current density does not preclude a correlation with past density. When the delay between past density and prevalence is short relative to the timescale of the host density fluctuations, then a positive correlation can still be expected. In contrast, when the delay is long, none or even an inverse relationship might occur. Such an inverse relationship can be the result of an influx of susceptible juveniles that enter the population (i.e. a juvenile dilution effect; (Mills et al. 1999). This situation is likely when host birth is limited to short breeding periods (birth pulses) and when the likelihood of becoming infected increases with age, thus when transmission is (mainly) horizontal (Adler et al. 2008). In contrast, a dilution effect is not predicted for vertically transmitted parasites, as infectious hosts immediately enter the population at birth, increasing host density and prevalence simultaneously (Davis et al. 2005).

Predictions concerning parasite persistence equilibria also depend on specific characteristics (Ryder et al. 2007). For parasites with density-dependent transmission, a density threshold (N_T) is predicted below which the parasite cannot persist in the host population (Begon et al. 2003; Lloyd-Smith et al. 2005a). Such density thresholds are more likely to be crossed for wildlife hosts that exhibit large density fluctuations in time or space, for example plague in great gerbils (*Rhombomys opimus*; Davis et al. 2004) and brucellosis in herds of bison (Davis et al. 2004; Dobson & Meagher, 1996). A high N_T is expected for host populations with tight birth pulses and low demographic turnover rates, and for parasites with short infectious periods (acute infections) that evoke a life-long immune response in the host (Peel et al. 2014). In contrast, for parasite-host systems with frequency-dependent transmission, long infectious periods (chronic infections) and/or waning immunity, no (or low) density thresholds are predicted and such infections are expected to persist even in very low-density populations (Anderson & May, 1981; Antonovics et al. 2017; Lloyd-Smith et al. 2005a). Given the difference in persistence probability, understanding the role of these different parasite-host characteristics is an important prerequisite for the development of wildlife disease control programs (e.g. to predict the effectiveness of culling to eliminate a disease; Potapov et al. 2012; Morters et al. 2012; Borremans et al. 2017).

Here, we investigate the relationship between population density and transmission characteristics for Morogoro arenavirus in a population of its reservoir host, the Natal multimammate mouse (*Mastomys natalensis*). The MORV-*M. natalensis* system is a suitable model for examining parasite-host interactions in natural conditions, as the ecology of the host and the virus have been studied intensively and the virus is not pathogenic for humans (Borremans et al. 2011; 2015; Gryseels et al. 2017; Günther et al. 2009; Leirs, 1994; Mariën et al. 2017; Sluydts et al, 2007). The system provides also a safe substitute for studying closely related but pathogenic arenaviruses such as Lassa virus (LASV), the etiologic agent of Lassa fever in humans (Monath 1987). MORV transmission is assumed to occur both horizontally and vertically in *M. natalensis*, based on the observations that seroprevalence increases with age, and virus RNA has been detected in very young individuals (Borremans et al. 2011, Mariën et al. 2017a). The available data suggest that most animals are acutely infected in natural conditions, shedding up to 30-40 days after infection (Borremans et al. 2011; Borremans et al. 2015; Mariën et al. 2017). Infected animals develop a lifelong antibody response and seem to experience no or only small pathogenic effects (Borremans et al. 2015; Mariën et al. 2017a Mariën et al. 2017b; Mariën et al. 2018).

The distribution of MORV is limited to *M. natalensis* in East Africa, where infection prevalence ranges from 0 to 20% (Goüy de Bellocq et al. 2010; Günther et al. 2009; Borremans et al. 2011; Gryseels et al. 2017). In this region, *M. natalensis* populations exhibit strong density fluctuations between seasons and years, generally ranging from 20-300 individuals per hectare but occasionally reaching outbreak densities of 600 individuals/ha or higher (Leirs, 1994; Sluydts et al. 2009). The seasonal fluctuations are the result of a bimodal rain pattern with long (March-May) and short (November-December) rains. Breeding is triggered by sprouting young grass at the end of the long rains and lasts until November, when the population size peaks. Shortly hereafter, the population decreases due to competition, food scarcity and other environmental conditions, reaching its lowest point around May (Leirs et al. 1990). *M. natalensis* has a promiscuous mating system and is not territorial or aggressive towards conspecifics (Kennis et al. 2008; Veenstra, 1958). Home range overlap is generally high and increases significantly with abundance, suggesting contact rates to be density-dependent, probably nonlinearly (Borremans et al. 2013; 2016; 2017). Given that MORV transmission is most likeley density-dependent, infection is predominantly acute, and the immune response is lifelong, it is surprising that MORV can persist during low host density periods, when host density would be expected to be below the N_T (Goyens et al. 2013).

In order to investigate if the seasonal *M. natalensis* fluctuations induce seasonal MORV seroprevalence cycles (i); transmission is affected by host density (ii); a juvenile dilution effect occurs at the end of the breeding season (iii); vertical transmission affects this dilution effect (iv); and chronic carriers are necessary to ensure viral persistence (v), we analysed a ten-year capture-mark-recapture time series and compared the results to simulations of an individual-based (mathematical) model. To further investigate whether chronically infected individuals are indeed present in natural conditions, we performed a small lab experiment in which wild-caught rodents were caged for eight weeks, sampled each week and afterwards dissected.

Methods

Study area and trapping

Between May 2007 and April 2017, a rodent capture-mark-recapture experiment was performed on the campus of the Sokoine university of Agriculture (Morogoro, Tanzania) on a rectangular grid of 300x100 m. The trapping area is a mosaic environment of maize field, wood and fallow land, in which *M. natalensis* is the dominant rodent species (>95% of all captures). A robust trapping design was used with trapping sessions conducted every month for three consecutive nights. Sherman live traps (Sherman Live Trap Co. Tallahassee, FL, USA) were placed evenly at 10m intervals (300 in total) and baited in the evening with a mixture of peanut butter and corn flour. Traps were checked the next morning and transported to the laboratory of the university, where species, weight, sex, and reproductive status were recorded (Leirs, 1994; Sluydts et al. 2007). Blood samples were taken with a heparinised microcapillary tube from the retro-orbital sinus and preserved on prepunched filter paper (±15 µL/punch; Serobuvard, LDA 22, Zoopole, France). Samples were taken only once per rodent per trapping session, so blood was not taken again for animals that were recaptured the same three-day session. Each rodent was individually marked by toe clipping (Borremans et al. 2015), and afterwards released at its capture site.

Serology

Filter papers were dried and stored in the dark at ambient temperature in a sealed plastic bag with dehydrating silica gel and since 2014 preserved at -20ºC. Dried blood spots were punched out of the filter paper and eluted in phosphate buffer saline and 0.25% NH_3 (Borremans 2014). Presence of anti-MORV antibodies in this solution was examined by indirect immunofluorescence assay (Günther et al. 2009). For this assay, Vero cells infected with

MORV were spread on immunofluorescence slides, air dried, and acetone-fixed. Antibodies in positive samples would then bind to antigens presented by the vero cells and be visualized with polyclonal rabbit anti-mouse IgG-FITC secondary antibodies (Dako, Denmark). Most samples were tested only once, but if they were uncertain, they were tested a second time for the final decision. Because the filter paper quality for the first three years of the time series was not sufficiently reliable, we removed these years from the dataset for the statistical analyses.

CMR data

The trapping effort resulted in 13,734 captures of 6,380 unique individuals during 111 trapping sessions. 950 individuals were positive at least once, of which 206 seroconverted from Ab-negative to positive in between primary trapping sessions. A small number of animals (n=58) showed an apparent loss of Ab. These negative samples were assumed to be false negatives due to Ab titers falling below the detection threshold of the assay, as *M. natalensis* normally develops a long-term Ab production after infection (Mariën et al. 2017). These samples were considered positive for further analyses, except for very young individuals (body weight at first capture < 15g) that still might have had maternally-derived Ab, which are likely to disappear after three weeks.

Ethics approval

All the procedures followed the Animal Ethics guidelines of the Research Policy of Sokoine University of Agriculture as stipulated in the 'Code of Conduct for Research Ethics' (Revised version of 2012) and the guidelines in (Sikes and Gannon 2007). The used protocol was approved by the University of Antwerp Ethical Committee for Animal Experimentation (2015–69) and adhered to the EEC Council Directive 2010/63/EU.

Seasonality and seroprevalence

In order to investigate how host density and seroprevalence correlate, we first decomposed each time series into a seasonal, trend and random component. Decomposition was necessary because the time series were variable (due to random noise and an overall increasing trend with time), and we were primarily interested in the seasonal pattern. Confidence intervals (95%) for the seasonal components were estimated by performing random permutations (5000 simulations), in which we changed the order of the years. June was selected as the onset of a biological year in these calculations, because that is when population density starts to increase. The possibility of time delays in the effect of density on seroprevalence was investigated by examining the cross-correlations at various time lags (-6 to 6 months).

To test whether the seasonal pattern in the seroprevalence time series was significant, we developed four generalized additive models (GAMs) with seroprevalence as a binomial response variable (logit-link function) and a seasonal and/or year component as explanatory variables (Voutilainen et al. 2016). GAM's were used because we expected a nonlinear response for the seasonal pattern of the seroprevalence. Model 1 (GAM1) was the intercept model to which we could compare the other models. Model 2 (GAM2) contained the seasonal effect in which seroprevalence was smoothed in a nonlinear way over the different months (k=12) using cyclic-cubic regression splines, which limited discontinuity between the end and the beginning of a new year. The optimal amount of smoothing was determined by cross-validation using the built-in function of the R-package (gamm4). Model 3 (GAM3) contained a fixed year effect (2010-2017), which allowed to investigate whether the overall seroprevalence varied significantly between years. Model 4 (GAM4) combined the smoothed seasonal and fixed year effects. Selection of the latter model would suggest a seasonal effect of which the average value changed between years. We did not include a model with an interaction between season and year because it would result in a perfect fit and is therefore not meaningful for comparison. Model selection was performed based on Akaike information criterion (AIC) and R^2-values. All analyses were performed in R using the R-packages: lubridate, mgcv, gamm4, ggplot2, scales and mvtnorm (Wickham 2009; Garrett 2011; Alan et al. 2017; Wickham 2017; Wood 2017; Wood and Scheipl 2017).

Mathematical modelling

Based on data from this and previous field and modelling studies, we built a stochastic individual-based model (IBM) that took into account host demography and MORV infection dynamics (Goyens et al. 2013). The model allowed to explore how the shape of the horizontal transmission function (frequency dependent, linearly or nonlinearly density dependent), the level of vertical transmission (presence or absence) and the percentage of chronically infected animals might influence MORV epidemics. The IBM is explained using the schematic depicted in Figure 1. Individuals are categorized into one of six states: susceptible (S), exposed but not infectious (E), acutely infectious (I), recovered (R), protected by maternal antibodies (M), and chronically infectious (C). Birth, death and state transition events were a function of time (unit of time is 1 day) and stochastic.

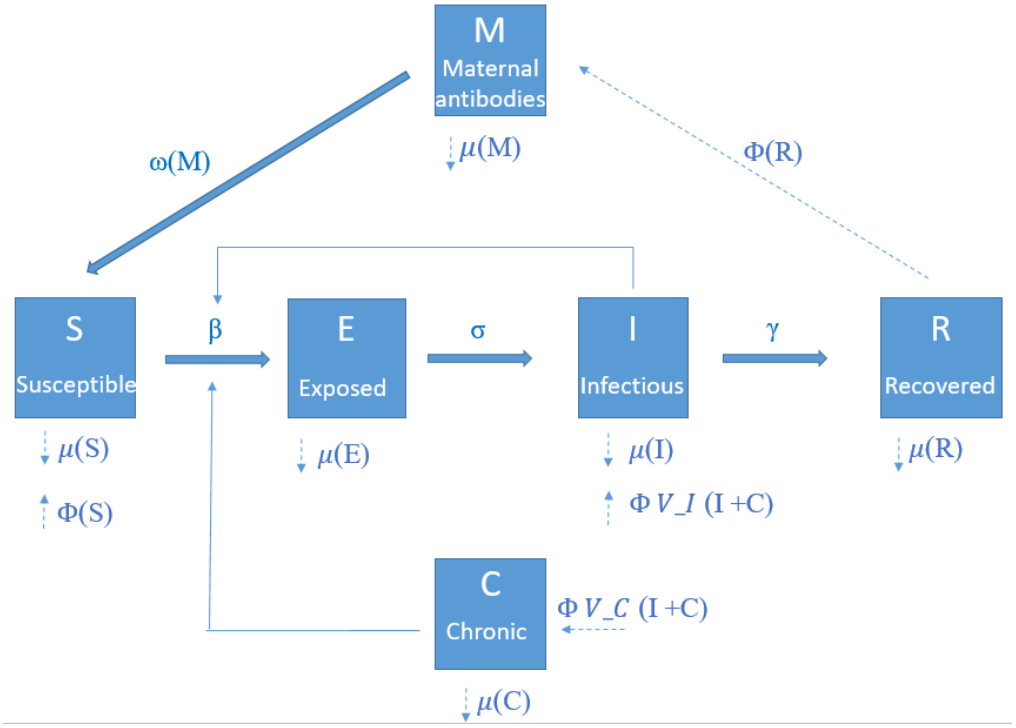

Fig 1: Schematic view of the individual-based model used to simulate the spread of Morogoro virus in populations of *M. natalensis* in Tanzania. Individual rodents are assigned to different states according to infection status: susceptible (S), exposed (E), acutely infectious (I), recovered (R), maternal antibody positive (M) and chronically infectious (C). State transition rates depend on the following parameters: transmission coefficient (β), latent period (σ^{-1}), infectious period (γ^{-1}), maternal antibody period (ω^{-1}). Fat solid arrows indicate possible transitions between different states. The dashed lines show the demographic parameters: Φ (birth rate) and μ (mortality rate). The probability to become acutely infected after vertical transmission is given by V_I and to become chronically infected by V_C. Thin solid arrows indicate that the rate at which individuals move from one state to another depends on the number of individuals in another state.

Demographic component

The main reason we used an IBM was that it allowed to assess the age distribution of animals in the population. Implementing a realistic age distribution was a prerequisite for comparing predicted and observed seroprevalence, as the age distribution of *M. natalensis* changes strongly during the year (due to seasonal birth pulses and high mortality) and the likelihood of being Ab-positive increases significantly with age. Both demographic parameters (birth = Φ and mortality = μ) were implemented to obtain a realistic monthly age distribution (similar to data of Leirs 1994) and abundance (similar to the abundance time series in this study) (Fig S1). The demographic parameters were allowed to change each time step as a function of time and individual age, which means that each individual could have a different birth and mortality rate each time step. Since we recently showed that MORV has no or only limited adverse effects on

M. natalensis (Mariën et al. 2017; 2018), the model assumes that the birth and death rates are equal for susceptible and infected animals. We highlight that modeling demography was not the prime goal of this study and that the parameters were optimized only to result in a realistic age distribution and rodent density, while being still biologically meaningful. A more detailed explanation of the demographic parameters is presented in the supplementary information.

Horizontal transmission: density-dependence

Horizontal (i.e. between individuals, as opposed to vertical transmission from mother to offspring) transmission of MORV was modeled as infection rate $\frac{\beta K^q S(I+C)}{N^q}$, following the implementation of Smith et al. (2009). This formulation allows simple comparison of the different shapes of the transmission-density relation by adjusting the parameter q: if q=1, transmission is independent of density (frequency- dependence); if q=0, transmission is linearly related to density (density-dependence); and if 0<q<1, transmission follows a power function (intermediate between frequency- and density-dependence). K is a rescaling constant (individuals) that allows comparison of the transmission-density functions at different host densities (N). The parameter $\beta (= k * v)$ represents the transmission coefficient, which is composed of k (a constant that links the contact rate to a given density) and v (probability of transmission when an I individual contacts an S individual) (Begon et al. 2002). The parameter β was allowed to change seasonally between three biologically meaningful periods of the year: β_1 = January-April (low-density, no-breeding period); β_2 =April-July (low-density, breeding period); and β_3 =August-December (high-density, no-breeding period). For each of three values of q (0, 0.5, 1), the best values of the three βs were obtained by fitting model outcomes to the seroprevalence field data (model inference is explained below). Given that *M. natalensis* is not territorial and its home range overlap is generally high, we assumed a homogeneously mixing community in which all individuals are also identical with respect to susceptibility and infectivity (Borremans et al. 2014)

Vertical transmission

Vertical transmission (mother-to-offspring) of MORV is implemented using infection rate: V_I [Φ (I+C)] + V_C [Φ (I+C)]. The model assumes that vertically infected juveniles can enter the population as either acutely (I) or chronically (C) infectious. The addition of the chronic infectious state was necessary to ensure viral persistence (explained below). The parameter V_C defines the proportion of juveniles that will become chronically infectious at birth through vertical transmission. This parameter was set so that approximately 10% of the overall

infectious individuals in the population (I+C) became a chronic carrier, which matches results of a modelling study on LASV and other field and laboratory data on MORV (Walker et al. 1975; Fichet-Calvet et al. 2014; Borremans et al. 2015; Mariën et al. 2017a). Chronic carriers were assumed to stay in this state lifelong and shed at the same transmission rate as acutely infectious animals. The parameter V_I defines the proportion of juveniles that will become acutely infectious at birth through vertical transmission. As we were interested in how vertical transmission may influence MORV transmission dynamics, we compared models in which 90% (V_I = 0.9) of vertically infected juveniles become acutely infected (and 10% chronically) and models where no juveniles (V_I = 0) become acutely infected through vertical transmission (10% still become chronically infectious).

Transitions

The model assumes that when a susceptible individual becomes acutely infected, it first passes a latency period (E stage) for an average period of 4 days (σ^{-1}) during which it is not yet infectious. It then becomes infectious for an average of 35 days (γ^{-1}), after which it recovers from the disease and develops lifelong immunity (antibody presence). The values of the latency (σ^{-1}) and infectious period (γ^{-1}) were derived from an inoculation experiment (Borremans et al. 2015). Furthermore, we assume that individuals born to recovered mothers (R) will enter the population carrying maternally derived antibodies (ΦR). Juveniles in this state (M) will be immune against MORV for an average of 30 days (ω^{-1}), after which the antibodies disappear and individuals move to the susceptible state (S). The presence of maternal antibodies in juveniles has been suggested for both LASV and MORV in field studies and observed for LCMV in laboratory experiments (Oldstone et al. 2002; Fichet-Calvet et al. 2014; Mariën et al. 2017a). The model assumes that each infected mouse remains in one state for a fixed amount of time (uniform distribution with probability of transition 0 for the interval 0 to t) and afterwards moves to a different state depending on a probability that follows the exponential distribution (e.g. all individuals remain infectious for at least 25 days, but the final day of recovery changes between individuals).

Statistical inference

After constructing six models that differed in how horizontal (q = 0, 0.5, or 1) and vertical (V_i = 0.0 or 0.9) transmission were implemented, three transmission coefficients (for each period of the year: β_1, β_2, and β_3) had to be inferred for each model. As too many possible combinations of βs would result in the same model fit (e.g. when using Deviance Information Criterion for model selection), we performed a more conservative approach by selecting the

model with the lowest relative differences between βs as the unique preferred model. The rationale was that larger differences between βs would suggest that a combination of model components (absence/presence of vertical transmission; density/frequency dependent horizontal transmission) was less likely and needed compensation by a relative change in βs to obtain the same likelihood (e.g. supplementary figure S2). Thus, when there are no differences between the three periods, a model with three identical β*s* would increase our confidence that all of the seasonal variation in seroprevalence can be explained by the model components alone. In contrast, differences between βs would suggest unexplained variation in seroprevalence by the model components (e.g. by external factors such as climate conditions).

Bayesian inference of the βs was performed by running 20,000 Markov Chain Monte Carlo chains in which the parameters were updated sequentially using a standard random-walk Metropolis–Hastings algorithm (Gibson and Renshaw 1998). The likelihood function in this algorithm calculated the probability that the seroprevalence field data (D) were derived from the model simulations given the set of transmission coefficients [P(D|β_1, β_2, β_3)]. To determine this, the log-likelihoods for all field observations (of the seasonal component) were calculated using a truncated normal distribution and summarized. We used weak uniform priors *U*(0, 100) for the different β*s*. Simulations were run for 12 years, discarding the initial five years. In order to mimic stochasticity by the capture process, the same number of individuals was drawn randomly from the model population as the number of individuals that were captured and sampled in the field.

Viral persistence and chronic infections

To investigate whether the presence of chronically infected individuals is necessary for MORV persistence, we carried out model simulations in which we varied the proportion of juveniles that is born as chronic carriers (V_C). We varied V_C from 0 (no chronically infectious individuals) to 1 (all juveniles born to infectious mothers become chronic carrier) by steps of 0.025. The proportion of vertical transmission resulting in acute infections (V_I) was equal to 1- V_C. A different set of βs was fitted for each V_C to keep the basic reproductive number constant. To limit the large number of different models that would arise from each parameter combination, this step was only done using the previous step's best fitting model. Simulations (n=500) were run for each V_C at different populations sizes [from 5ha to 50ha fields], as the likelihood of persistence correlates positively with population size (Lloyd-Smith et al. 2005). Although the total population size could increase (equivalent to increasing the occupied area in

hectares), the range of population densities was kept constant at a maximum of 250 individuals/ha. If the virus did not go extinct within ten years, a model run was considered to be persistent (Bartlett 1957). The proportion of persistent simulations was used as a measure of persistence probability.

Cage experiment

In order to investigate if chronic infections are indeed present in natural conditions (as was suggested by the modelling results), we performed a small field experiments in which we caged wild *M. natalensis* for eight weeks. The higher number of sampling occasions (once per week in contrast to once per month for the CMR) allowed us the better examine how long animals remain infectious in nature.

From 6 July to 8 August 2016, 270 *M. natalensis* were captured using Sherman live traps in different maize fields surrounding the campus of the Sokoine University of Agriculture, Morogoro (Tanzania). Captured *M. natalensis* were transported to the laboratory of the university, where body weight, sex and reproductive status were determined, and mice were individually marked using toe clipping (Borremans et al. 2015). Animals were housed per two in cages (28 x 11.5 x 12 cm) where shelter and ad libitum food and water were provided. Blood, urine and saliva samples were taken the day of capture and each consecutive week. Saliva was collected by forcing the animal to chew on a small filter paper slip for a few seconds. Animals were kept in a plastic bag until they urinated. Urine was also preserved on filter paper. Filter papers were dried and stored as described previously. Maximally eight weeks after capture, the mice were euthanized using chloroform, humanely killed by cervical dislocation and dissected. The kidneys, liver and spleen were stored in RNAlater.

Weekly blood samples of all captured mice were screened for antibodies against MORV as described previously. For all captured mice, viral RNA was extracted from kidney biopsies using the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. As kidney samples usually give a higher RNA yield than filter paper samples (simple because there is more RNA material), we assumed that when kidney samples were negative filter paper samples would be negative too. Therefore, we only screened blood, urine and saliva samples for mice that contained MORV RNA positive kidneys. For the filter paper samples, viral RNA was extracted using the QIAmp vRNA Mini Kit (Qiagen, Hilden, Germany). RT-PCR was done on the L-segment using MoroL3359-forward and

MoroL3753-reverse primers following the protocol described in (Vieth et al. 2007; Günther et al. 2009). All amplicons were confirmed by Sanger-sequencing in both directions at the Vlaams Instituut voor Biotechnologie (Antwerp, Belgium).

Ethics approval

All the procedures followed the Animal Ethics guidelines of the Research Policy of Sokoine University of Agriculture as stipulated in the 'Code of Conduct for Research Ethics' (Revised version of 2012) and the guidelines in (Sikes and Gannon 2007). The used protocol was approved by the University of Antwerp Ethical Committee for Animal Experimentation (2015–69) and adhered to the EEC Council Directive 2010/63/EU.

Results

Seasonality and seroprevalence

There were clear seasonal cycles for both rodent abundance and seroprevalence (Fig 2). Peaks in rodent abundance occurred in November and troughs in May, while seroprevalence peaks occurred in January-April (26%; 95%CI 12-36%) and troughs in June-August (5%; 95%CI 1-12%) (Fig 3). This result matches the outcome of the cross-correlation analysis, which supported strong positive correlations between seroprevalence and density one to four months earlier (Fig 4). It also matches the results of the GAM analyses, as the model with the lowest AIC and highest adjusted R^2 (GAM4) supported a seasonal pattern in the seroprevalence time series (par=16, AIC=405,R^2=0.55) (table 1). This model was clearly preferred to the same model (GAM3) without seasonal component (par=6, AIC=456, R^2=0.33), suggesting that the month in which an individual is trapped is an important predictor of antibody status (df=6, chisq=60.87, $p < 0.0001$). The best fitting model also suggests that the overall seroprevalence differs significantly between years (df=5, chisq=93.06, $p < 0.0001$).

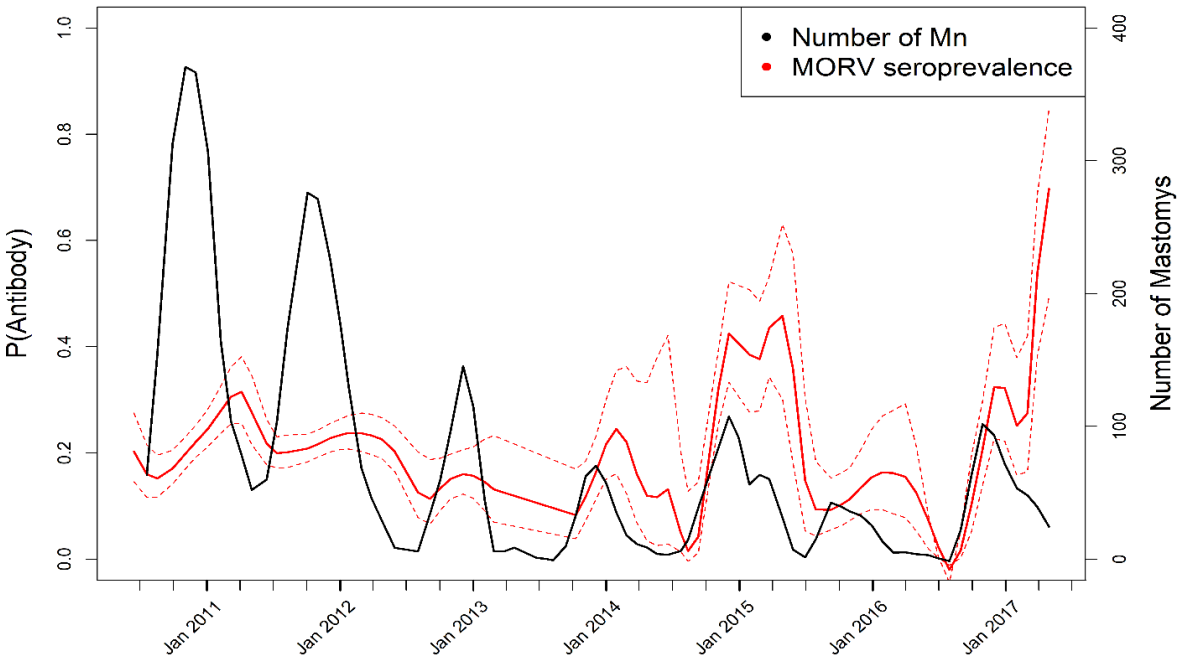

Fig 2: Time series (using cyclic-cubic regression splines) of *M. natalensis* density and MORV seroprevalence in function of time. The bold lines represent mean monthly seroprevalence (red) and number of *M. natalensis* captured each month (black). The dotted lines represent 95% confidence intervals on the seroprevalence estimation.

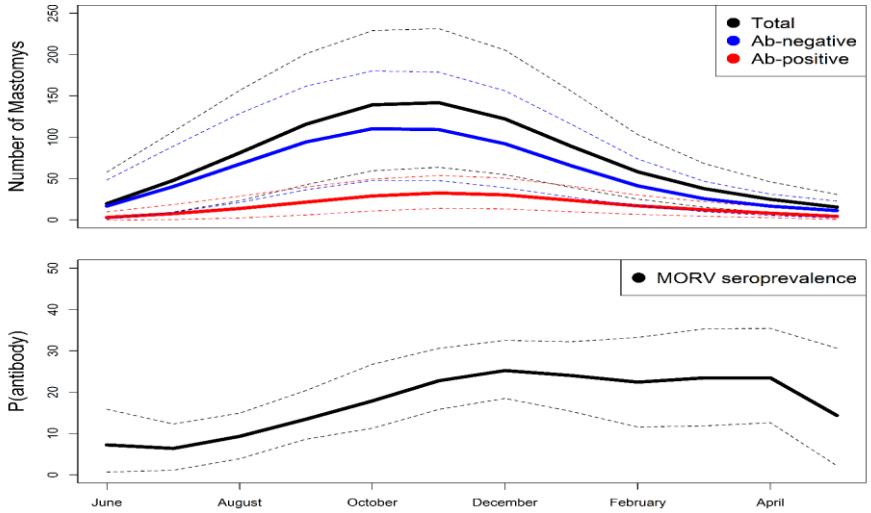

Fig 3: Seasonal component of *M. natalensis* density (upper) and MORV seroprevalence (lower) time series. The bold lines represent means and dotted lines 95% confidence intervals. June was selected as the onset of a biological year, because that is when population density starts to increase.

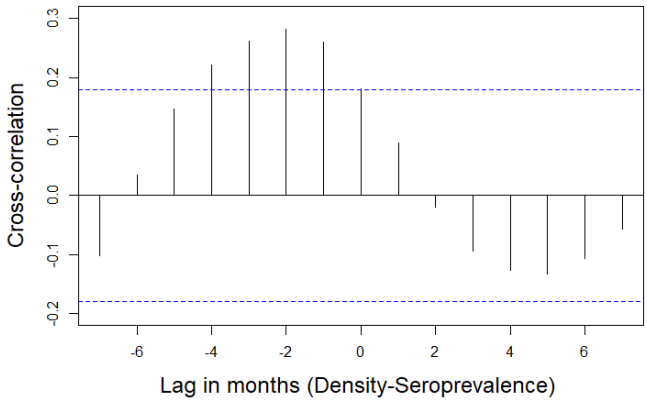

Fig 4: Cross-correlation analysis between *M. natalensis* density (number of uniquely captured individuals per month) and MORV seroprevalence. The blue dotted lines represent 95% confidence intervals which need to be exceeded for correlations to be significant at a given time lag.

Mathematical modelling

Horizontal and vertical transmission

Mathematical models that included a combination of vertical and horizontal (with q=0-0.5) transmission reduced most variation between the transmission coefficients (β_1, β_2, β_3) (table 2). The true value of the horizontal transmission parameter q is likely somewhere between 0 and 0.5, as models with q=0 (model 3) and q=0.5 (model 5) could minimize the difference in βs equally well. These two models required a sevenfold (e^2) increase of β_2 and no compensation for β_3 (zero was located within the credible intervals), while all other models needed a stronger increase of β_2 or β_3. Relative differences between βs were smaller for all models that included vertical transmission (for both β_2 and β_3). This suggests that the seasonal variation in seroprevalence can be best explained by a transmission mechanism that includes vertical and horizontal transmission (q between 0 and 0.5). However, the seasonal variation cannot be explained by the transmission mechanisms alone (the median of β_2 was still larger than zero for the best fitting models; table 2), indicating that other factors are also important to explain the seroprevalence patterns observed in the field data (e.g. seasonal climate variables).

Chronic infections

As expected, the percentage of persistent infections in the model increased with population size and the proportion of chronically infected animals (Fig 5). Interestingly, the model shows that MORV can never persist when there are no chronically infected animals (even at a population

size corresponding to 50ha). At least a few chronic carriers (5-10%) are needed in the population to ensure viral persistence throughout the low host density period (Fig 5: right).

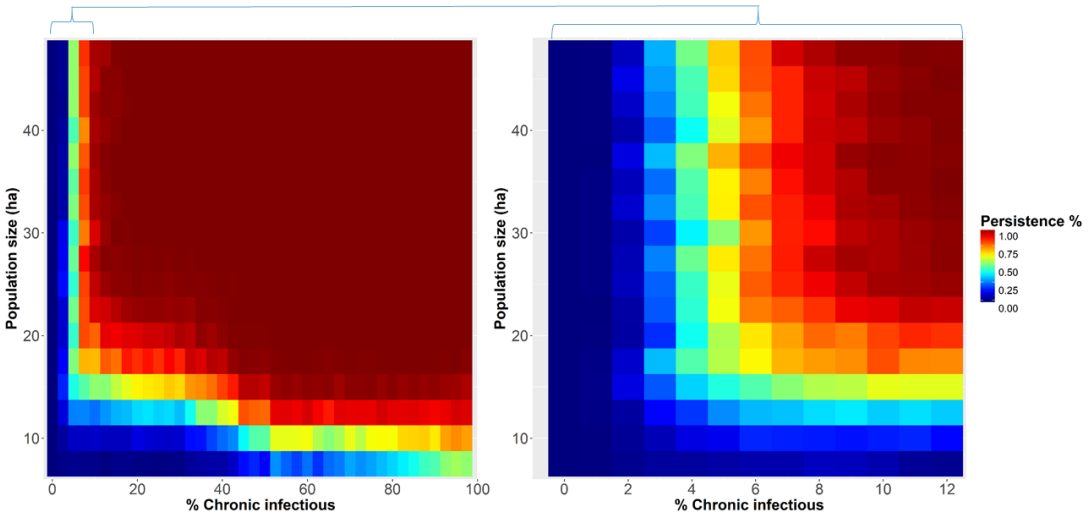

Fig 5: Persistence probabilities for Morogoro virus in populations of *Mastomys natalensis* as a function of the population size (ha) and the proportion of animals that becomes chronically infected at birth (V_c) in the mathematical model.

Cage experiment

From the 270 captured *M. natalensis*, 41 (15%) were at least once antibody positive and seven (3%) contained MORV RNA in their kidneys. Six mice with RNA-positive kidneys were at least once antibody positive. Two of the mice with RNA-positive kidneys showed signs of chronic infections with continuous presence of viral RNA in excretions for eight weeks (S Fig 4 and 5), one of the mice showed signs of chronic infection with virus retreated into the organs (S Fig 6), while the other four mice showed signs of more recent infection. This suggests that 8% (3/38) of the infected animals became a chronic carrier, or contained MORV RNA in the body for a time period of at least 8 weeks. The four mice that showed signs of recent infections were not included in this sum because we cannot determine whether they were chronically or acutely infectious.

Discussion

The seasonal pattern of MORV seroprevalence reflected the population demography of *M. natalensis*, peaking after the high density season (January-March) when the population consists of predominantly older animals. The most straightforward conclusion is that MORV

transmission is positively correlated with *M. natalensis* density. Although several studies have investigated relationships between host density and (sero)prevalence, significant positive correlations were only rarely observed for parasites in rodent populations (Davis et al. 2005). Begon et al. 2009 argued that the lack of significant correlations might have been caused by the short time periods of most studies (not enough data to observe significant pattern), as in their long-term time series they did observe clear positive correlations between cowpox virus prevalence and density of field voles (*Microtus agrestis*). This, together with the results of our long-term study, suggest that positive correlations between parasite prevalence and host density may be more common than expected.

Our mathematical model suggests that the seasonal seroprevalence pattern is best explained by a density-dependent transmission component that follows the power law (q=0-0.5). Density-dependent transmission was already expected for *M. natalensis*, as it is a non-territorial species of which the contact rate is likely to increase with density (Borremans et al. 2014). However, we emphasize that it remains difficult to infer the exact transmission-density function based on serology data alone and that a combination with semi-controlled field experiments may provide more definite answers. For example, an enclosure experiment found that contact rates of *M. natalensis* increase with density in a sigmoid pattern, arguing that MORV transmission might be a nonlinear function of density (Borremans et al. 2016). Another enclosure experiment found that Sin Nombre hantavirus transmission increased with deer mice (*Peromyscus maniculatus*) density, and that the effect was heavily dependent on the season (Bagamian et al. 2012). Similarly, a seasonal effect on the shape of the transmission-density relation was observed for cowpox virus dynamics in field voles (*M. agrestis*), in which transmission appeared to be density-dependent during winter, but frequency-dependent in summer (Smith et al. 2009). We could not observe a significant difference in the transmission-density function between (climatic) seasons, as host density in itself is highly correlated to seasonal climate and thus impossible to disentangle.

We had expected a strong juvenile dilution effect after the breeding season (October), when most newborn animals have entered the population, but found no convincing support of this in the field data. The mean seroprevalence reached its lowest point during the end of the breeding season (July), suggesting a small dilution effect, which increased steeply afterwards (August-November). This steep, almost immediate increase was unexpected, given the high rate at which juveniles entered the population. The short time-lag between density and seroprevalence (1-4 months) suggests that transmission rates are high during this period. One possibility to explain

these results is that the proportion of vertically infected juveniles is higher than previously expected, which is supported by the mathematical models. This possibility is also supported by the relatively high seroprevalence (15%) of very young individuals (<20g) (supplementary material: S.Fig. 3), suggesting high levels of vertical transmission or maternally derived antibodies. Vertical transmission is also suggested for LASV transmission in *M. natalensis* and Junin arenavirus in *Calomys musculinus*, while it has been directly observed in the laboratory for LCMV in *Mus musculus* (Vitullo and Merani 1988; Demby et al. 2001; Oldstone et al. 2002). Another possibility that may explain the lack of a clear dilution effect is a seasonal change in the transmission coefficient β. For example, the steep increase in seroprevalence might be the result of increased indirect transmission due to drier environmental conditions during the period following the breeding season (August-October), which has been shown to enhance survival of arenaviruses (Stephenson et al. 1984).

Evidence from experimental infections and natural observations suggests that acute infections with virus shedding of a few weeks and lifelong immunity is most standard among arenavirus infections in adult natural hosts (Murphy et al. 1976; Gonzalez et al. 1983; Demby et al. 2001; Fulhorst et al. 2001; Borremans et al. 2011; Milazzo and Fulhorst 2012; Fichet-Calvet et al. 2014; Borremans et al. 2015). Given that MORV transmission is furthermore not frequency-dependent, an interesting question that remains is how the virus could survive the low density periods of its host. Our mathematical model suggests that this may be due to the presence of a small number of chronically infected animals (5-10% of the total infected population), which could bridge the transmission chain during the critical density periods. Interestingly, when monitoring wild caught *M. natalensis* in the lab for eight weeks, about 8% of total MORV infected animals had a long term infection with the rest being short term. This proportion of chronic-acute MORV infections is thus consistent with the prediction of our mathematical models. Chronic infections have also been directly observed after inoculation of newborn hosts in laboratory conditions, while other field studies have suggested their presence alongside acute infections in natural conditions (Borremans et al. 2015; Fichet-Calvet et al. 2014; Mariën et al. 2017; Oldstone, 2002; Walker et al. 1975). Furthermore, it is possible that some animals develop latent (chronic) infections, in which the virus retreats to organs from where it might be reactivated under stressful conditions (Plowright et al. 2016). For example, viral reactivation could be triggered by increased testosterone levels in males and by pregnancy or offspring care in females during the breeding season (Altizer et al. 2006). Besides chronic and latent infections, other factors that could increase the persistence probability of MORV include

indirect transmission (e.g. prolonged survival outside the host), behavioral changes (e.g. in social/sexual activity or host manipulation), metapopulation dynamics and spatial heterogeneity (Borremans, 2015, PhD thesis). These factors remain to be investigated.

Overall, the results from this study give mixed support for the development of rodent control programs with the aim of reducing arenavirus spillover to humans. Given the positive correlation between MORV transmission and host density and the similarities between MORV and LASV, it is likely that LASV prevalence would decrease if *M. natalensis* density would be artificially reduced (e.g. by rodent elimination). A decrease in density may even have an additional negative effect on the spillover rate to humans, as this is a function of both rodent density and LASV prevalence (Davis et al. 2005). In other words, killing rodents in LASV-endemic villages could reduce LASV transmission to humans because (i) there are less rodents and (ii) less rodents are infectious. However, rodent control is unlikely to completely eliminate LASV from the rodent population, as the survival of only a few chronically infected animals seems sufficient for viral persistence and complete removal of rodents is practically impossible. Given the presence of chronic carriers and the high breeding and recolonization capacity of *M. natalensis*, occasional rodent control is unlikely to be effective in eliminating LASV as *M. natalensis* densities would quickly return to their carrying capacity and LASV prevalence to its original prevalence (Mariën et al. 2018).

Conclusion

In this study, we investigated how host demography and transmission mechanisms may affect the dynamics of an arenavirus. We observed seasonal cycles of MORV seroprevalence in a highly fluctuating population of *M. natalensis*, and argued that density-dependent horizontal in combination with vertical transmission could explain the observed patterns. In addition, we highlighted that the presence of a small number of chronically infected animals can explain the persistence of the virus at low host density periods. An interesting next step would be to investigate how climate affects MORV transmission, as effects can be expected for both the survival probability of the virus and the host. For example, a prolongation of the rainy season is known to result in larger numbers of *M. natalensis*, while drier conditions may increase indirect transmission (Stephenson et al. 1984; Sluydts et al. 2007). Future research could therefore investigate whether climate variables are indeed useful predictors of arenavirus outbreaks by relating these variables to both transmission mechanisms and host demography. These new insights, together with the results from this study, would provide a solid framework for developing our understanding of when arenavirus outbreaks can be expected.

Acknowledgements

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Availability of data and materials

Data supporting the conclusions of this article are included in the article and its additional files. The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceived the study: JM, BB and HL. Wrote the paper: JM and BB. Performed the experiments: JM, BB, CV, SGr, JG-B. Performed the analyses: JM, BB, JR and LK. Supervised field and laboratory work: JG-B, CS, SGu, AM, and HL. All authors read and approved the final manuscript.

Tables

Table 1: Comparison of generalized additive models investigating seasonal and year effects in the MORV seroprevalence time series.

Model	Time component	Par	AIC	R2
GAM1	intercept	1	556	0.00
GAM2	s(month)	10	487	0.22
GAM3	year	6	456	0.33
GAM4	s(month)+year	16	405	0.55

Abbreviations: s(), smoothed parameter; AIC, Akaike information criterion; Par, number of identifiable parameters; Adj R^2 = adjusted R^2 value;

Table 2: Comparison of mathematical models investigating the effect of the shape of the transmission-density relation and the level of vertical transmission on MORV seroprevalence.

Model	Horizontal	Vertical	*β_1*	*β_2*	*β_3*
M1	Frequency (q=1)	Yes	0 [-3,1]	2 [1,3]	2 [0,3]
M2	Frequency (q=1)	No	0 [-2,2]	3 [2,4]	3 [1,4]
M3	**Density (q=0)**	**Yes**	**0 [-3,2]**	**2 [1,3]**	**0 [-2,1]**
M4	Density (q=0)	No	0 [-3,2]	3 [1,3]	1 [-1,2]
M5	**Freq-Dens (q=0.5)**	**Yes**	**0 [-3,1]**	**2 [1,3]**	**1 [-1,3]**
M6	Freq-Dens (q=0.5)	No	0 [-3,1]	3 [2,4]	2 [1,3]

Numbers indicate median relative log-transformed βs $[\log(\beta_x/\beta_1)]$ and 95% credible intervals between brackets. Models with βs closer to zero (or that contain zero within the credible intervals) are preferred to models with higher values, as these models needed less compensation to simulate data similar to the field data. The two most preferred models are indicated in bold. We assume that no correction was needed when the credible interval contains zero. *β_1* = January-April (low density period); *β_2* = April-July (low density and breeding period); *β_3* = August-December (high density period).

Supplementary figures

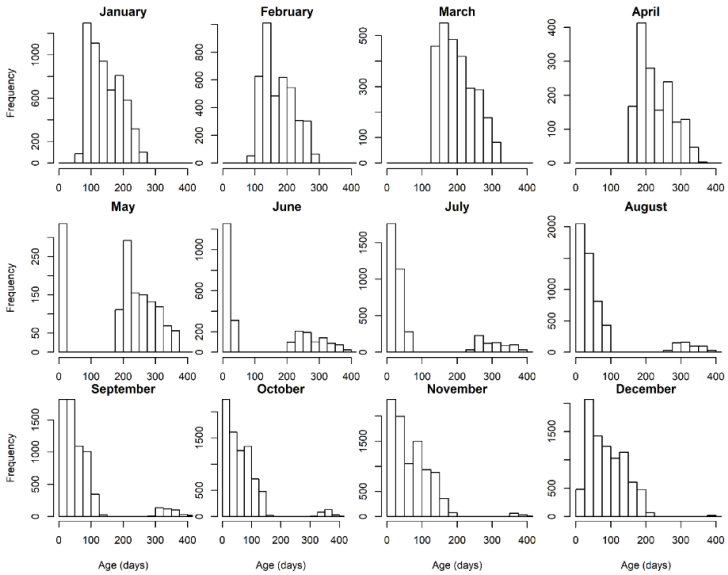

Fig S1: Age distribution (days) of *M. natalensis* in the model simulations per month. Age distributions were simulated as closely as possible to field data (Leirs 1994).

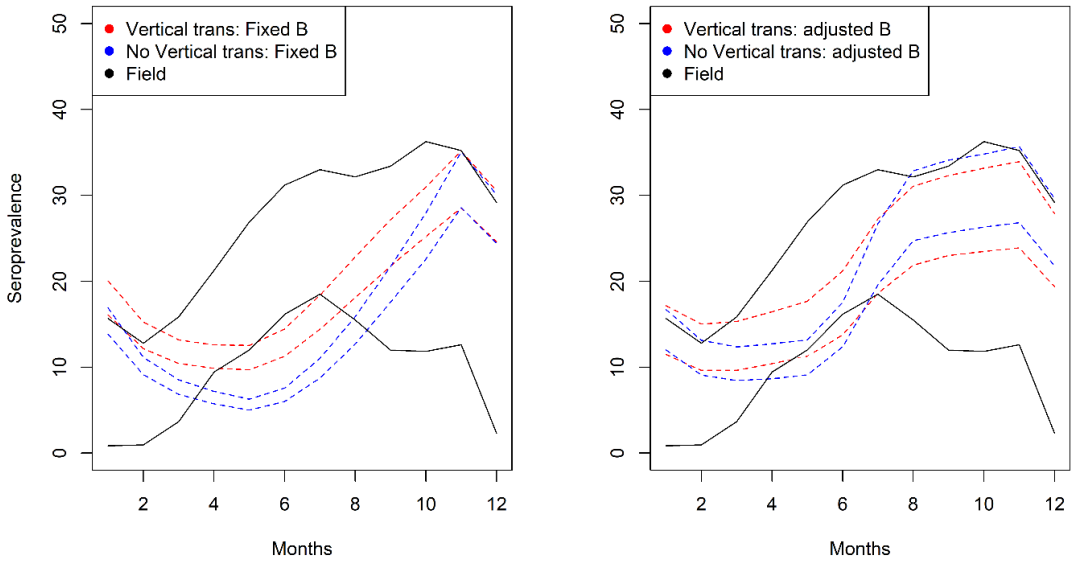

Fig. S2: Fit of two mathematical models with frequency-dependent transmission of MORV in a population of *M. natalensis*. Model fits for a model with (red) and without (blue) vertical transmission to the seroprevalence field data (black). *Left*: Both models assume that the transmission coefficient β remains constant during the year. *Right:* Both models allow β to change between three predefined time-periods of the year (January-April, April-July and August-December). The incorporation of season-specific βs clearly improves the model fit. Red and blue dotted lines indicate 95% confidence intervals of the model results. Black solid lines indicate 95% confidence intervals on the seasonal pattern of the field data.

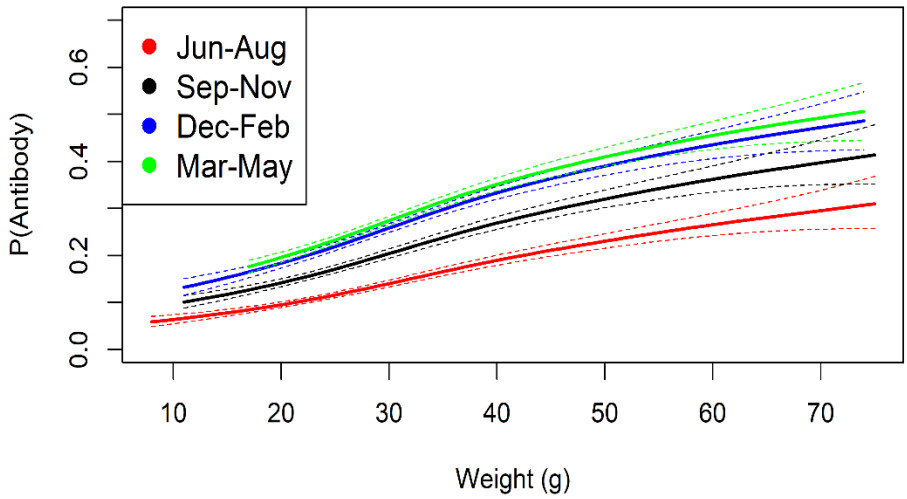

Fig S3: Correlations between body weight of *Mastomys natalensis* (as a proxy for age) and probability of being Morogoro virus-antibody positive at different periods of the year (June-August, September-November, December, February and March-May). The figure suggests that both season (density) and age are predictors of antibody presence in *M. natalensis*.

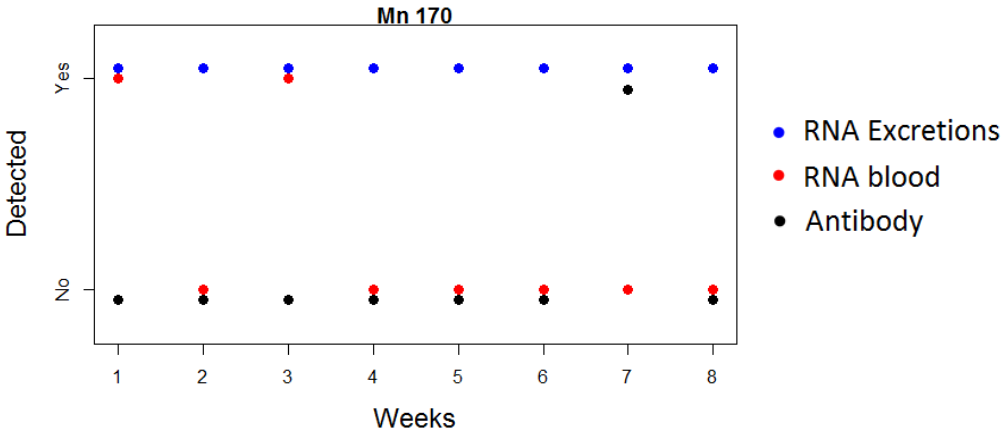

Fig S4: Individual 170: MORV RNA was detected in excretions (saliva and urine) every week, in blood on week one and three, and in the kidneys (week eight). Antibodies were only detected on week seven.

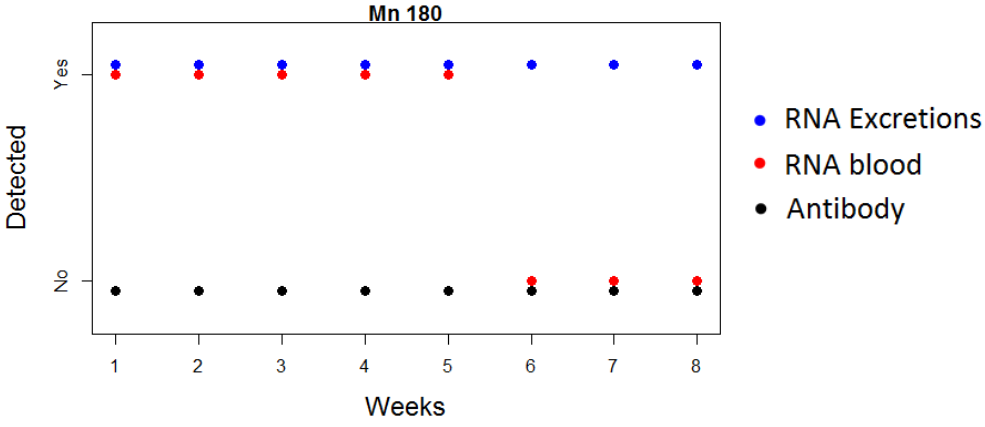

Fig S5: Individual 180: MORV RNA was detected in excretions (saliva and urine) every week, in blood the first five weeks, and in the kidneys (week eight). Antibodies were not detected.

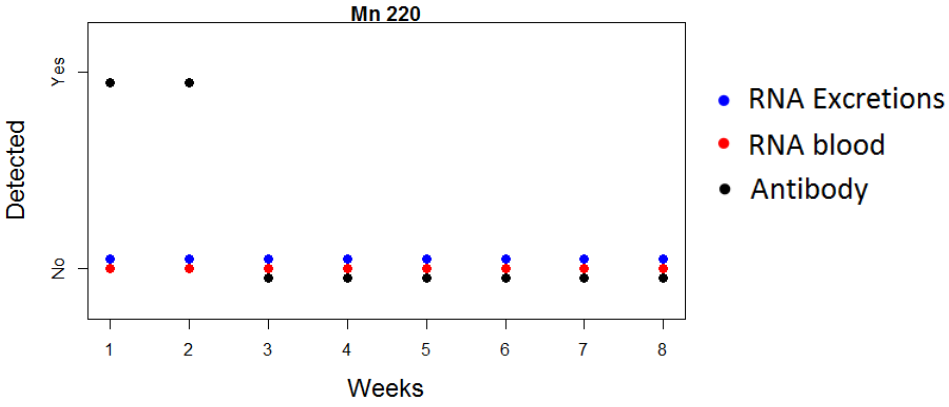

Fig S6: individual 220: MORV RNA was detected in the kidneys only (week eight). Antibodies were detected in week one and two, suggesting that this individual became infected before it was captured, removed the virus from blood and excretions, but retained the virus in its organs.

Additional information on the demographic component of the mathematical model

As explained in the main article, the demographic parameters were dependent on the day of the year (day 1 = 1 January) and the age of an individual (in days). They were optimized in order to simulate realistic population density fluctuations and age distributions, while being also biologically realistic. The population densities were compared to the longitudinal field data of the CMR experiment. The age distributions were compared to the field data of Leirs (1994), who measured the eye lens weight of *M. natalensis* throughout the year as a proxy of the animals' age (Fig S1).

Individuals live for an exponentially distributed amount of time depending on the mortality parameter (μ), which was modelled by the following equations:

μ (day < 15 & age < 366) = 0.004 lifespan^{-1}

μ (14 < day < 74 & age < 366) = 0.013 lifespan^{-1}

μ (74 < day < 120 & age < 366) = 0.0064 lifespan^{-1}

μ (day >120 & age < 366) = 0.004 lifespan^{-1}

μ (age > 365) = 0.09 lifespan^{-1}.

As an example, an individual of 100 days old on January 1 had a probability of 0.4% to die before the next time step, which is one day later. Given that animals older than one year did not occur in the CMR dataset, we increased the mortality rate once individuals reached that age in the model.

The following equations model the birth rate of *M. natalensis* (Φ), which was also a function of time and age:

Φ (day < 111 or day > 329 & age > 120) = 0 [births/(individual*time interval)]

Φ (110 < day < 256 & age > 120) = 0.0012 * (day – 110) [births/(individual*time interval)]

Φ (255 < day < 330 & age > 120) = 0.16 * (day – 255) [births/(individual*time interval)]

Φ (age < 121) = 0 [births/(individual*time interval)].

We assumed that individuals younger than 120 days could not give birth. Furthermore, Φ was corrected each year by a factor in order to simulate variation in peak densities similar to the densities observed in the field data.

Movement patterns of small rodents in Lassa fever-endemic villages in Guinea

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Abstract

The Natal multimammate mouse (*Mastomys natalensis*) is the reservoir host of Lassa arenavirus, the etiological agent of Lassa fever in humans. Because there exists no vaccine for human use, rodent control and adjusting human behavior are currently considered to be the only options for Lassa fever control. In order to develop efficient rodent control programs, more information about the host's ecology is needed. In this study, we investigated the spatial behavior of *M. natalensis* and other small rodents in two capture-mark-recapture and four dyed bait (Rhodamine B) experiments in Lassa fever-endemic villages in Upper-Guinea. During the capture-mark-recapture studies, 23% of the recaptured *M. natalensis* moved between the houses and proximate fields. While *M. natalensis* was found over the entire study grid (2ha), other rodent species (*Praomys daltoni, Praomys rostratus, Lemniscomys striatus, Mus* spp.) were mostly trapped in the surrounding fields. Distances between recapture occasions never exceeded 100m for all rodent species. During the dyed bait experiments, 11% of *M. natalensis* and 41% of *P. daltoni* moved from the fields to houses. We conclude that commensal *M. natalensis* easily moves between houses and proximate fields in Guinea. We therefore consider occasional domestic rodent elimination to be an unsustainable approach to reduce Lassa virus transmission risk to humans, as *M. natalensis* is likely to reinvade houses quickly from fields in which rodents are not controlled. A combination of permanent rodent elimination with other control strategies (e.g. make houses rodent proof or attract predators) could be more effective for Lassa fever control, but must be further investigated.

Introduction

The Natal multimammate mouse, *Mastomys natalensis* (Smith, 1834), is the natural host of Lassa virus (LASV), an arenavirus that causes Lassa hemorrhagic fever (LF) in humans (Monath 1987). In West Africa, these rodents thrive in houses and surrounding fields of rural villages where they shed the virus through feces, urine and saliva (Mccormick et al. 1987; Walker et al. 1975). Humans can become infected by ingestion of contaminated food or water, inhalation of virus particles, touching contaminated objects or by direct consumption of the rodents (Stephenson et al. 1984; McCormick JB 1999; Bonwitt et al. 2016). Secondary human-to-human transmission is also possible and occurs typically within households or health care facilities (Carey et al. 1972; Fisher-Hoch et al. 1995; Lo Iacono et al. 2015). Annually around 200,000 people are affected, with a fatality rate of 1–2% (Monath 1987; McCormick JB 1999; World Health Organization 2016). Since no human vaccine exists and therapeutic options are

limited to the broad-spectrum antiviral ribavirin, rodent control and adjusting human behavior are currently considered to be the only options for LASV prevention (Bausch et al. 2010; Fisher-Hoch et al. 2000; Hastie et al. 2017).

In order to develop sustainable rodent control programs, basic knowledge of the rodent's spatial behavior is essential to determine when and where to control and to anticipate recolonization events (Singleton et al. 1999; 2010). Since *M. natalensis* is the most important rodent pest species in sub-Saharan Africa, plenty of information can be found in literature and outbreak forecast models have been developed (Leirs 1994; Leirs et al. 1997; Monadjem and Perrin 1998; Massawe et al. 2011; Borremans et al. 2015). While most of these studies investigated *M. natalensis* living in large agricultural fields or natural savannah habitat in East or Southern Africa, little attention has been paid to its ecology in LF-endemic areas in West Africa (Swanepoel et al. 2017). Here, the rodent flourishes in houses and surrounding cultivations of rural villages, where it reaches between 95-98% of all indoor captures (Demby et al. 2001; Fichet-calvet et al. 2009). The reason why *M. natalensis* is found in houses in remote areas in West Africa could be the absence of competing *Rattus* species (Demby et al. 2001), which are suggested to scare away *M. natalensis* from buildings in East and South Africa (Monadjem et al. 2011). Because several environmental factors (e.g. resource availability, predation risk or mating system) can affect rodent behavior considerably, additional research is needed to better understand *M. natalensis'* ecology in these commensal environments (Mulungu et al. 2015; Fichet-Calvet et al. 2007).

To our knowledge, only one longitudinal survey investigated the spatial behavior of *M. natalensis* in LF-endemic areas. Fichet-Calvet et al.(2007) trapped rodents inside houses and surrounding cultivations in three villages in Upper-Guinea. They found clear seasonal fluctuations in abundance according to habitat type. While *M. natalensis* was as numerous inside as outside houses during the rainy season, its abundance increased significantly inside but decreased outside houses during the dry season. This pattern suggests a change in habitat preference driven by fluctuations in food availability. When the dry season starts, the harvest is cut and the crops are stored inside the houses. At the same time, fields are left fallow or burned for the cultivation of crops the following year. These environmental conditions might attract *M. natalensis* from the surrounding fields to the houses where they find food and shelter. Because houses in these villages typically consist of one room in which the harvest is stored close to the bed, the living space of humans and *M. natalensis* seems to overlap almost completely during the dry season (Bonwitt et al. 2017). An increased transmission risk of LASV to humans can

therefore be expected and is also noted by clinicians who observed more LF cases in local hospitals during this period (Mccormick et al. 1987; Bausch et al. 2001; Asogun et al. 2012).

In this study, we examined the spatial behavior of *M. natalensis* in LF-endemic villages during the dry season at three levels. First, we investigated if *M. natalensis* moves from the surrounding fields to the houses, as hypothesized by Fichet-Calvet et al. 2007. Secondly, we reported the mean and maximum distances that this rodent moved between captures, which we used as an indicator of its home range size. Finally, we investigated the rodent's microhabitat choice within and close to the village. Although we were mainly interested in the spatial behavior of *M. natalensis* as the reservoir host of LASV, we included other captured rodent species in our analysis as well. These rodents are not reservoirs of LASV, but spillover infections to these animals (*Praomys daltoni, Praomys rostratus, Lemniscomys striatus, Mus matthei/minutoides*) have been observed (Fichet-Calvet et al. 2014). We performed two types of experiments: capture-mark-recapture (CMR) experiments in which traps were set in both houses and surrounding fields, and rhodamine B (RB) experiments in which colored bait was set in the fields and rodents were trapped inside houses.

Methods

Selection of study sites

Because we wanted to build on the results of Fichet-Calvet et al 2007, we chose the same area used in that study for our experiments, which is the prefecture of Faranah in Upper Guinea. This area was chosen for its high mean human LASV seroprevalence (approx. 35%) and the abundance of *M. natalensis* in the houses (Demby et al. 2001; Lukashevich et al. 1993). In Faranah, the rainy season starts in April and continues until October with an annual mean rainfall of 1458mm. Four rural villages were selected: Tambaya (10°18'25''N; 10°51'45''W), Silimi (09°58'32''N, 10°39'07''W), Brissa (10°13'00''N, 10°41'20''W) and Yarawalia (9°57'18''N, 10°43'56''W). They were chosen because of their remote location from a paved road, a size not exceeding 1000 inhabitants (Tambaya 1000, Silimi 960, Brissa 655, and Yarawalia 570 inhabitants), and less than 45 min driving time from Faranah. Experiments were performed during the beginning of the dry season in the months October and November of the years 2015 and 2016. This period was chosen because the harvest is then recently stored inside the houses and the fields are left fallow, which we hypothesized to be the main drivers for *M. natalensis* to enter the houses.

Capture mark recapture studies

Two capture-mark-recapture (CMR) experiments were performed: one in Tambaya (14/10/2015–6/11/2015*)* and a second in Silimi (17/10/2016–8/11/2016). The two villages were chosen for biosafety reasons, as the LASV prevalence in the rodent population was zero in Tambaya and low in Silimi (1/42) in comparison to other villages in this area (Fichet-Calvet et al. 2016).

During one month, rodents were live-trapped every week for three consecutive nights using Sherman live traps (Sherman Live Trap Co. Tallahassee, FL, USA). Trapping was done in 2-ha rectangular grids of 20x10 traps spaced 10m apart. The grids were located on the edge of the villages in a way that both houses and surrounding fields were included. The habitat type was noted for each location where a trap was set: inside a house, or outside in bush, grass, cultivations or open space (public square) habitat (Fig 1). The traps were baited (with a mixture of peanuts, dry fish and wheat flour) in the evening and checked in the morning. Captured rodents were taken to a small building, where species, sex, weight and reproductive status were recorded (Leirs, 1994; Sluydts et al. 2007). Identification of rodent species was based on external morphology following Kingdon et al. 2013. Mice were considered to be adults if signs of sexual activity were observed (scrotal testes in males; perforated vagina, lactating nipples or pregnancy in females). All animals were individually marked by toe clipping or tattooing (Leirs 1994; Hess 2009; Borremans et al. 2015), and released at the same location as where they were previously captured (even if they were captured inside a house).

The mean distance between successive captures (MDSC) and maximum distance between all captures (MDAC) were calculated for each recaptured individual based on the Euclidian distance between trapping locations. The MDSC was used as an indicator of home range size which could not be calculated directly due to the low number of recaptures per animal (Monadjem and Perrin 1998; Slade and Russell 1998). The R-packages ‘colorRamps’ and ‘fields’ were used for the plotting of habitat types, rodent movements and capture locations in the village (R Core Team 2016).

Microhabitat choice was analyzed by relating habitat type per trapping station to trapping success per station (i.e. total number of rodents captured on that station / number of trapping occasions). The habitat type around each station was determined and divided in five categories: house (indoor), cultivations (outdoor, surrounded by agricultural plants), bush (outdoor, surrounded by trees or shrubs > 1m), grass (outdoor, surrounded by *Poaceae* plants) or open space (outdoor, no vegetation). We did not include the habitat ‘open space’ in the analyses

because there were only a few stations placed in this habitat. The relation was analyzed with a generalized linear model (GLM) with negative binomial distribution (*glm.nb* function in R), as there was considerable evidence of overdispersion when we used a Poisson distribution. Multiple comparisons (*glht* function in R) between the different vegetation types were analyzed by a Tukey test using the Rpackage 'Multcomp'(R Core Team 2016). Separate analyses were performed for the two villages, as rodent diversity and abundance differed considerably between them. We only retained rodent species of which there were at least 25 captures (i.e. *M. natalensis, P. daltoni, P. rostratus, Mus* spp. and *L. striatus*).

Rhodamine B experiments

Rhodamine B (RB) is a dye that, once ingested, becomes incorporated into keratinous structures of animals where it is detectable for up to several weeks under fluorescent light (Jacob et al. 2002). The dye is often used to examine dispersal of mammals and was previously shown to be effective to study rodent movements (Mohr et al. 2007; Monadjem et al. 2011; Rahelinirina et al. 2009). Four RB experiments were performed: two in Brissa (first 23/10/2015-11/11/2015; second 26/10/2016-14/11/2016) and two in Yarawalia (first 1/11/2015-18/11/2015; second 4/11/2016-23/11/2016). These villages were chosen for practical reasons, as they are part of ongoing studies in which LASV viral prevalence was followed in the rodent population for several years.

For the preparation of the bait, 2g RB powder (Sigma-Aldrich) was mixed in 1kg of conventional bait (Monadjem et al. 2011). Bait stations were filled with 50g RB bait and placed in (one to three) fields close to the village border in a grid formation spaced 5m apart (Fig 3). The number of bait stations differed between the two years: while 100 bait stations were placed in both villages in the year 2015, 200 stations were placed in both villages in 2016. The stations stayed in the field for eight consecutive days, where they were rebaited each two days and checked for signs of consumption. Eight days after removal of the stations, 120 traps were placed in 60 houses that were randomly chosen along a transect from the RB fields to the village center. In order to be sure that rodents were willing to eat the bait, 50 additional traps were placed in the RB fields. All traps were baited during three consecutive evenings and checked every morning. Captured rodents were humanely killed, measured morphometrically (as described in Fichet-Calvet et al. 2007) and checked for external signs of RB. The whiskers (including the follicle) and a piece of the fur were removed, put in small Ziploc bags and stored

in a -20°C freezer until further analysis followed. Coordinates of the houses where rodents were trapped were recorded with a GPS.

*A*t least six whiskers and a piece of the fur were eventually placed on a microscope slide in a drop of water and covered with a coverslip. Slides were examined for signs of RB under a fluorescence microscope (UV light at 530–585 nm) at low magnification and always compared with a positive and negative control sample. Distances between the houses and the fields were estimated based on the GPS coordinates and calculated with the 'Vincenty (ellipsoid)' method from the Rpackage 'geosphere'. Locations of the RB fields and houses were plotted in Google Maps (with the Rpackage 'ggmap') (R Core Team 2016).

Ethical statement

During all manipulations, standard procedures for BSL3 work in the field were followed (Mills et al. 1995). The investigation and permission to conduct research on wild animals were approved by the National Ethics Committee of Guinea (permit n° 12/CNERS/12 and 129/CNERS/16) and carried out in accordance with the approved guidelines. The experiments were performed in collaboration with the local health authorities (Prefecture de Faranah) and in agreement with the village chiefs. Trapping and releasing of rodents in houses was only performed if permission was obtained from the individual house owners.

Results

Capture mark recapture study

During 4,800 trap nights, we captured 378 animals of which 109 individuals were recaptured a total of 203 times (table 1). The exact number of times that individuals were recaptured is presented per species in the additional information table 1.

The species richness and evenness differed considerably between the two villages (table 1). Almost all animals trapped in Tambaya were *M. natalensis* (94%) and only one other rodent (*P. daltoni)* and *Crocidura* species were found. *M. natalensis* was also the most abundant rodent in Silimi (37%) but other rodents [*P. daltoni* (5%), *P. rostratus* (20%), *L. striatus* (11%); and *Mus* spp. (14%)]. were captured frequently as well. Other trapped species in Silimi were *Grammomys butingi, Uranomys ruddi, Lophuromys sikapusi, Gerbilliscus guineae, Rattus rattus* and *Crocidura* spp. Although we could not identify all individuals to the species level, we know from previous studies that three sibling species of *Mus* (*M. mattheyi, M. minutoides*

and *M. baoulei*) and *Crocidura* (*C. theresae*, *C. buettikoferi* and *C. lamottei*) can occur in this area (Fichet-calvet et al. 2009).

Direct movements into or out of houses or recaptures in the same house were mainly found for *M. natalensis* (Fig 1 and additional information table 2). We captured three *M. natalensis* (two in Tambaya and one in Silimi) first outside and afterwards inside a house, eight *M. natalensis* (five in Tambaya and three in Silimi) first inside and afterwards outside a house, one *M. natalensis* (in Silimi) in one house and later in another house, and six *M. natalensis* (four in Tambaya and two in Silimi) always in the same house. In addition, we captured two *P. daltoni*, one *P. rostratus*, one *L. striatus* and two *M. musculus* (all in Silimi) first inside and later outside a house, two *P. daltoni* and one *L. striatus* first outside and later inside a house, and one *P. daltoni* two times in the same house. Outdoor movements between different vegetation types were observed for fifteen *M. natalensis*, seven *P. daltoni*, twelve *P. rostratus*, eleven *L. striatus*, three *Crocidura* spp and seven *Mus* spp. captures (additional information: table 2).

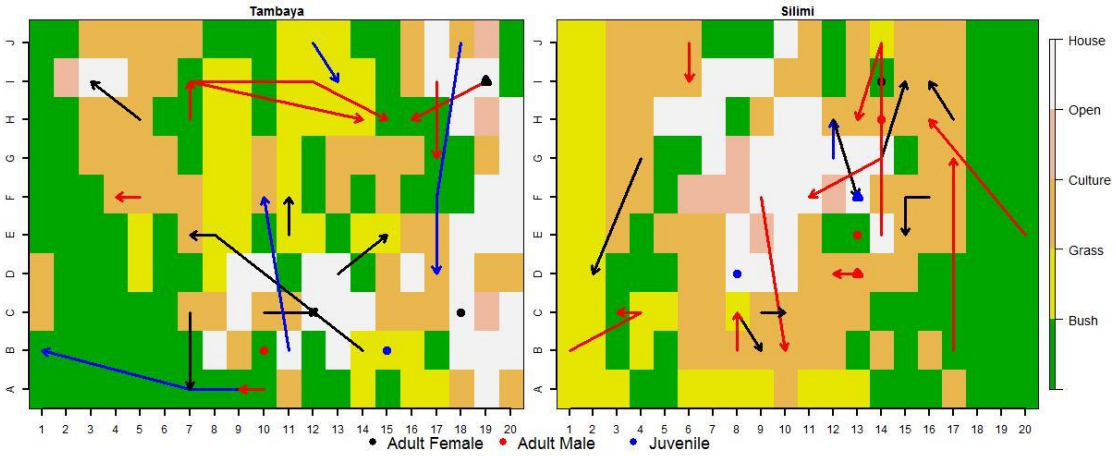

Fig 1: Movements of recaptured *M. natalensis* during the capture-mark-recapture experiments in Tambaya (left) and Silimi (right). Traps were spaced 10m apart. Arrows represent the direction and minimum distance that an individual moved between two successive captures (they indicate the change from first to following captures, not the actual path that the animal moved). Points (two times) and triangles (three times) represent recaptures on the same location. Colors of the arrows indicate sex and reproductive status of the animals (red=male and adult, black=female and adult, blue=juvenile). The colors of the different coordinates represent the habitat type where the trap was placed (white=house, pink= open space, orange=cultivations, yellow=grass, green=bush).

Distances between captures were small for all rodent and shrews: the mean distance between successive captures (MDSR) lay between 10.0m and 40.6m and maximum distance between all captures (MDAR) between 10.0m and 60.4m depending on the species (table 1). Some

individuals moved larger distances, but the MDAR never exceeded 100m for all species (Fig 2).

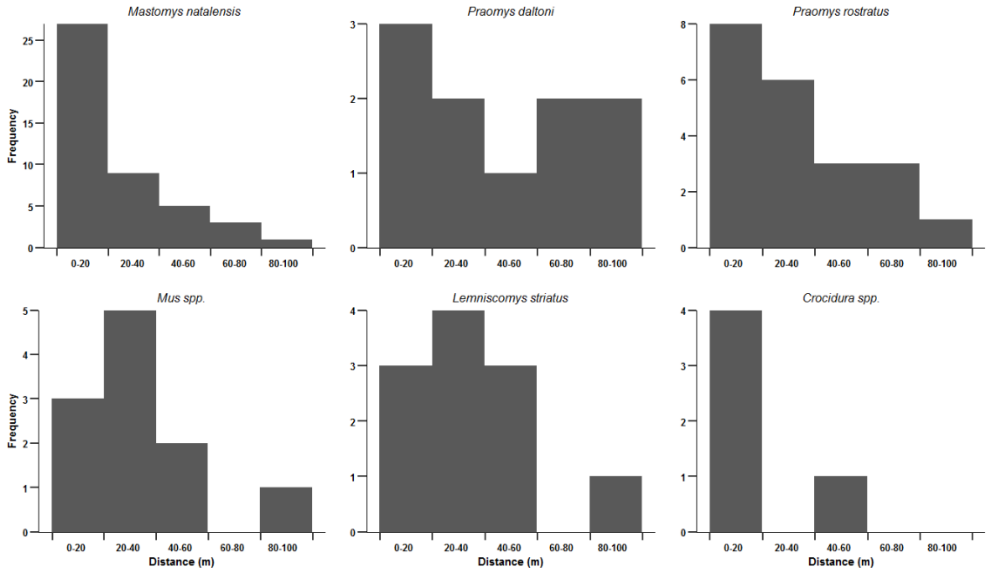

Fig 2: Maximal distances between all recaptures (MDAR) for *M. natalensis*, *P. daltoni*, *P. rostratus*, *Mus* spp. *L. striatus* and *Crocidura* spp during the capture-mark-recapture experiments in Tambaya and Silimi. Bars represent the number of individuals that moved between a certain MDAR interval.

Differences in microhabitat choice were observed for all rodent species but significant differences were only found for *M. natalensis* and *P. rostratus* (table 2; additional information table 3 for the p-values). In both villages, we trapped significantly more *M. natalensis* inside houses than in bush vegetation patches (z-value=0.35, p=<0.01). We also trapped more *M. natalensis* in houses compared to cultivations in Tambaya (z-value: 3.22, p=<0.01), and in houses compared to grass in Silimi (z-value=0.38, p<0.01). We trapped almost no other rodent species inside houses, but only *P. rostratus* was captured significantly more frequently in grass patches compared to houses (z-value=-2.59, p=<0.01). Significant differences between vegetation types were only found for *M. natalensis*, which we trapped more often in cultivations compared to bush vegetation in Silimi (z-value=0.31, p=<0.01).

Rhodamine B experiment

During 2,040 trap nights, we captured 223 animals of which 174 individuals were trapped inside a house and 49 in a RB field (table2). The most dominant species in both villages was *M. natalensis* (86%) followed by *P. daltoni* (13%). Other trapped species were *P. rostratus* , *L. striatus*, *Mus* spp. , *R. rattus* and *Crocidura* spp..

We found 65 animals with signs of RB (table 3). The majority of trapped rodents in the RB fields were RB positive, indicating that these animals were willing to eat the dyed bait. In the houses, 18 *M. natalensis* and 7 *P. daltoni* were found to be RB positive, which means that these individuals had moved from the fields to the houses during our study (thus within maximally 16 days) (Fig 3). Most of these RB positive individuals were found in houses that were close (<25m) to a RB field (Fig 4). The number of RB positive animals decreased with distance from the RB fields and no positive individuals were found in houses that were more than 100m from the closest RB field.

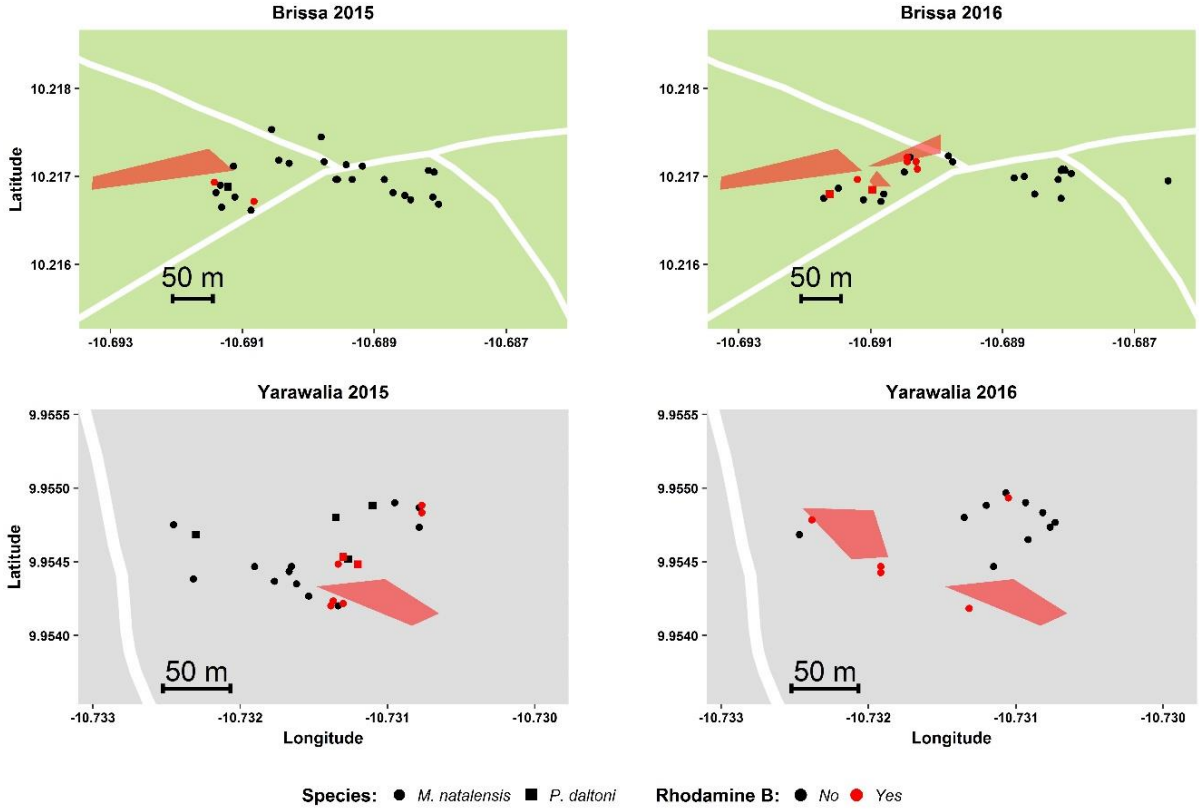

Fig 3: Overview of the villages (Brissa and Yarawalia) were Rhodamine B experiments were performed per year (google maps image). Red polygons represent Rhodamine B fields. Points and squares represent houses where rodents were captures (Colour: black= Rhodamine B negative rodents, red= Rhodamine B positive rodents; symbol: points=*M. natalensis*; squares=*P. daltoni*).

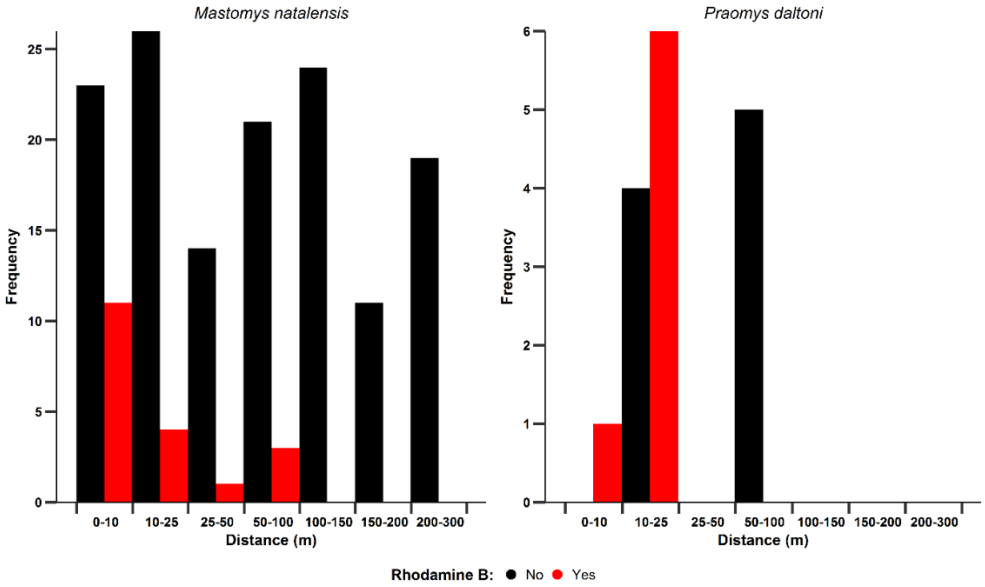

Fig 4: Minimal distances between Rhodamine B fields and houses where rodents (left=*M. natalensis* and right=*P. daltoni*) were trapped. Bars represent the number of trapped individuals per distance interval away from the closest Rhodamine B field (black= rodents without Rhodamine B signs, red=rodents with RB signs).

Discussion

We hypothesized that *M. natalensis* moves from the surrounding fields to the houses during the dry season to search for food and shelter, which is abundant inside but scarce outside houses at that time (Fichet-Calvet et al. 2007). While we observed direct movements from the fields to the houses in all experiments, we did not find overall movements in this direction only. In fact, more individuals moved from the houses to the fields during the CMR experiments. The low number of recaptured animals in the CMR experiments and the fact that we only checked for movements from the fields to the houses in the RB experiments made it impossible to statistically investigate the overall direction in which *M. natalensis* actually migrated.

Although we could not statistically confirm the movement hypothesis, our study shows that *M. natalensis* can easily enter and leave houses, stay for a long time inside the same house or move from one house to another. These results suggest that an individual home range of *M. natalensis* can include both houses and outdoor fields in rural villages. Therefore, it remains likely that *M. natalensis* stays for longer periods inside houses when food availability is limited outdoor, which might explain the increased indoor rodent abundance and LASV transmission risk to humans during the dry season (Bausch et al. 2001; Fichet-Calvet et al. 2007). Similar results were found in other field studies performed in Tanzania and Swaziland, where *M. natalensis* visited houses after which it retreated up to 100m into the surrounding fields (Monadjem et al.

2011). Our results are also consistent with a telemetry study performed in Namibia in which radio-tracked *M. natalensis* were found to enter houses regularly during the post-harvest season, but not during the pre-harvest or pre-planting seasons (Monadjem et al. 2011). Half of the radio-tracked individuals remained inside the same house after entering, while the other half returned to the fields. In contrast, radio-tracked *M. natalensis* stayed predominantly in the fields in rural villages in Swaziland, probably due to the abundant presence of competing *Rattus* species in the houses (Monadjem et al. 2011).

Most *M. natalensis* moved little between captures and distances presented here are similar to those observed in other CMR studies performed in agricultural fields in Africa. Since the MDSC was suggested to be a good indicator of *M. natalensis'* home range size (Monadjem & Perrin, 1998; Slade & Russell, 1998), we can assume that sizes in Guinea are similar to those observed in South-Africa (±680m^2) and Tanzania (±650m^2) (Borremans et al. 2013; H. Leirs et al. 1997; Monadjem & Perrin, 1998). These sizes could however be underestimations as home ranges of radio-tracked *M. natalensis* were considerably larger in agricultural fields in Tanzania (±1200m^2), rural villages in Swaziland and Namibia (±4300m^2) and natural vegetation in Uganda (±8400m^2) (Hoffmann & Klingel, 2001; Leirs et al. 1997; Monadjem et al. 2011). Animals with home ranges of this magnitude would indeed be able to move easily between houses and surrounding fields.

The patchy vegetation structure of the fields allowed us to assess microhabitat choice of *M. natalensis* within and close to the villages. Overall, *M. natalensis* seems to move easily among vegetation types without showing clear preferences between grass, bush or cultivations. There were however some differences between the two villages. *M. natalensis* was trapped more frequently in cultivations than in bush and grass in Silimi, although the opposite was true in Tambaya. More vegetables were still present on the cultivations in Silimi compared to Tambaya, which might explain this result. In Tambaya, *M. natalensis* was most often trapped in grass, which is also assumed to be its natural habitat (Leirs 1994). In East Africa, abundances are generally lower in grasslands compared to agricultural fields, except during the early breeding season (Leirs 1994). This suggests that *M. natalensis* migrates from agricultural fields to grasslands for reproduction, probably because juveniles are better protected against predators in the denser grass vegetation. Since *M. natalensis* breeds throughout the year in Guinea (Fichet-Calvet et al. 2008), abundances in grass vegetation might be generally higher here.

We also captured several other rodent and shrew species in this study besides *M. natalensis*. These species were predominantly captured outdoors and assumed not to be reservoirs of LASV. Nevertheless, we included these animals in this study for two reasons. First, antibodies against LASV, or LASV-like arenaviruses, were found previously in some of these species, indicating that spillover infections could occur (Fichet-Calvet et al. 2014). Secondly, according to our knowledge, no other studies investigated the spatial behavior of these animals in rural villages. Although observed habitat choices and distances between captures were not exceptional for most species (Happold 1977), a high proportion of *P. daltoni* trapped in houses close to the RB field (<25m) showed signs of RB consumption. This proportion was considerably higher compared to *M. natalensis* (63% versus 30% respectively), suggesting that *P. daltoni* visits houses for short periods after which it returns to the fields, where it is trapped most often. In other countries such as Ghana, Senegal or Nigeria, *P. daltoni* can represent a larger proportion of the commensal rodent populations (Bâ et al. 2012; Kronmann et al. 2013; Olayemi et al. 2017). *P. daltoni* seems to become the main commensal species when *M. natalensis* is rare or the other way around.

Overall results from this study are rather discouraging for the development of simple rodent control programs. Occasional rodent elimination seems to have only limited succes, as *M. natalensis* is likely to reinvade houses or cultivations rapidly from fields (e.g. bush or grass patches) where rodents are not controlled. In support of this reasoning, local villagers in Sierra-Leone (a neighboring country) had the impression that rodents returned quickly after they set poison or traps, especially in houses close to the village border (Bonwitt et al. 2017). We therefore recommend that, in Guinea, rodents must be controlled on a permanent basis in order to be effective, at least during the dry season when *M. natalensis* abundances are high in the houses. Similar suggestions were made during experimental studies performed in rural villages in Namibia where contamination and loss of grain were clearly lower in year-round controlled than in non-controlled villages (Taylor et al. 2012). We also recommend combining rodent elimination with other control strategies. Rodent proofing of houses and storing food in airtight containers were found to be highly effective in discouraging rodents from entering houses, while keeping cats and dogs or attracting wildlife predators (e.g. owls, civets) might scare them away (Hopkins et al. 2002; Labuschagne et al. 2016; Mahlaba et al. 2017; Mdangi et al. 2013). Future research is necessary to see if these ecologically-based control strategies can indeed decrease LASV transmission risk to humans in Guinea.

Authors contributions

Conceived the study: JM, HL and EFC. Wrote the paper: EFC. Performed the experiments: JM, FK, EFC. Performed the analyses: JM. Supervised field and laboratory work: EFC and NM. All authors read and approved the final manuscript.

Tables

Table 1: Data by species and village on the number of unique and recaptured individuals, number of total recapture occasions, mean distance between successive recaptures (MDSR), mean distance between all recaptures (MDAR), sex (males:females) and reproductive activity (active:non-active) ratios of recaptured individuals.

Village	Species	Unique individuals	Recaptured individuals	Total recaptures	MDSR (m)	MDAR (m)	Sex ratio	Reproductive ratio
Tambaya	*Mastomys natalensis*	123	22	31	19.7 ± 3.7	25.1 ± 5.4	1:1.4	1:0.3
	Praomys daltoni	4	2	7	40.4 ± 9.7	60.4 ± 10.4	1:0	1:0
	Crocidura spp.	4						
Silimi	*Mastomys natalensis*	91	23	28	17.2 ± 3.4	18.6 ± 3.6	1:1.1	1:0.2
	Praomys rostratus	49	22	64	23.8 ± 4.3	32.4 ± 5.0	1:6.1	1:0
	Praomys daltoni	13	8	21	27.5 ± 7.6	40.8 ± 11.8	1:1.8	1:0.3
	Lemniscomys striatus	27	12	18	40.6 ± 9.8	49.4 ± 13.8	1:1.8	1:0.3
	Mus spp.	35	11	18	27.2 ± 4.4	34.7 ± 7.4	1:1.8	1:0.1
	Grammomys butingi	2	1	4	22.5	41.2	0:1	1:0
	Uranomys ruddi	2	1	1	20	20	1:0	1:0
	Lophuromys sikapusi	2	1	1	10	10	1:0	1:0
	Gerbilliscus guineae	2	1	1	31	31	1:0	0
	Rattus rattus	2						
	Crocidura spp.	22	5	9	14.0 ± 5.1	16.5 ± 7.2	1:0.3	1:0

Table 2: Average number of captured individuals per trap over all trapping nights. Results are given per habitat type for the five most abundant rodent species in the villages Tambaya and Silimi.

	Tambaya					Silimi				
Species	House	Open	Cultivations	Bush	Grass	House	Open	Cultivations	Bush	Grass
Mastomys natalensis	1.42		0.54	0.62	0.89	1.35	0.50	0.76	0.23	0.36
Praomys rostratus						0.15	0.17	0.48	0.70	0.88
Praomys daltoni	0.06		0.02	0.05	0.11			0.28	0.20	0.06
Lemniscomys striatus						0.04	0.17	0.25	0.22	0.33
Mus spp.						0.12	0.33	0.30	0.28	0.27
Number of traps	33	4	50	77	36	26	6	71	64	33

Table 3: Number of rhodamine B positive individuals per total number of individuals that were captured during the rhodamine B experiments in the villages Brissa and Yarawalia

	2015				2016			
	Brissa		Yarawalia		Brissa		Yarawalia	
Species	Houses	Field	Houses	Field	Houses	Field	Houses	Field
Mastomys natalensis	2/60	6/8	7/35	4/5	4/36	4/6	5/25	9/9
Praomys daltoni	0/1	6/6	5/14	1/1	2/2	3/3		2/2
Praomys rostratus								1/1
Rattus rattus					0/1			1/1
Mus spp.		0/2				0/1		
Lemniscomys striatus				1/1				1/1
Crocidura spp.				0/1				1/1

Supplementary Tables

S. Table1: Number of individuals that were captured x (one to ten) times during the capture-mark-recapture experiments in Tambaya and Silimi

Species	One	Two	Three	Four	Five	Six	Seven	Eight	nine	Ten
M. natalensis	159	33	11		1					
P. rostratus	27	7	4	2	3	3		1		1
P. daltoni	7	2	3	2	1	1	1			
L. striatus	15	1	7	4	1					
Mus spp.	24	6	3	2						
Grammomys butingi	1			1						
U. ruddi	1	1								
L. sikapusi	1	1								
G. guineae	1	1								
R. rattus	2									
Crocidura spp.	21	4			1					

S. Table2: Number of movements between habitat types or within the same type during the capture-mark-recapture experiments in Tambaya and Silimi for all recaptured individuals

	M. natalensis	*P.daltoni*	*P.rostratus*	*L. striatus*	*Mus* spp.	*Crocidura* spp.
House-House	12	1			1	
House-Grass	2		1			
House-Cultivation	4	1			1	
House-Bush	2		2	1		
Grass-Grass	5		11	1	2	3
Grass- Cultivation	3		3	1		
Grass-Bush	3		1	1		1
Cultivation - Cultivation	9	11	14	3	4	
Cultivation -House	4	1			0	
Cultivation -Grass	2	1	4	2	0	
Cultivation -Bush	2	2	2	2	3	
Cultivation Village				1		
Bush-Bush	7	7	22	1	3	2
Bush-House				1		
Bush-Grass	2		2			1
Bush- Cultivation	3	4	1	3	3	1
Village-House		1				
Village-Bush			1			
Village- Cultivation				1	1	
Total	60	29	64	18	18	8

S. Table 3: Pairwise comparisons of microhabitat preference between different habitat types during the capture-mark-recapture studies in Tambaya and Silimi

Species and village	Vegetation	Estimate	z-value	P value
	Cultivation - Bush	-0.14	-0.51	0.95
	Grass - Bush	0.35	1.23	0.58
M. natalensis	House - Bush	0.82	3.12	<0.01*
Tambaya	Grass - Cultivation	0.49	1.58	0.38
	House - Cultivation	0.96	3.22	<0.01*
	House - Grass	0.47	1.57	0.39
	Cultivation - Bush	1.17	0.31	<0.01*
	Grass - Bush	0.44	0.42	0.71
M. natalensis	House - Bush	1.7	0.35	<0.01*
Silimi	Grass - Cultivation	-0.73	0.35	0.15
	House - Cultivation	0.58	0.27	0.15
	House - Grass	1.3	0.38	<0.01*
	Cultivation - Bush	0.36	0.79	0.85
	Grass - Bush	-0.32	-0.53	0.94
P. daltoni	House - Bush	-1.26	-1.49	0.43
Silimi	Grass - Cultivation	-0.69	-1.33	0.66
	House - Cultivation	-1.63	-1.92	0.209
	House - Grass	-0.94	-1.02	0.739
	Cultivation - Bush	-0.38	-1.09	0.68
	Grass - Bush	0.22	0.53	0.94
P. rostratus	House - Bush	-1.5	-2.39	0.07
Silimi	Grass - Cultivation	0.67	1.46	0.45
	House - Cultivation	-1.14	-1.77	0.27
	House - Grass	-1.74	-2.59	0.04
	Cultivation - Bush	0.15	0.37	0.98
	Grass - Bush	0.42	0.91	0.78
L. striatus	House - Bush	-1.74	-1.63	0.34
Silimi	Grass - Cultivation	0.27	0.61	0.92
	House - Cultivation	-1.88	-1.78	0.26
	House - Grass	-2.16	-1.94	0.17
	Cultivation - Bush	0.05	0.12	0.99
	Grass - Bush	-0.03	-0.06	1
Mus spp.	House - Bush	-0.89	-1.25	0.58
Silimi	Grass - Cultivation	0.08	-0.16	0.99
	House - Cultivation	-0.94	-1.34	0.524
	House - Grass	-0.86	-1.12	0.67

Evaluation of rodent control to fight Lassa fever based on field data and mathematical modelling

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(Manuscript in preparation)

Abstract

The Natal multimammate mouse (*Mastomys natalensis*) is the reservoir host of Lassa virus, an arenavirus that causes Lassa haemorrhagic fever in humans in West Africa. Because no vaccine exists and therapeutic options are limited, preventing infection through rodent control and human behavioural measures is currently considered to be the only option. In order to assess the potential of rodent control, we performed a four-year field experiment in rural Upper Guinea and developed a mathematical model to simulate different control strategies (annual density control, continuous density control and rodent vaccination). For the field study, rodenticide baits were placed each year in three rural villages, while three other villages were used as controls. Rodents were trapped before and after every treatment and their antibody status and age were determined. Data from the field study were used to parameterize the mathematical model. In the field study, we found a significant negative effect of rodent control on seroprevalence, but this effect was small (5% reduction per year), especially given the effort. Furthermore, the rodent populations recovered rapidly after rodenticide application, leading us to conclude that an annual control strategy is unlikely to significantly reduce Lassa virus spillover to humans. In agreement with this finding, the mathematical model suggests that the use of continuous population control or rodent vaccination are the only strategies that could lead to Lassa virus elimination from the rodent population. These field and model results can serve as a guide for determining how long and frequent rodent control should be done in order to eliminate Lassa virus in rural villages.

Introduction

Lassa fever is a viral haemorrhagic fever caused by Lassa arenavirus (LASV), which is endemic in West Africa (Buckley et al. 1970). The main reservoir of the virus is the Natal multimammate mouse (*Mastomys natalensis*), a rodent species that thrives in rural villages and agricultural habitats, where it sheds the virus in its droppings and urine (Walker et al. 1975; Monath 1987; Lecompte et al. 2006). Humans mainly become infected through ingestion of contaminated food or water, inhalation of aerosolized virus particles, or direct consumption of an infected rodent (Stephenson et al. 1984; McCormick JB 1999; Bonwitt et al. 2017). Human-to-human transmission is thought to be rare, and mainly occurs in households and hospitals (Carey et al. 1972; Fisher-Hoch et al. 1995; Lo Iacono et al. 2015). By extrapolation from a seroepidemiological survey from 1987, it is estimated that between 100.000 and 300.000 infections could occur each year, with a fatality rate of 1-2% (Mccormick et al. 1987). However,

recent incidence reports suggest a substantial increase in the number and geographical extent of cases, exemplified by an unprecedented 2017-2018 outbreak in Nigeria (Safronetz et al. 2010; Kouadio et al. 2015; Roberts 2018). Although this can partly be explained by the availability of better diagnostic tools and increased public awareness (especially after the 2014-2016 West-Africa Ebola epidemic), increased spillover rates and transmission are likely, and can be driven by changes in climate, land use, and human mobility (Redding et al. 2016; Gibb et al. 2017). Because of the absence of a human vaccine or efficacious drug, the World Health Organization added LASV to its list of priority pathogens of epidemic potential for which there are no, or insufficient, countermeasures (World Health organisation, 2018). The options for LASV prevention are currently limited to rodent control and changes in human awareness and behaviour (Fichet-Calvet et al. 2007; Mariën et al. 2018). Here, we investigate the feasibility of rodent control for managing the spillover of LASV from rodents to humans.

Rodent control is expected to reduce LASV spillover risk based on two independent assumptions: (i) the contact rate between rodents and humans is positively related to rodent population density; and (ii) LASV prevalence in rodents is positively related to rodent population density (Davis et al. 2005). We consider the first assumption to be valid, as long as *M. natalensis* densities remain effectively lower in the houses (e.g. no direct recolonization of houses, but see chapter 6 of this thesis). The second assumption is a consequence of host and virus characteristics. For directly transmitted microparasites, two contrasting transmission modes are typically considered: density-dependent transmission when host contact rate (and transmission) increases linearly with host density; and frequency-dependent transmission when host contact rate remains constant with density (McCallum et al. 2001; Begon et al. 2002). For parasites with density-dependent transmission, a density threshold is predicted below which the parasite cannot invade in the population (Bartlett 1957; Begon et al. 2003; Lloyd-Smith et al. 2005). Rodent control measures, aiming at reducing the density of rodents below this threshold, would then be highly effective for LASV elimination even if it does not lead to complete eradication of the rodents (Davis et al. 2005). In contrast, parasites with frequency-dependent transmission are predicted to persist in very low-density populations, and (rodent) control measures aimed at reducing densities would be useless for LASV elimination. In this case, viral extinction can only be achieved if the proportion of susceptibles is too low for viral transmission (e.g. because most animals have become immune due to vaccination) (Morters et al. 2013; McCallum 2016).

For wildlife diseases, it is often difficult to determine direct relationships between parasite prevalence and host density because the ecology of the host is not well known (Morters et al. 2013; Borremans et al. 2017). However, as *M. natalensis* is the most important rodent pest species in sub-Saharan Africa, a lot of information about its ecology can be found in the literature (Leirs 1994; Fichet-Calvet et al. 2008; Sluydts et al. 2009). *M. natalensis* has a promiscuous mating system and is not territorial or aggressive towards conspecifics (Veenstra 1958; Kennis et al. 2008), and two studies independently found evidence for a strong positive relationship between population density and contact rates (Borremans et al. 2014; 2016). Furthermore, the analysis of a 10-year capture-mark-recapture time-series of a population of *M. natalensis* in Tanzania found that transmission of Morogoro virus, an arenavirus genetically closely related to LASV, is probably density-dependent (Chapter 5 of thesis). It therefore seems safe to assume that transmission of LASV in *M. natalensis* is density-dependent.

As density-dependence implies the existence of a density threshold for viral transmission, we expect that a reduction in rodent density will decrease and eventually prevent LASV transmission in the rodent population. In this study, we evaluate the effectiveness of this approach in rural villages in West Africa. First, we performed a four-year field experiment in Upper Guinea, in which rodents were eliminated annually in three villages using rodenticides, while three other villages were used as control. Rodent seroprevalence and age distribution were monitored during the entire experiment. Using information obtained from this field experiment we then developed a mathematical model to simulate LASV transmission. The model was used to estimate the effect of different control strategies (annual density control, continuous density control and rodent vaccination) on LASV invasion and extinction probability. The outcomes of the models can serve as a guide for how long and frequent rodent control should be done in order to eliminate LASV in a rural village.

Methods

Field experiment

Study sites

The field experiment was performed in the prefecture of Faranah (Upper Guinea), which was chosen for its high mean human LASV seroprevalence (35%) and the abundant presence of *M. natalensis* in the houses (>95% of captures is *M. natalensis*) (Lukashevich et al. 1993; Demby et al. 2001). In this area, six rural villages were selected based on the presence of LASV

(seroprevalence in the rodent population > 20%), their remote location from a paved road, a size not exceeding 1000 inhabitants and less than 45 min driving time from Faranah (Saez et al under review). Rural villages in this area typically consist of groups of houses clustered within small agricultural or fallow land patches, which is optimal habitat for a commensal species such as *M. natalensis* (Mariën et al. 2018). The villages themselves lie within a matrix of tropical dry forest (within or closely located to the National Park of the Upper Niger), in which *M. natalensis* is absent (Fichet-calvet et al. 2009). This means that we can consider the villages to be effective islands in which *M. natalensis* can thrive, connected only by human traffic routes. The six villages were randomly grouped into control (Brissa, Dalafilani and Yarawalia) and treatment (Damania, Sokourala and Sonkonia) villages.

Rodenticide Treatment

Rodenticide treatment was performed once a year (for 10 days) over a period of four years. The interventions were carried out during the dry season (November-April) when rodents were assumed to aggregate in houses to search for food and shelter. Anticoagulant baits (Bromadiolone or Difenacoum) were distributed in baiting stations (Coral, 158 Ensystex Europe) and were both placed in each open door house of the village, resulting in 300-600 baiting stations per village. We refer to Sáez et al (2018) for a more detailed explanation of the intervention and its effect on rodent abundance.

Rodent trapping

Rodents were trapped during three consecutive nights using Sherman live traps (Sherman Live Trap Co. Tallahassee, FL, USA), which were placed in pairs in 60 houses that were randomly chosen along a transect in the village. Traps were baited (with a mixture of peanuts, dry fish and wheat flour) in the evening and checked the next morning. Trapped rodents were humanely killed and necropsied in situ according to BSL3 procedures (Mills et al. 1995; Fichet-Calvet et al. 2007). Blood was drawn from the hart with a syringe and preserved on prepunched filter paper (±15 µL/punch; Serobuvard, LDA 22, Zoopole, France). Eyes were preserved in 10% formalin. Trapping sessions were performed before and after intervention in the treatment villages and once a year in the control villages. Due to personnel safety issues, it was not possible to trap rodents in the villages Sokourala (year 2 and 3) and Sonkonia (year 2) during the Ebola epidemic (Saez et al. 2018). In total, we analysed 14,394 trap nights. Trapping data are available at DOI 10.6084/m9.figshare.5545267.

Serology

Filter paper was stored in small re-sealable zipper bags with desiccant silica gel at -20ºC. Dried blood spots were punched out of the filter paper and eluted in phosphate buffer saline and 0.25% NH_3 (Borremans 2014). Presence of anti-LASV IgG antibodies in this elution was examined by indirect immunofluorescence assay (Wulff and Lange 1975; Hufert et al. 1989; Fichet-Calvet et al. 2014). Mouse antibodies were visualized using polyclonal rabbit anti-mouse IgG-FITC secondary antibodies (Dako, Denmark).

Eye lens weight

The age of individuals was estimated using eye lens weight (ELW), a known proxy for age in small mammals (Morris P, 1973). Eye lenses were extracted with forceps, cleaned, dried for 2 h at 100 °C and weighed to the nearest 0.1 mg (Fichet-Calvet et al. 2014). Raw ELW data were used as an age proxy for the statistical analysis of the field data. In order to parametrize the demographic component of the mathematical models (see below), we estimated age using the published function: age = e^(10.46088 +ELW/2)/4.35076 (Leirs 1994). This conversion was not used for the statistical analyses because the estimate error becomes very large for ELW values above 25 mg.

Statistical analyses

In order to assess the population recovery rate after intervention, we tested the effect of rodenticide treatment on ELW distribution in the villages. For this analysis, we used a linear mixed model with treatment status (treatment vs control village) and year (one to four as a factor) as independent fixed effects, village as random effect and ELW as dependent variable.

We were also interested to see whether rodenticide treatment affects the force of infection (FOI) in the rodent population. The FOI is an important epidemiological parameter that expresses the rate at which susceptible individuals become infected. Under the assumption of lifelong immunity, this parameter can be derived from age-specific seroprevalence data (Hens et al. 2012). In order to estimate FOI and test whether it is affected by treatment, we fitted generalized linear mixed models with ELW (proxy for age), treatment and year as fixed independent variables, village as random effect and antibody status (positive or negative) as dependent variable, assuming a binomial distribution with logit-link function. For this analysis we removed the youngest individuals from the dataset (ELW<15mg) because antibodies in these young animals could have been maternal.

We used the lmer and glmer functions of the lme4 package (version 1.1-7) of the R statistical software version 3.3.0 (R Core Team 2016). When fitting the models, we started with the fully parameterised models (all two-way interactions between the independent fixed variables) and sequentially dropped variables that had the highest insignificant p-values.

Ethics statement

All experiments were approved by the National Ethics Committee of Guinea (permit n° 12/CNERS/12 and 129/CNERS/16), performed in collaboration with the local health authorities (Prefecture de Faranah) and in agreement with the village chiefs. Rodenticide and traps were only placed in a house if permission was obtained from the individual house owner.

Modelling LASV transmission

Using data from the field experiment and previous studies, we developed a stochastic individual-based model (IBM) to simulate the spread of LASV in a population of *M. natalensis* in Upper Guinea. The central aim of the modelling study was to investigate the effectiveness and sustainability of different control methods (annual density control, continuous density control, or rodent vaccination) to eliminate LASV from a rural village. The IBM is explained using the scheme depicted in Figure 1. Individuals are categorized in six compartments: susceptible (S), exposed but not infectious (E), acutely infectious (I), recovered (R), maternal antibodies (M), and chronically infectious (C). Both demographic and transition (movement of individuals between states) events were a function of time (unit of time is 1 day) and stochastic. The model is similar to the model described in chapter 5 of this thesis, but some of the demographic and transition parameters differ.

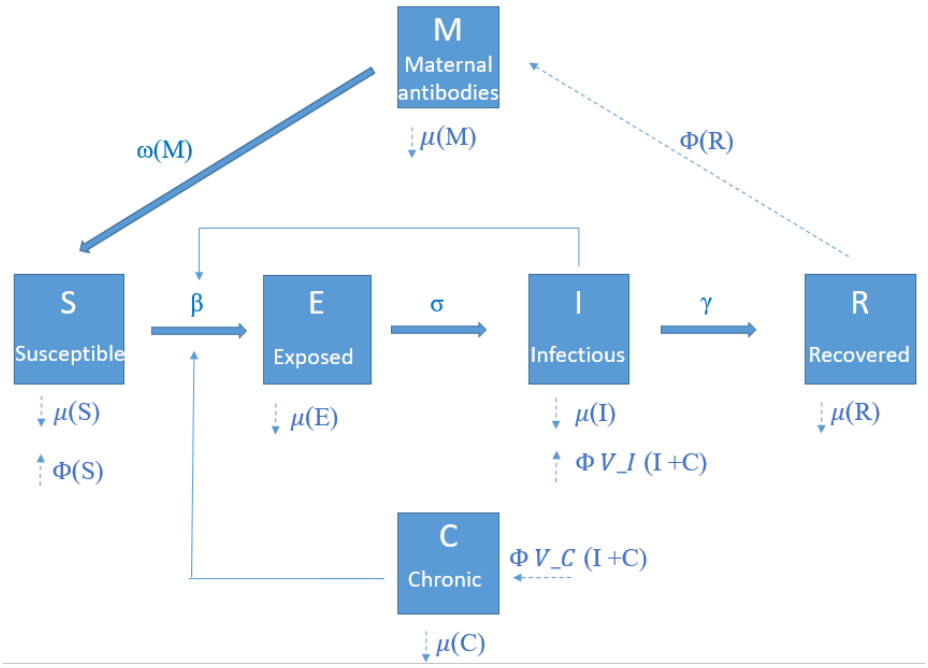


Fig 1: Schematic view of the individual-based model used to simulate the spread of Morogoro virus in populations of *M. natalensis* in Tanzania. Individual rodents are assigned to different states according to infection status: susceptible (S), exposed (E), acutely infectious (I), recovered (R), maternal antibody positive (M) and chronically infectious (C). State transition rates depend on the following parameters: transmission coefficient (β), latent period (σ^{-1}), infectious period (γ^{-1}), maternal antibody period (ω^{-1}). Fat solid arrows indicate possible transitions between different states. The dashed lines show the demographic parameters: Φ (birth rate) and μ (mortality rate). The probability to become acutely infected after vertical transmission is given by V_I and to become chronically infected by V_C. Thin solid arrows indicate that the rate at which individuals move from one state to another depends on the number of individuals in another state.

Demographic component

In order to test the impact of rodent control, the average rodent density in a typical rural village was implemented in the model. The best possible estimations on *M. natalensis* densities in Upper Guinea can be derived from a capture-mark-recapture study, indicating a rough estimate of 80 *M. natalensis* per hectare over the course of one month in a rural village (Mariën et al. 2018). Another field study showed that the overall trapping rate remained constant over the years (Fichet-Calvet et al. 2007). Based on these studies, we considered 80 *M. natalensis* per hectare to be a realistic average density (N_d) and assumed that it remains constant over time. To estimate the total rodent population size in a village, we measured the average surface of villages in this area using google earth (±25ha) and multiplied it with the average rodent density (25ha x 80 mice/ha = 2000 mice per village).

It was also important to implement a realistic age distribution in the model, as age-specific seroprevalence was used to estimate the FOI of the model. Optimization of birth (Φ) and mortality (μ) parameters was done by comparing the age distribution of the model to the field data (ELW data from trapping sessions before intervention). Birth (Φ) was a function of density (*N*) and modelled using the following equations:

$\Phi (N > 0.75 * N_d) = 0.0027$ [births/(individual*time interval)]

$\Phi (N \leq 0.75 * N_d) = 0.0044$ [births/(individual*time interval)].

If host density was below 0.75*(N_d) (e.g. after intervention), we increased the birth parameter so that the population would recover at the same rate as observed in the field experiment. Age of individuals followed an expontentially decreasing distribution based on the mortality parameter μ, which was age dependent and modelled by these equations:

$\mu\ (age < 50) = 0.002\ lifespan^{-1}$

$\mu\ (49 < age < 100) = 0.003\ lifespan^{-1}$

$\mu\ (99 < age < 366) = 0.007\ lifespan^{-1}$

$\mu\ (age > 365) = 0.02\ lifespan^{-1}$.

Because arenaviruses have no or limited adverse effects on their reservoir hosts, we assume that the birth and death rates are unaffected by infection (Mariën et al. 2017; 2018), so infectious individuals have the same demographic parameters as other individuals in the model.

Transmission component

Transmission in this study can be divided into a horizontal and vertical component. Horizontal transmission of MORV occurs with an infection rate $\frac{\beta Sk\hat{}q(I+C)}{N^q}$, following the implementation of Smith et al. (2009). This formulation allows to easily compare the different shapes of the transmission-density relation by adjusting the parameter q: if q=1, transmission is independent of density (frequency-dependence); if q=0, transmission is linearly related to density (density-dependence); and if 1>q>0, transmission follows a power function (intermediate between frequency- and density-dependence). In chapter 5, we suggested that q is (close to) zero for transmission of MORV in *M. natalensis*. However, as the commensal *M. natalensis* populations in West Africa might differ from the wild populations in East Africa, we implemented four different q values during the model simulations (q=0, 0.25, 0.50, 0.75). The parameter β represents the transmission coefficient, which is composed of k (the contact rate at a given q)

and v (probability of transmission between an infectious and a susceptible individual if they make a contact), and can be derived from the FOI (β=FOI/I). Optimization of β was done by comparing the FOI of the model to the field experiment (age and seroprevalence data from trapping sessions before intervention) for different values of q (Hens et al. 2012). Given that *Mastomys* is not territorial and seems to move randomly across the (relatively small) villages (Mariën et al. 2018), we assumed a homogeneously mixing community in which all individuals are also identical with respect to susceptibility and infectivity.

Vertical transmission occurs at the following infection rate: V_I [Φ (I+C)] + V_C [Φ (I+C)]. The model assumes that juveniles can enter the population as acutely (I) or chronically (C) infectious. The parameter V_C defines the proportion of juveniles that will become chronically infectious at birth. This parameter was fixed so that approximately 10% of the infectious individuals in the population became chronically infected, which matches findings of previous studies on LASV and MORV (Walker et al. 1975; Fichet-Calvet et al. 2014; Borremans et al. 2015; Mariën et al. 2017a). These chronic carriers were assumed to stay infectious for the rest of their lives. The parameter (V_I =1-V_C) defines the proportion of juveniles that will become acutely infectious by vertical transmission.

The model assumes that when a susceptible individual becomes infected, it first passes a latency state (E) for an average of 4 days (σ^{-1}) during which it is not infectious. Subsequently, it becomes acutely infectious (I) for an average of 35 days (γ^{-1}), after which it recovers from the disease and develops lifelong immunity (R). The average latency and infectious lengths were derived from inoculation experiments of LASV and MORV in *M. natalensis* (Walker et al. 1975; Borremans et al. 2015). We finally assume that offspring from antibody-positive mothers (R) will acquire maternal antibodies (M). These are present for an average of 30 days (ω^{-1}), after which they disappear and the individual becomes susceptible. Direct evidence of maternal antibodies is not available for LASV in *M. natalensis*, but the decrease in seroprevalence from newborns to juveniles in the field data suggests that maternal antibodies indeed occur (supplementary figure 2; and see Demby et al. 2001). Furthermore, maternal antibodies were directly observed for lymphocytic choriomeningitis virus (LCMV) in laboratory experiments (Oldstone et al. 2002). The assumption that we make in the model is that every infected mice remains for a fixed amount of time in one state (uniform distribution with probability of

transition 0) and afterwards moves to a different state depending on a probability that follows the exponential distribution.

Implementation of control methods

We investigated three potential LASV control methods:

Annual density control represents a situation similar to the interventions of the field experiment. The rodent population was reduced once a year by random removal of individuals in the model. The population has completely recovered by ten months after the intervention because of a birth rate (Φ) increase, which was forced on the model.

Continuous density control represents a situation in which the average rodent density (N_d) was reduced for a long period. This could be implemented by continuously distributing rodent traps or poison in houses and proximate cultivations, storing food into rodent-proof containers, or attracting more domestic or wildlife predators to the village. This situation was simulated by reducing N_d of the model over a period of 10 years.

Rodent vaccination represents a situation in which animals become immune due to an oral vaccine. This situation was simulated by changing the infection status of a random subset (50-90%) of susceptible individuals (S) to recovered (R). Different scenarios were simulated for distribution of an oral vaccine between once and four times per year.

For all control methods, we investigated whether they could eliminate LASV from the rodent population, how long (years) this would take and what the required mortality and vaccinated rate would be. We considered an elimination successful if the infection went extinct from the population within 11 years after the start of a simulation. We also investigated the **invasion probability** of LASV in a completely susceptible rodent population. An invasion was considered successful if the seroprevalence was higher than 10% at any moment after one infectious individual entered the population. We ran 1000 simulations for each control method and transmission-density coefficient (q=0, 0.25, 0.50, 0.75). The R code of the model with additional information can be found in the supplementary materials.

Results

Field experiment statistics

The trapping rate of *M. natalensis* was higher before than after rodenticide treatment (80% reduction), but there was no decrease in pre-treatment abundance over time (years) nor did we find a difference between treatment and control villages (supplementary figure 1 and Sáez et al. 2018 for P-values). In accordance, the mean ELW of pre-treatment individuals did not differ significantly between treatment and control villages (χ^2=0.89, df=1, p=0.34), and actually increased over time in both types of villages (χ^2=26.58, df=1, p<0.0001) (Fig. 2). Together, these results suggest that the rodent populations were strongly affected by rodenticide treatment in the short-term, but could easily recover within a period of at most one year.

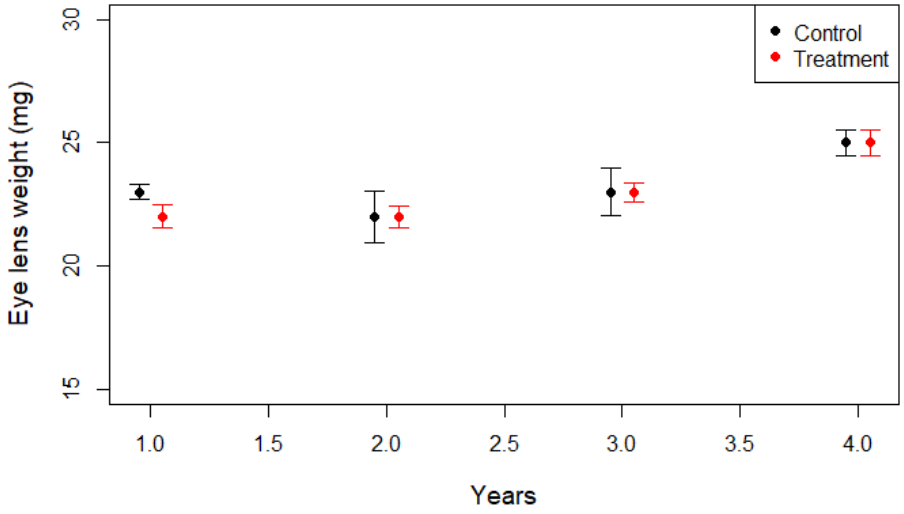


Fig 2: Mean eye lens weight (ELW) of *Mastomys natalensis* for the control (black) and treatment villages (red) in function of year. The ELW is used as a proxy for age in the rodents. The error bars indicate standard errors on the means.

Antibody presence increased significantly with rodent age (χ^2=71.30, df=1, p<0.0001) with an interaction between treatment and time (χ^2=15.69, df=1, p<0.0001), where seroprevalence decreased over time in the treatment villages (reduction of 5% per year over the whole age distribution), while remaining constant in the control villages (Fig 3). We also observed that the average seroprevalence (corrected for age effects using the slope of the ELW~seroprevalence correlation in year 1) decreased over time in all treatment villages, but not in the control villages (Fig 4). These results suggest that the FOI decreased in the rodent population because of the rodenticide treatment.

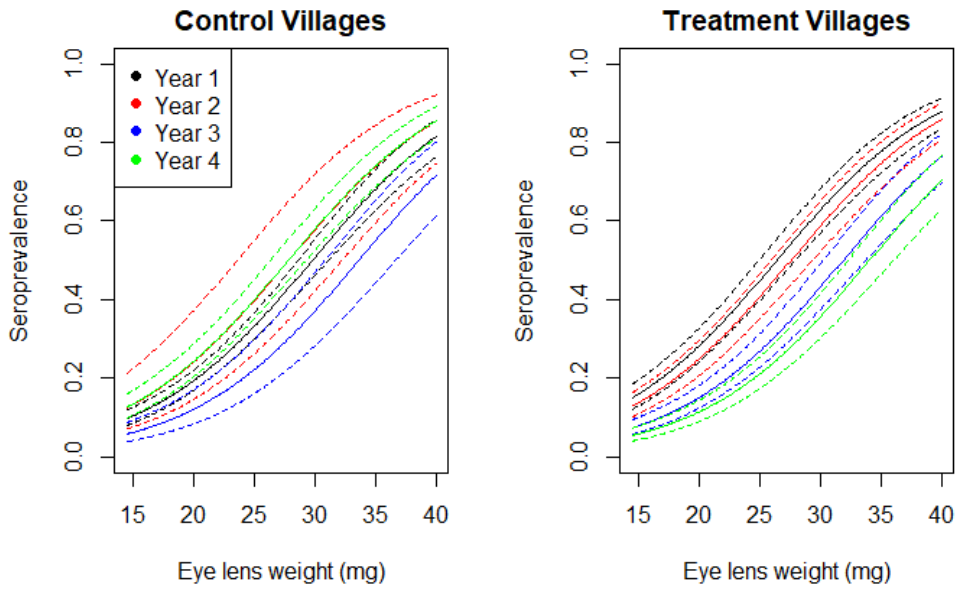


Fig 3: Proportion of LASV antibody-positive *Mastomys natalensis* as a function of the eye lens weight (proxy for age) for the control (left) and treatment (right) villages. The different colours represent the different years when rodents were captured. The solid lines represent mean estimated seroprevalence values (derived from a generalized linear model) and dashed lines standard errors.

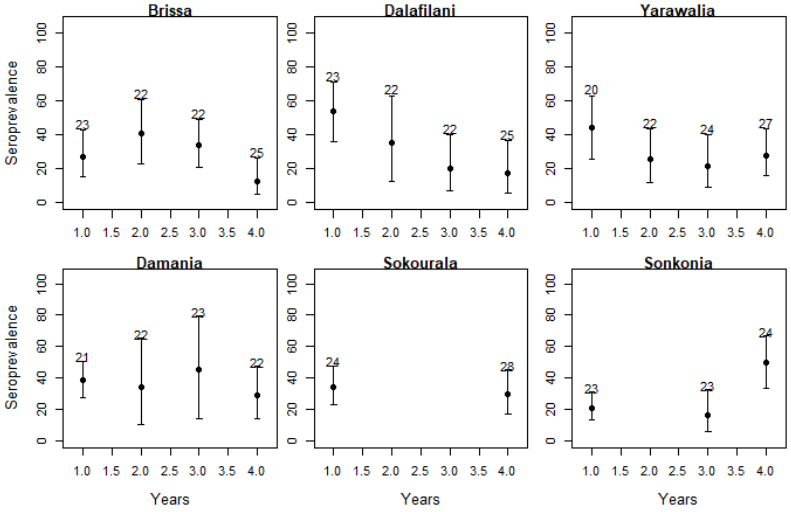


Fig 4: Mean LASV seroprevalence of *Mastomys natalensis* in the treatment (Brissa, Dalafilani and Yarawalia) and control (Damania, Sokourala and Sonkonia) villages over time (years). The seroprevalence was corrected by the mean eye lens weight (number on top of the bars). Bars indicate 95% (binomial) confidence intervals on the mean seroprevalence.

Simulation results

Annual rodent control

For annual rodent control, the simulation model suggests that viral extinction only occurs if the population density is reduced by at least 70% for ten consecutive years (Fig 5). However, the extinction probability strongly depends on the transmission-density coefficient (q). For example, if 70% of the population is killed each year, the predicted extinction probability varies from 1% for q=0.75 (little density-dependence) to 22% when q=0 (full linear density-dependence) (Fig 5 left). If we assume that the true value of q is around 0.25 (based on MORV data), annual rodent control is predicted to ensure LASV elimination (i.e. extinction probability ≥95%) only if more than 90% of the rodents are eradicated for a period of at least four years (Fig 5 right).

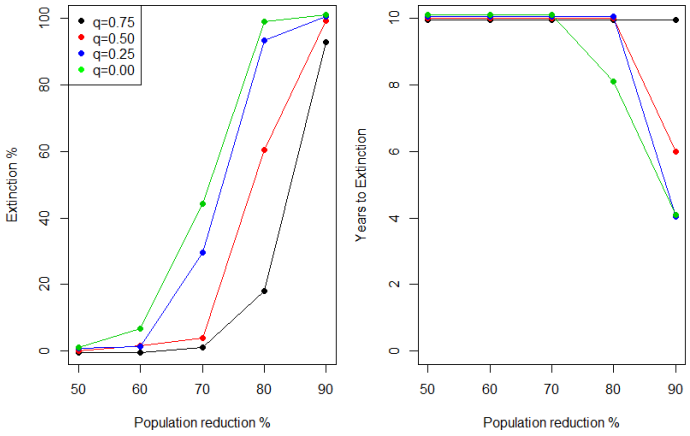


Fig 5: Model simulations to predict the effect of annual rodent control on LASV extinction probability in a *M. natalensis* in a rural village in Upper Guinea. The left figure shows the extinction probability in function of population reduction (e.g. 90% reduction = 10% of the population at carrying capacity) if rodent control was performed annually for ten consecutive years. The right figure shows the number of consecutive years that rodents need to be controlled to ensure LASV extinction (>95 % of simulation extinct). If years to extinction = ten years, at least ten years or more will be necessary to ensure extinction. The different colours represent simulations at different values of the transmission-density coefficient (q=0 is density-dependent transmission; q=1 is frequency- dependent transmission).

Continuous rodent control

In comparison with annual control, continuous control is more effective at eliminating LASV from the rodent population (Fig 6). Although the extinction probability again strongly depends on the assumed transmission-density coefficient (q), viral extinction can already be achieved at 50% population reduction (q<0.75) in ten years' time. If we again assume that q=0.25, rodent control is predicted to ensure LASV extinction when densities are reduced by 60% for four years (Fig 6 right).

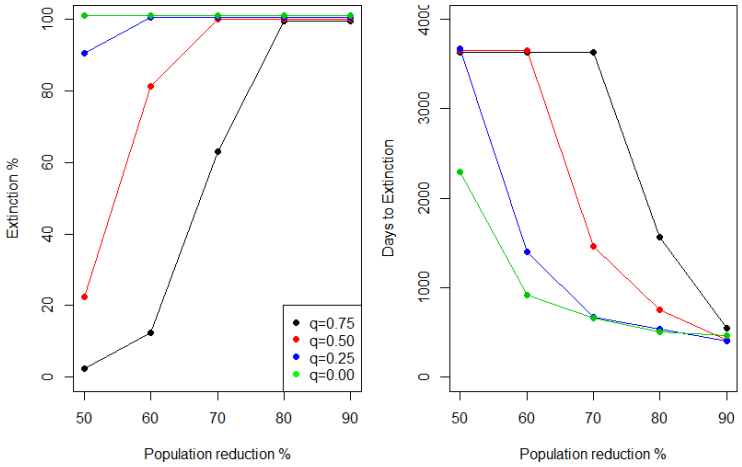


Fig 6: Model simulations to predict the effect of continuous rodent control on LASV extinction probability in a *M. natalensis* in a rural village in Upper Guinea. The left figure shows the extinction probability in function of population reduction (e.g. 90% reduction = 10% of the population at carrying capacity) if rodent control was performed continuously for ten consecutive years. The right figure shows the number of consecutive days that rodents need to be controlled to ensure LASV extinction (>95 % of simulation extinct). If days to extinction = 3650 days, at least 3650 days or more will be necessary to ensure extinction. The different colours represent simulations at different values of the transmission-density coefficient (q=0 is density-dependent transmission; q=1 is frequency-dependent transmission).

Rodent vaccination

The simulations predict that a rodent vaccine could effectively eliminate LASV if it is distributed more than once per year and when more than 70% of rodents become vaccinated (Fig 7 left). For example, if the vaccine is distributed three times per year and 70% is vaccinated, viral extinction is predicted to happen within three years, regardless of which q is assumed (as rodents densities remain constant in this scenario) (Fig 7 right). In contrast, when the vaccine is distributed only once per year, viral extinction is never predicted even at high vaccination rates (90%).

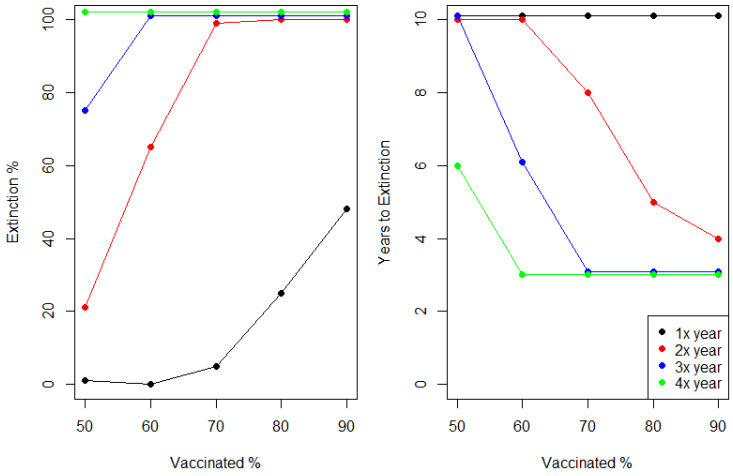


Fig 7: Model simulations to predict the effect of rodent vaccination on LASV extinction probability in a *M. natalensis* in a rural village in Upper Guinea. The left figure shows the extinction probability in function of vaccination rate (e.g. 90% vaccinated = 90% of the susceptible population is vaccinated). The right figure shows the number of consecutive years that rodents need to be vaccinated to ensure LASV extinction (>95 % of simulation extinct). If years to extinction = ten years, at least ten years or more will be necessary to ensure extinction. The different colours show the number of times per year the vaccine was distributed in the village.

Simulation results - Invasion probability

LASV invasion probability after introduction of one infected individual in a fully susceptible population increased positively with population density and the transmission-density coefficient (q). For q=0.25, successful LASV invasion can be expected at population densities reduced by 60% (population density of 32 *Mastomys*/ha) or less. At higher values of q (i.e. larger contribution of frequency-dependence), the population would need to be reduced by at least 90% to prevent LASV invasion (Fig 8).

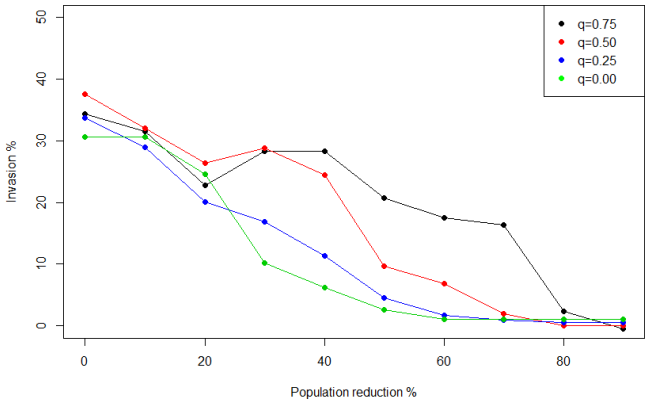


Fig 8: Model simulations to predict the invasion probability of LASV when one LASV positive *M. natalensis* enters a completely susceptible population in a rural village in Upper Guinea. The invasion probability is given in function of population density (e.g. 90% population reduction = 10% of the population at carrying capacity remains). The different colours represent simulations at different values of the transmission-density coefficient (q=0 is density-dependent transmission; q=1 is frequency-dependent transmission).

Discussion

We hypothesized that rodent control can be used to reduce LASV spillover risk to humans through a decrease in rodent density and/or LASV prevalence in the rodent population. Our field experiment shows that an annual rodenticide treatment can indeed strongly reduce rodent density in a rural village, but also that the population quickly returns to levels equivalent to those of the previous year (Saez et al. 2018; and ELW data). We also found that annual control can reduce LASV prevalence in the rodent population, but this reduction is likely too small (± 5% per year) to be a cost-effective and feasible strategy, given the high workload and financial costs. After four years of rodent density control, we can conclude that annual control is unlikely to significantly reduce LASV spillover risk to humans, as both rodent density and LASV prevalence rapidly return to pre-treatment levels. The inability to eliminate a sufficiently large proportion of the population (only rodents in houses) and the rapid recolonization and birth rate of *M. natalensis* are the main reasons for the limited success (Leirs 1994). Although the treatment effectively killed rodents in the houses (based on the carcasses that were found), we placed the baiting stations indoor and in open houses only. Rodents that lived outside or in closed houses remained unaffected and could continue breeding and transmitting the virus, before recolonizing the previously treated houses. In support of this reasoning, local villagers had the impression that rodents returned quickly after the treatment, especially in houses close to the village border (Saez et al. 2018).

Nevertheless, even though only a part of the total rodent population was eliminated, we did observe a small significant negative effect (5% per year) on the seroprevalence. This suggests that LASV transmission is density-dependent and that a density threshold exists below which the virus cannot persist in the rodent population (Davis et al. 2005). However, the mathematical model suggests that the threshold is low, probably due to a small subset of chronically infectious animals that can continue the transmission chain at low densities. If rodent control is performed once per year, the model indicates that rodent densities need to be reduced by 90% for at least four years to ensure virus extinction. This would be very difficult to achieve given that eradication would need to happen both indoors and outdoors, while it was already challenging to eliminate rodents in open houses only. Furthermore, if rodent control would stop after LASV extinction and rodent densities would return to pre-treatment levels, LASV could re-invade rapidly in the susceptible population (invasion probability is ±40% for even a single infectious rodent). Successful LASV invasion could be the result of an infectious rodent that arrives from a neighbouring village using human traffic routes (e.g. in food trucks) or could be the result of

a reverse zoonosis (human infects rodent) (Fichet-Calvet et al. 2016). The latter transmission route is not documented in the literature, but was earlier hypothesized to explain the transmission dynamics of the disease: humans become vectors by excreting the virus in urine and saliva on the ground, infecting rodents through contact with this contaminated soil (Fichet-Calvet et al. 2014).

In contrast to annual density control, continuous density control would be a more promising strategy for eliminating LASV from the rodent population. The model predicts that reducing rodent densities by 60% can ensure LASV extinction if it is maintained for at least four years. Continuous control however is labour-intensive and demands a human behavioural shift that includes the participation of the whole village community (Taylor et al. 2012). We recently proposed the development of an integrated control system by combining poisoning with regular trapping (Saez et al 2018). In this scenario, poisoning during the dry season would be combined with indoor trapping during the remainder of the year. In addition to active rodent control, specific changes in human behaviour could also reduce rodent densities, and are likely to be a more sustainable strategy. The hygienic state of houses in these rural villages is currently so low that even simple interventions (e.g. rodent proofing of houses or storing food in airtight containers) could have a big impact on the indoor rodent abundance (Bonwitt et al. 2017). In contrast, outdoor control could be more difficult to implement. Outside rodenticide treatment is less advised due to the potentially negative effects on other wild or domestic animals, while traps are often stolen (Taylor et al. 2008). An alternative for outside lethal control is fertility control through the distribution of hormones (or other chemical compounds) in baits (Humphrys and Lapidge 2008). This approach is not only suggested to be safer, but also to be more cost-effective and sustainable because it prevents compensatory reproduction and increased survival of rodents, which is often observed after rodenticide treatments (Hone 1992; Singleton et al. 2007; Stenseth et al. 2010). Preliminary studies with synthetic steroid hormones (quinestrol and levonorgestrel) in wild *M. natalensis* in Tanzania show higly promising results (Massawe et al. 2018). In addition to lethal and fertility control, biological control (e.g. attracting predatory birds to the village) might also work outdoors and could have long-lasting effects, which are not dangerous for humans or domestic animals (Labuschagne et al. 2016). Currently, predatory bird densities are very low in these areas because villagers kill these animals for food and superstitious reasons (e.g. owls are believed to represent bad ghosts), which might contribute to high rodent densities (Krebs et al 2013).

We also assessed whether rodent vaccination could be an efficient alternative to eliminate LASV. The models suggest that this approach could work if it is performed more than once per year and more than 70% of the population is vaccinated during three years. Although an oral vaccine has not yet been developed, it might be easier and cheaper to produce than a human vaccine, as it will be possible to skip the expensive and time-consuming clinical test phases (Cross et al. 2007; Hallam et al. 2018; Mendoza et al. 2018). The vaccine could be distributed to rodents in the form of bait pellets, similar to how rodents consume rodenticide. According to our knowledge, no attempts have been made to vaccinate rodent populations, and the elimination of rabies in foxes is the only example where a vaccine was successfully used to control a wildlife disease (Smith and Cheeseman 2002; McCallum 2016). Nevertheless, the delivery of an oral vaccine was also proposed for other wildlife diseases, including chlamydial infection in koalas, Tasmanian devil facial tumour disease and chytridiomycosis in frogs (Woods et al. 2007; Kollipara et al. 2012; Cashins et al. 2013).

We highlight that we had to make some assumptions in the model, which might reduce its prediction power, but creates opportunities for future research. First, more information is needed about *M. natalensis* densities in these rural villages, so that we know at least how many rodents need to be eliminated. Such quantitative information is available for many areas of sub-Saharan Africa, but not for the Lassa fever-endemic region (Swanepoel et al. 2017; Mariën et al. 2018). Other important aspects that still need to be addressed are the transmission-density relation and the percentage of chronically infected animals in the wild (chapter 5 of thesis). Recent data about MORV in *M. natalensis* improved our understanding of arenavirus ecology, but additional studies on LASV are necessary to compare these results.

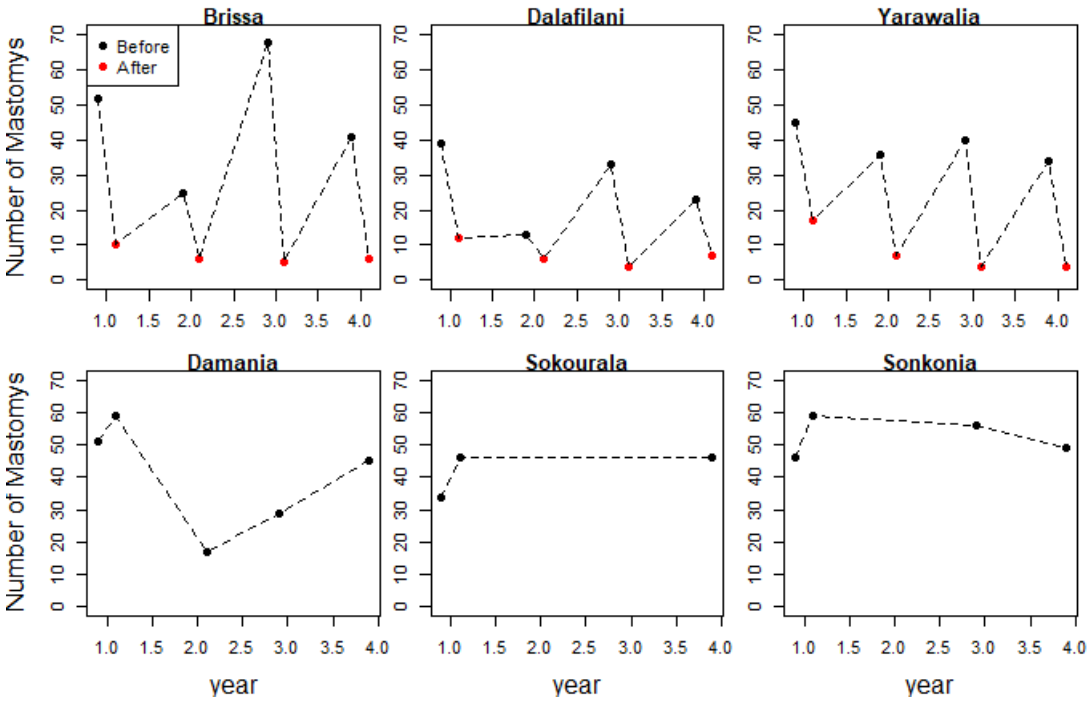
Conclusions

We conclude that an annual rodenticide treatment is unlikely to be a sufficient strategy for managing LASV spillover risk, because rodent populations returned rapidly to pre-treatment levels in our field trail and the reduction in seroprevalence was low (only 5% per year). In accordance, the mathematical model predicts that an annual treatment is an ineffective and unsustainable approach to eliminate LASV from the rodent population and prevent re-invasion. The models suggest that continuous density control or rodent vaccination would be better approaches, and should be considered by policy makers.

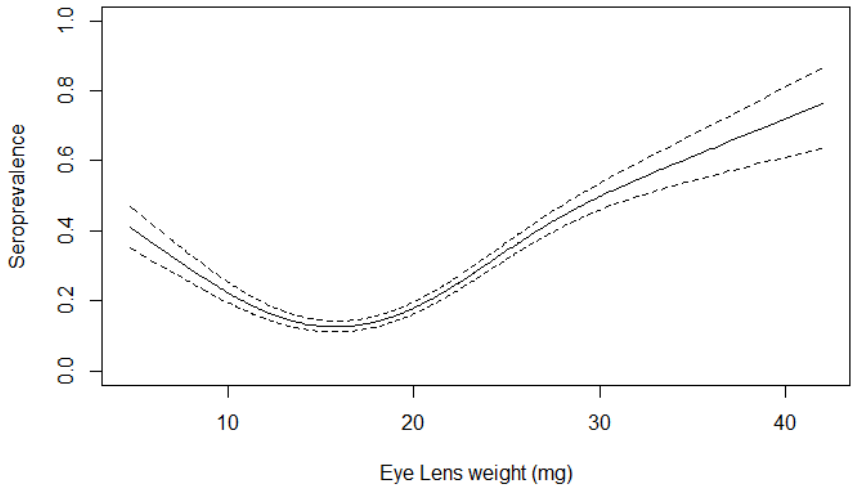
Author contributions

Conceived the study: JM, EFC and HL. Wrote the paper: JM. Performed the experiments: JM, FK, EFC. Performed the analyses: JM and BB. Supervised field and laboratory work: EFC, SG, and TR. All authors read and approved the final manuscript.

Supplementary Figures



Supplementary Fig 1: Number of *Mastomys natalensis* captured per trapping session before (black) and after (red) rodenticide treatment (Saez et al 2018).



Supplementary Fig 2*:* Proportion of LASV antibody-positive *Mastomys natalensis* as a function of the eye lens weight (proxy for age) modelled by a generalized additive model. Antibodies of individuals younger than 15mg were considered to be derived from the mother.

Discussion

1. Arenavirus persistence in highly fluctuating populations of the reservoir host

Parasites that occur in highly fluctuating host populations face two important problems. First, they must persist during low host density periods when transmission may cease due to insufficient contacts between hosts. Second, they must persist during periods following high host density when transmission may cease due to depletion of susceptible or infected hosts, as too many hosts might have become immune. However, parasites can overcome these challenges by evolving adaptive strategies. In order to explain how Morogoro virus (MORV) can persist in highly fluctuating populations of its reservoir host (*Mastomys natalensis*), we developed nine not mutually exclusive hypotheses (H1-H9) at the beginning of my PhD (chapter 1). Although I was not able to test all the proposed hypotheses, I will discuss all factors that might be important and include the knowledge that became available during my PhD in the following paragraphs.

Based on morphological characteristics (chapter 2) and survival data (chapter 3) of rodents, this thesis suggests that **MORV virulence is low in *M. natalensis***, which could be an adaptation of the virus to survive the yearly population bottlenecks of the host (H1). The result is in line with the trade-off hypothesis, which states that it is impossible for a parasite to increase its infection potential without paying a cost (Anderson and May 1982b; Alizon et al. 2009). For example, while highly virulent parasites (high β) can infect many other hosts in a short time-span (e.g. due to shedding of higher viral loads), they are also likely to increase the host's mortality rate. The reduced lifespan will then result in a shorter infectious period (γ^{-1}) and eventually a lower R_0. Low virulence towards *M. natalensis* was not only observed for MORV but also for Lassa virus (LASV) and Gairo virus (GAIV) in chapter 2. These viruses occur in populations with smaller density fluctuations, suggesting that low pathogenicity is not only the result of host density fluctuations but might be a general trend for arenavirus infections in the reservoir host. Nevertheless, we highlight that it was impossible to rule out the possibility that a small subset of infected animals may experience extreme pathogenic effects that lead to their immediate death (± between 1 and 5 days after infection), a situation that we could not test in our experimental setup. However, even if MORV would cause such fatal effects in some individuals, we argue in chapter 3 that the overall disease effect on host mortality (and thus on

the persistence probability of the virus) would be small in comparison to the effect of other factors (such as predation, climate variables or scramble competition for food).

Low virulence towards the reservoir host was also observed in laboratory experiments where mice were inoculated with arenaviruses. For example, inoculations with MORV or LASV caused no long-term effects on the morphology or survival of *M. natalensis* (Walker et al. 1975; Borremans et al. 2015). In contrast, two recent field studies suggested strong teratogenic effects of arenaviruses in their reservoir hosts: LASV in *M. natalensis* (Lalis et al. 2015) and Wēnzhōu arenavirus in *Rattus exulans* (Artois et al. 2017). Nevertheless, these field studies had two important limitations. First, their sample sizes were extremely low (100 and 43 animals respectively, whereas it was >1000 in our studies). Second, the authors did not appropriately correct for age effects, which is important as age correlates with both morphometric and infection parameters. For example, a the significant negative correlation between arenavirus presence and rodent age may easilty lead to a negative correlation between infection and body mass, when not correcting for age effects).

A small proportion of chronically infectious animals is probably the most important factor contributing to the persistence of MORV in populations of *M. natalensis* (H2). Because most individuals in the field are either PCR or antibody-positive (not both), arenavirus infections are generally assumed to be acute (Borremans et al. 2011; Fichet-Calvet et al. 2014; Olayemi et al. 2018). Nevertheless, laboratory experiments showed that animals can also become chronically infected when they are inoculated at very early age (as newborns) (Walker et al. 1975; Oldstone 2002; Borremans et al. 2015). Such chronic infections were never observed in natural conditions, possibly because they are difficult to detect in the field due to several reasons (Buhnerkempe et al. 2017). For example, animals need to stay alive and located at approximately the same place to be recaptured repeatedly after the initial sampling occasion. This might be especially a problem for arenaviruses, given that we only expect a small proportion to be chronically infected, thus the sample size needs to be sufficiently high. Another reason is that viruses might not be constantly present in blood or excretions but instead retreat into organs that are difficult to sample non-lethally (Plowright et al. 2016).

In chapter 4, we describe a first attempt to find such chronic carriers in longitudinal samples that we collected during three months of bi-weekly trapping in Morogoro. We found evidence for chronic infections in only one out of 39 infected animals. This individual was MORV RNA-positive for at least 55 days in blood and excretions. We also observed evidence for temporary flare-ups of excretion in six other animals, suggesting that some animals develop latent

infections in which the virus retreats to organs from where it might be reactivated under stressful conditions (Plowright et al. 2016). To further investigate this possibility, we performed an experiment in which wild infected *M. natalensis* were caged for eight weeks and afterwards dissected (chapter 5). Thereby, it was not only possible to screen blood and excretion samples for viral RNA but also organs. We observed that 8% (3/38) of the infected animals were a potential chronic carrier, or at least contained MORV RNA in the body for a time period of eight weeks. Then, using simulations of a mathematical model, we investigated the importance of these carriers for long-term persistence in the host population (chapter 5). When animals could not become chronically infected, MORV could simply not persist in these simulations, not even when the overall population size (the sampling area over which we assumed homogeneous mixing) corresponded to an area as high as 50 ha (Fig 5 chapter 5). We therefore conclude that a low number of chronic carriers (>5%) is necessary to ensure MORV persistence during the critical low host density period. A similar situation has been found for Puumala hantavirus infections in bank voles (*Myodes glareolus*) (Forbes et al. 2018). After an initial peak in viral shedding of one month, a subset of voles continues to shed and transmit hantavirus probably throughout their lifetime (Bernshtein et al. 1999). These chronic infections were suggested to be a means for hantavirus to persist during host populations bottlenecks and to recolonize fragmented areas where temporal extinction may occur more easily (Voutilainen et al. 2015)

Long-time survival outside the host and indirect transmission are two additional factors that could increase the persistence probability of MORV (H3). Although outside survival is arguably important to explain intraspecies transmission for many arenaviruses (e.g. for LASV or LCMV), empirical data to support a free-living stage are rare (Oldstone 2002; Bonwitt et al. 2017). According to my knowledge, the only available data comes from laboratory experiments where LASV was deposited on solid surfaces in the dark (90% inactivation at 60h) or aerosolized (50% inactivation at 60h) (Stephenson et al. 1984; Sagripanti et al. 2010). While these data provide a first indication of how long arenaviruses can survive outside the host, the situation in the natural environment can differ substantially (e.g. UV-radiation or humidity are known to severely affect the biological half-life of RNA viruses) (Lytle and Sagripanti 2005; Tang and Tang 2009). The importance of indirect transmission for viral persistence has been more extensively investigated for hantaviruses (Forbes et al. 2018). Here, the existence of a free-living viral stage (e.g. two weeks for Puumala hantavirus in *Myodes glareolus*) has been shown to significantly increase persistence during the low-density period of the host population

(Sauvage et al. 2003; Eva R. Kallio et al. 2006). Furthermore, based on model simulations, it was observed that even a short free-living stage can significantly decrease stochastic extinction risk (Sauvage et al. 2003). To explore the longevity of MORV once shed into the environment, we performed two transmission experiments in the laboratory during my PhD. Unfortunately, both experiments failed to provide clear results for several reasons. For example, two out of ten receptor animals seroconverted after exposure to infectious material in the first experiment, but we did not manage to recover viral RNA by PCR. Therefore, we could not exclude the possibility that the seroconversion was due to aspecific binding of the antibody assay. The performed experiments are explained in more detail by B. Borremans (2015, PhD thesis) and S. Van Den Panhuyzen (2018, master thesis).

MORV might **manipulate the behaviour** of *M. natalensis* in order to increase its persistence probability (H4). Some RNA viruses are indeed known to alter their host's behaviour. For example, rabies virus induces aggressive (biting) behaviour in its host, as this is an effective way of propagating itself (high viral load in saliva) (Thomas et al. 2005; Nagaraj et al. 2006). Because such manipulative effects have been observed for other arenaviruses (e.g. LCMV can impair the learning capacity of laboratory mice), it is not unreasonable to hypothesize that MORV could affect the behaviour of *M. natalensis* too (Gold et al. 1994; De La Torre et al. 1996; Kunz et al. 2006; VanDen Broecke et al. 2017). We briefly discussed this possibility in chapter 3, where we found a significant positive correlation between MORV antibody-status and *M. natalensis*' recapture probability. One explanation for this correlation was that MORV decreases neophobic behaviour in the rodent, a phenomenon which is described for brown rats (*Rattus norvegicus*) infected with *Toxoplasma gondii* (Webster et al. 1994). In the same way, it might be possible that MORV stimulates social or sexual behaviour of its host, thereby increasing the host's contact rate and perhaps even its own transmission.

Sexual behaviour could increase the persistence probability of MORV during the breeding season, which largely coincides with the low-density period (H5). Indeed, sexual behaviour may increase transmission in two ways. On the one hand it may increase the number of direct contacts between mice of different sexes, which is very likely for *M. natalensis* as it is a promiscuous rodent that actively searches for mates during the breeding season (Kennis et al. 2008). On the other hand it may facilitate transmission during contact, given that reproductive organs often contain high arenaviral loads (Webb et al. 1975). The possibility of sexual transmission for arenaviruses was briefly discussed in chapter 2, where we found that sexual maturity of *M. natalensis* correlates positively with LASV and GAIV (but not MORV) antibody

presence. Sexual transmission was also suggested for other arenaviruses. For example, Machupo virus transmission in vesper mice (*Calomys callosus*) was suggested to occur at least partly by sexual contacts, as transmission rates were considerably higher in opposite- than same-sex pairs during a cage experiment (Webb et al. 1975).

Vertical transmission is known to efficiently contribute to the intergenerational maintenance of viruses (e.g. Bhatta et al. 2018; Contopoulos-Ioannidis et al. 2018). Therefore, it might also prevent extinction of MORV during the low-density (breeding) season (H6). Although direct evidence of this mechanism does not exist for MORV, some information can be derived from the available field data. For example, the fact that MORV-RNA positive animals usually belong to younger age classes indicates that some individuals may become vertically infected (Borremans et al. 2011). In chapter 5, we also show that ecological models with vertical transmission (in combination with horizontal transmission) better fit to the seasonal seroprevalence pattern observed in the field than models without vertical transmission. More direct evidence was observed in laboratory experiments where offspring of LCMV inoculated house mice (*M. musculus*) were all LCMV positive, and high viral loads were detected in the placenta, ovaries, uterus and mother milk of female mice (Mims 1970; 1981; Oldstone 2002). Furthermore, vertical transmission was also suggested to be important for the persistence of Junin arenavirus in fluctuating populations of vesper mice (*Calomys musculinus*) (Vitulla and Merani 1988; 1990). It therefore seems that vertical transmission is a common mechanism for arenaviruses to spread and perhaps persist in populations of the reservoir host.

Maternal antibodies may prevent extinction of MORV by temporally reducing the transmission rate during the high-density period (H7). The rationale behind this hypothesis is as follows. Individuals may lose maternal antibodies at varying rates, so susceptible individuals will enter the population over an extended period, in contrast to their immediate influx. This gradual influx might lower the transmission rate during the high-density period, resulting in a larger pool of susceptibles that can maintain infection the following low-density period (Boulinier and Staszewski 2008). Besides two potential observations of maternal antibodies against MORV in the longitudinal field data (discussed in chapter 4), we do not have any indications of their existence. However, maternal antibodies were also suggested for LCMV infections in house mice and LASV in *M. natalensis*, indicating that they might be generally transferred after arenavirus exposure (Baldridge and Buchmeier 1992; Demby et al. 2001; Fichet-Calvet et al. 2014). The ecological importance of maternal antibodies has been more extensively investigated for hantaviruses (Kallio et al. 2006). Based on longitudinal field data

and modelling techniques, it seems that maternal antibodies lower the transmission rate of Puumala hantavirus during cycle peak years of their reservoir host (*M. glareolus*), which might prevent virus extinction the following year (Kallio et al. 2010; Voutilainen et al. 2016). In the same way, maternal antibodies might significantly affect the transmission dynamics of arenaviruses.

There are also some aspects of the ecology of the host that could increase the persistence probability of MORV. We initially assumed that contact rates of *M. natalensis* are linearly related to density due to the lack of territoriallity (Veenstra 1958; Borremans et al. 2014). However, a more recent field experiment revealed instead that contact rates of *M. natalensis* scale non-linearly with density, in a sigmoid fashion, where **contact rates remain relatively high at low densities** (Borremans et al. 2016). This may prevent MORV from disappearing during the low-density season, especially since this period also corresponds to the breeding season when sexual behaviour may further increase the number of contacts (H8). In chapter 5, we explored this possibility based on the long-term field data and model simulations. These simulations suggest that the relation between MORV transmission and host density is most likely density-dependent, although there was equal support for a linear and non-linear density-dependent model. Since there is no support for frequency-dependent transmission, it seems that the contact-density relation of the host would rather prevent than facilitate MORV persistence. Nevertheless, we highlight that it was impossible to infer the exact transmission-density relation based on the serology data alone and that better (semi-controlled) field experiments are necessary to provide more definite answers.

Metapopulation dynamics may be a final factor that can contribute to the long-term persistence of MORV (H9). If host densities would fluctuate asynchronously in time or space, and when subpopulations are in regular contact due to migration of individuals, extinction-recolonization dynamics may allow host densities to locally fall below the critical population density (N_T) (Foley et al. 1999; Hanski 1999; Guivier et al. 2011). According to my knowledge, metapopulation dynamics have never been investigated for arenaviruses. However, they could be examined by looking at the spatiotemporal genetic pattern of these viruses. Indeed, due to their high mutation rate, RNA viruses will likely contain information about their location's origin at a fine spatial scale (Rambaut et al. 2009). By genotyping all MORV strains in the long-term capture-mark-recapture data, it would be possible to compare the strain composition before and after the high-density season, and to relate it to the strain composition observed in nearby fields. Such a genetic study was recently performed for Puumala hantavirus in

populations of bank voles (*M. glareolus*) in Germany (Weber de Melo et al. 2015). However, as the genetic analyses suggested strong geographical structuring at the very local scale and the persistence of strains throughout multiple years, the conclusion was that transmission between neighbouring host populations contributes little to the persistence of this virus.

2. Rodent control to reduce LASV spillover risk to humans

The second goal of this thesis was to investigate the efficiency of rodent control to reduce and eliminate LASV in *M. natalensis* populations. This is relevant for the development of public health strategies as rodent control is currently suggested to be the only option to reduce LASV spillover risk to humans. We first investigated the transmission dynamics of MORV, which given its similarities to LASV (e.g. 62% nucleotide similarity for L-gene and same reservoir host), is considered a safe and appropriate substitute for testing hypotheses related to LASV ecology (chapters 2-5). Then, we performed field experiments to gain more insight in how *M. natalensis* moves in LASV-endemic villages (chapter 6). Finally, we analysed data from a rodent control experiment performed in six Lassa fever-endemic villages in Guinea, and combined all the available information into a mathematical model, which made it possible to answer quantitative questions on how rodent control should be performed to eliminate LASV (chapter 7).

Overall results in this thesis are equivocal for the use of rodent control techniques to manage LASV. Because we found that MORV and LASV transmission rates decrease at lower *M. natalensis* densities (chapter 5 and 7), it was expected that killing rodents would reduce LASV spillover because of two reasons: (i) there are fewer rodents and (ii) proportionally fewer rodents are infectious (Davis et al. 2005). However, rodent control is unlikely to completely eliminate LASV from the rodent population, as the survival of only a few chronically infected animals seems sufficient for viral persistence and the reduction in rodent density is likely too small for a rapid elimination of the virus. Indeed, we only observed a 5% reduction in seroprevalence per year in Guinea, although the rodent population was reduced by 80% on a yearly basis (hypothesis H11 in chapter 1). Furthermore, as population densities returned quickly to pretreatment levels in the experiment in Guinea, we can assume that LASV prevalence will do the same, which was clearly supported by simulations of the mathematical model (chapter 7). We therefore conclude that occasional (e.g. annual) rodent control is an unsustainable strategy to reduce LASV spillover risk to humans (H13).

Although the annual rodent control experiment was clearly unsuccessful, other control strategies can be more effective to eliminate LASV in rural villages. Model simulations suggested that both continuous density control and vaccination of rodents could more effectively eliminate LASV (chapter 7). For example, extinction of infection was predicted in four years' time if the rodent population was permanently reduced by 60% or if more than 70% of animals were vaccinated. Unfortunately, these control methods are more labour-intensive, expensive and demand the participation of the whole village community (Taylor et al. 2012). For these reasons, we recommend the use of an integrated control system in which poisoning is combined with year-round rodent trapping, human behavioural changes (e.g. rodent proofing of houses, or storing food in airtight containers) and more ecologically based methods (e.g. attracting wildlife predators or keeping cats). Furthermore, as we showed that *M. natalensis* often moves between the houses and the outside fields in rural villages (chapter 6), we highlight that rodents should not only be controlled inside houses but also in the fields surrounding the village (hypothesis H10 in chapter 1). While outside poisoning is less advised due to the potentially negative effects on other wild or domestic animals, fertility control could be an interesting alternative to keep rodent populations low in the fields. This technique is not only suggested to be safer but also to be more sustainable because it prevents compensatory reproduction, a phenomenon often noticed after poisoning rodents in agricultural fields (Hone 1992; Singleton et al. 2007; Stenseth et al. 2010). Future studies could investigate if the proposed integration of different control techniques can indeed decrease LASV transmission risk to humans in a more sustainable way.

3. Future research

The work in this thesis focussed on the transmission of arenavirus at the population level (the population averaged approach) and put little emphasis on how individual characteristics might affect transmission. Nevertheless, simulation studies have shown that individual heterogeneity in the acquisition of infectious diseases can have a large impact on the estimation of model parameters and overall conclusions (Abrams & Hens, 2014; McDonald et al. 2016). While we considered the MORV-*M. natalensis* to be a single parasite-host system, we cannot exclude that other parasites influence the host's individual likelihood to become infected, as they may hinder or facilitate MORV entry or replication within the host. For example, new malaria infections are six times more likely to occur in HIV-positive compared to HIV-negative humans (Kamya et al. 2006). In turn, effects of co-infection at the scale of the individual may affect host parasite

dynamics at the population level (Telfer et al. 2008). Similarly, infection heterogeneity can be attributed by consistent behavioural differences between individuals across time and contexts, also knowns as animal personality (Réale *et al.* 2007; Carere & Maestripieri 2013). For example, bolder deer mice (*Peromyscus maniculatus*) are three times more likely to be infected with the Sin Nombre virus and known to cause most of the transmission events (Clay et al. 2009; Dizney & Dearing, 2013). These results suggest that both co-infections and personality might be incorporated into future models to get a better understanding of the overall transmission dynamics of rodent-borne diseases.

Studying how climate variables affect arenavirus prevalence in rodents is an important next step for research on LASV, as effects can be expected on both the survival probability of the virus and the host. Relating climate variables to host and virus characteristics may help to predict LASV spillover risk in the near or more distant future. For example, given that extreme weather events may significantly impact rodent densities, it might be possible to predict and anticipate LASV outbreaks a few weeks in advance (Yates et al. 2002; Demissie and Mengisitie 2017). Similarly, as climate models predict hotter and wetter conditions in West Africa the coming decades, it is suggested that the climatic suitability for *Mastomys* will increase and thus also the overall Lassa fever burden (Redding et al. 2016).

Another question that needs further investigation is how easily rodents migrate between villages in West Africa. In the mathematical models developed in this thesis (chapter 7), we considered rural villages to be islands on which *M. natalensis* can thrive surrounded by inhabitable tropical forest. We therefore assumed that these villages are not connected to each other and that there is no (or limited) migration of *M. natalensis* between villages. However, villages are connected to each other by roads on which human traffic might facilitate LASV gene flow, e.g. by passive transportation of infected rodents in food trucks. A recent study found that LASV strains in villages are diverse and polyphyletic, suggesting that gene flow between villages is more common than previously thought (Fichet-Calvet et al. 2016). Therefore, it might be interesting to further explore how and at which rate LASV strains move between villages, as this will give an indication of how fast susceptible villages might become invaded and which villages are currently at the highest risk. Given that human displacements will increase significantly the coming decades, we can hypothesise that LASV will spread more rapidly than ever to currently unaffected regions in West Africa.

A question related to the previous one is how important humans are themselves as vectors of LASV. In the mathematical model of this thesis (chapter 7) we assumed that rodents are the

primary reservoirs of LASV. However, given that LASV is highly transmissible from contact with body fluids of infected humans, it is likely that humans can also infect rodents (e.g. by urinating on the ground). Although there is no empirical evidence of this 'reversed zoonosis' hypothesis, this transmission route could explain several important aspects of the epidemiology of the disease. For example, spatial investigations in the Lassa fever countries showed that few localities have LASV positive *M. natalensis* (Fichet-Calvet et al. 2014). This patchy distribution may be explained by infected humans that spread the virus by trade or travel to rodent populations in new villages (in combination with passive transportation of infected rodents). While the majority of reversed spillover events may go extinct rapidly, some spillover events may successfully invade a rodent population. If an invasion has successfully spread, infected rodents may act in turn as amplifiers of LASV transmission to humans.

4. Overall conclusions

In this thesis, we present new insights into the transmission dynamics of Old World arenaviruses in populations of their reservoir hosts. By using the MORV-*M. natalensis* system as a model, our main results suggest that arenaviruses exhibit several characteristics that can prevent extinction at low densities, such as their avirulent residence and ability to initiate chronic infections in the host. The importance of other characteristics for viral persistence was also suggested (e.g. host manipulation and indirect, sexual or vertical transmission), although they were not investigated in detail in this thesis. Then, based on the insights derived from MORV and additional field experiments in Guinea, we developed a mathematical model to predict the effectiveness of rodent control techniques to manage LASV spillover risk. Both field experiments and model simulations suggest that annual rodent elimination is pointless, as *M. natalensis* will reinvade houses quickly and LASV prevalence will return quicly to levels before control. In contrast, the models suggest that continuous elimination or vaccination of rodents are more sustainable approaches, and should be considered by policymakers in combination with other prevention strategies, such as rodent proofing of houses.

Acknowledgments ~ Dankwoord

Mijn naam mag dan wel op de voorpagina van dit doctoraat staan, eigenlijk was de realisatie hiervan één groot groepswerk. Ik zou dan ook enkele mensen expliciet willen erkennen die hiertoe hebben bijgedragen.

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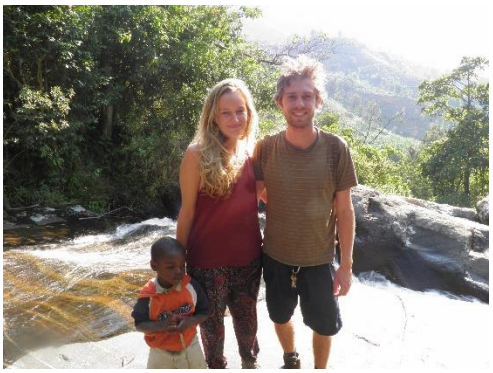

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References

Abbott KD, Ksiazek TG, Mills JN (1999) Long-term hantavirus persistence in rodent populations in central Arizona. Emerg Infect Dis 5:102–12. doi: 10.3201/eid0501.990112

Abrams S, & Hens N (2014). Modeling individual heterogeneity in the acquisition of recurrent infections: An application to parvovirus B19. *Biostatistics*, *16*(1), 129–142. doi:10.1093/biostatistics/kxu031

Adler FR, Pearce-Duvet JMC, Dearing MD (2008) How host population dynamics translate into time-lagged prevalence: An Investigation of Sin Nombre virus in deer mice. Bull Math Biol 70:236–252. doi: 10.1007/s11538-007-9251-8

Alan G, Frank B, Tetsuhisa M, Xuefei M, Friedrich L, Fabian S (2017) mvtnorm: Multivariate Normal and t Distributions. R package version 1.0-8. URL http://CRAN.R-project.org/package=mvtnorm

Alizon S, Hurford A, Mideo N, Van Baalen M (2009) Virulence evolution and the trade-off hypothesis: History, current state of affairs and the future. J Evol Biol 22:245–259. doi: 10.1111/j.1420-9101.2008.01658.x

Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P (2006a) Seasonality and the dynamics of infectious diseases. Ecol Lett 9:467–84. doi: 10.1111/j.1461-0248.2005.00879.x

Amstroing C, Lillie RD (1934) Experimental Lymphocytic Choriomeningitis of Monkeys and Mice Produced by a Virus Encountered in Studies of the 1933 St . Louis Encephalitis Epidemic. Public Health Rep 49:1019–1027.

Anderson R, May R (1978) Regulation and Stability of Host-Parasite Population Interactions : I . Regulatory processes. J Anim Ecol 47:249–267.

Anderson R, May R (1979) Population biology of infectious diseases: Part I. Nature 280:361–367.

Anderson RM, May R (1979) Population biology of infectious diseases: Part II. Nature 280:361–7.

Anderson R, May R (1981) The Population Dynamics of Microparasites and Their Invertebrate Hosts. Philos Trans R Soc B Biol Sci 291:451–524. doi: 10.1098/rstb.1981.0005

Anderson R, May R (1982) Coevolution of hosts and parasites. Parasitology 85:411–426. doi: 10.1017/S0031182000055360

Antonovics J, Wilson AJ, Forbes MR, Hauffe HC, Kallio ER, Leggett HC, Longdon B, Okamura B, Sait SM, Webster JP (2017) The evolution of transmission mode. Philos Trans R Soc B Biol Sci 372:20160083. doi: 10.1098/rstb.2016.0083

Artois J, Blasdell K, Duong V, Buchy P, Hul V, Morand S, Claude J (2017) Effects of mammarenavirus infection (Wēnzhōu virus) on the morphology of Rattus exulans. Infect Genet Evol. doi: 10.1016/j.meegid.2017.05.023

Asano BMS, Ahmed R (1996) CD8 T Cell Memory in B Cell-deficient Mice. Journal of Experimental Medicine 183:2165–2174.

Asogun DA, Adomeh DI, Ehimuan J, Odia I, Hass M, Gabriel M, Olschlager S, Becker-Ziaja B, Folarin O, Phelan E, Ehiane PE, Ifeh VE, …, Akpede GO, Günther S (2012) Molecular Diagnostics for Lassa Fever at Irrua Specialist Teaching Hospital, Nigeria: Lessons Learnt from Two Years of Laboratory Operation. PLoS Negl Trop Dis. doi: 10.1371/journal.pntd.0001839

Augusteyn RC (2014) Growth of the eye lens : I . Weight accumulation in multiple species. Mol Vis 20:410–426.

Awosanya EJ (2018) Post-epidemic awareness and knowledge of Lassa fever among residents in affected community in Ibadan, Oyo State, Nigeria. Vet World 11:1059–1063. doi: 10.14202/vetworld.2018.1059-1063

Bâ K, Kane M, Gauthier P, Granjon L (2012) Ecology of a typical West African Sudanian savannah rodent community. Afr J Ecol 51:447–455.

Bagamian KH, Towner JS, Kuenzi AJ, Douglass RJ, Rollin PE, Waller LA, Mills JN (2012) Transmission Ecology of Sin Nombre Hantavirus in Naturally Infected North American Deermouse Populations in Outdoor Enclosures. PLoS One 7:1–10. doi: 10.1371/journal.pone.0047731

Bagamian KH, Towner JS, Mills JN, Kuenzi AJ (2013) Increased detection of Sin Nombre hantavirus RNA in antibody-positive deer mice from Montana, USA: Evidence of male bias in RNA viremia. Viruses 5:2320–2328. doi: 10.3390/v5092320

Baldridge JR, Buchmeier MJ (1992) Mechanisms of antibody-mediated protection against lymphocytic choriomeningitis virus infection: mother-to-baby transfer of humoral protection. J Virol 66:4252–4257.

Barber I, Dingemanse NJ (2010) Parasitism and the evolutionary ecology of animal personality. Philos Trans R Soc B Biol Sci 365:4077–4088. doi: 10.1098/rstb.2010.0182

Bartlett MS (1957) Measles Periodicity and community size. J R Stat Soc 120:48–70.

Barton LL, Mets MB, Beauchamp CL (2002) Lymphocytic choriomeningitis virus: Emerging fetal teratogen. Am J Obstet Gynecol 187:1715–1716. doi: 10.1067/mob.2002.126297

Bausch DG, Demby AH, Coulibaly M, Kanu J, Goba A, Bah A, Condé N, Wurtzel HL, Cavallaro KF, Lloyd E, Baldet FB, Cissé SD, Fofona D, Savané IK, Tolno RT, Mahy B, Wagoner KD, Ksiazek TG, Peters CJ, Rollin PE (2001) Lassa Fever in Guinea: I. Epidemiology of Human Disease and Clinical Observations. Vector borne zoonotic Dis 1:269–281.

Bausch DG, Hadi CM, Khan SH, Lertora JJL (2010) Review of the Literature and Proposed Guidelines for the Use of Oral Ribavirin as Postexposure Prophylaxis for Lassa Fever. Clin Infect Dis 51:1435–1441. doi: 10.1086/657315

Begon M, Bennett M, Bowers RG, French NP, Hazel SM, Turner J (2002) A clarification of transmission terms in host-microparasite models: numbers, densities and areas. Epidemiol Infect 129:147–53.

Begon M, Hazel SM, Telfer S, Bown K, Carslake D, Cavanagh R, Chantrey J, Jones T, Bennett M, Journal S, Mar N, Bown K, Carslake D, Cavanagh R (2003) Rodents , Cowpox Virus and Islands : Densities , Numbers and Thresholds. 72:343–355.

Begon M, Telfer S, Burthe S, Lambin X, Smith MJ, Paterson S (2009a) Effects of abundance on infection in natural populations: field voles and cowpox virus. Epidemics 1:35–46. doi: 10.1016/j.epidem.2008.10.001

Begon M, Telfer S, Smith MJ, Burthe S, Paterson S, Lambin X (2009b) Seasonal host dynamics drive the timing of recurrent epidemics in a wildlife population. Proc R Soc B-Biological Sci 276:1603–1610. doi: 10.1098/rspb.2008.1732

Beldomenico PM, Telfer S, Lukomski L, Gebert S, Bennett M, Begon M (2009) Host condition and individual risk of cowpox virus infection in natural animal populations: cause or effect? Epidemiol Infect 137:1295–1301. doi: 10.1017/S0950268808001866

Benavides JA, Caillaud D, Scurlock BM, Maichak EJ, Edwards WH, Cross PC (2017) Estimating Loss of Brucella Abortus Antibodies from Age-Specific Serological Data In Elk. Ecohealth 14:234–243. doi: 10.1007/s10393-017-1235-z

Bennett M, Crouch AJ, Begon M, Duffy B, Feore S, Gaskell RM, Kelly DF, McCracken CM, Vicary L, Baxby D (1997) Cowpox in British voles and mice. J Comp Pathol 116:35–44. doi: 10.1016/S0021-9975(97)80041-2

Bernshtein A, Apekina N, Mikhailova T, Myasnikov Y, Khlyap L, Korotkov Y, Gavrilovskaya I (1999) Dynamics of Puumala hantavirus infection in naturally infected bank voles (Clethrinomys glareolus). Arch Virol 144:2415–2428. doi: 10.1007/s007050050654

Bhatta AK, Keyal U, Liu Y, Gellen E (2018) Vertical transmission of herpes simplex virus: An update. JDDG - J Ger Soc Dermatology 685–692. doi: 10.1111/ddg.13529

Billings AN, Rollin PE, Milazzo ML, Molina CP, Eyzaguirre EJ, Livingstone W, Ksiazek TG, Fulhorst CF (2010) Pathology of Black Creek Canal virus infection in juvenile hispid cotton rats (Sigmodon hispidus). Vector borne zoonotic Dis 10:621–628. doi: 10.1089/vbz.2009.0156

Black FL (1966) Measles endemicity in insular populations: Critical community size and its evolutionary implication. J Theor Biol 11:207–211. doi: 10.1016/0022-5193(66)90161-5

Bonthius DJ (2012) Lymphocytic Choriomeningitis Virus: An Underrecognized Cause of Neurologic Disease in the Fetus, Child, and Adult. Semin Pediatr Neurol 19:89–95. doi: 10.1016/j.spen.2012.02.002

Bonwitt J, Kelly AH, Ansumana R, Agbla S, Sahr F, Saez AM, Borchert M, Kock R, Fichet-Calvet E (2016) Rat-atouille: A Mixed Method Study to Characterize Rodent Hunting and Consumption in the Context of Lassa Fever. Ecohealth 13:234–247. doi: 10.1007/s10393-016-1098-8

Bonwitt J, Sáez AM, Lamin J, Ansumana R, Dawson M, Buanie J, Lamin J, Sondufu D, Borchert M, Sahr F, Fichet-Calvet E, Brown H (2017) At home with mastomys and rattus: Human-rodent interactions and potential for primary transmission of lassa virus in domestic spaces. Am J Trop Med Hyg 96:935–943. doi: 10.4269/ajtmh.16-0675

Boots B, Mealor M (2007) Local Interactions Select for Lower Pathogen Infectivity. Science (80) 315:1284–1287.

Borremans B, Leirs H, Gryseels S, Günther S, Makundi R, de Bellocq JG (2011a) Presence of Mopeia virus, an African arenavirus, related to biotope and individual rodent host characteristics: implications for virus transmission. Vector Borne Zoonotic Dis 11:1125–31. doi: 10.1089/vbz.2010.0010

Borremans B (2014) Ammonium improves elution of fixed dried blood spots without affecting immunofluorescence assay quality. Trop Med Int Health 19:413–6. doi: 10.1111/tmi.12259

Borremans B, Hughes NK, Reijniers J, Sluydts V, Katakweba A a. S, Mulungu LS, Sabuni C a., Makundi RH, Leirs H (2014) Happily together forever: temporal variation in spatial patterns and complete lack of territoriality in a promiscuous rodent. Popul Ecol 56:109–118. doi: 10.1007/s10144-013-0393-2

Borremans B, Sluydts V, Makundi R, Leirs H (2015) Evaluation of short-, mid- and long-term effects of toe clipping on a wild rodent. CSIRO Wildl Res 42:143–148. doi: 10.1071/WR14109

Borremans B, Vossen R, Becker-ziaja B, Gryseels S, Hughes N, Gestel M Van, Houtte N Van, Günther S, Leirs H (2015) Shedding dynamics of Morogoro virus , an African arenavirus closely related to Lassa virus , in its natural reservoir host Mastomys natalensis. Nat Publ Gr 1–8. doi: 10.1038/srep10445

Borremans B (2015) Transmission ecology of Morogoro arenavirus in the multimammate mouse *Mastomus natalensis* in Tanzania. PhD dissertation at University of Antwerp.

Borremans B, Hens N, Beutels P, Leirs H, Reijniers J (2016) Estimating Time of Infection Using Prior Serological and Individual Information Can Greatly Improve Incidence Estimation of Human and Wildlife Infections. PLoS Comput Biol 12:e1004882. doi: 10.1371/journal.pcbi.1004882

Borremans B, Reijniers J, Hughes NK, Godfrey SS, Gryseels S, Makundi RH, Leirs H (2016) Nonlinear scaling of foraging contacts with rodent population density. Oikos 1–9. doi: 10.1111/oik.03623

Borremans B, Reijniers J, Hens N, Leirs H (2017) The shape of the contact–density function matters when modelling parasite transmission in fluctuating populations. R Soc Open Sci 4:171308. doi: 10.1098/rsos.171308

Borucki MK, Boone JD, Rowe JE, Bohlman MC, Kuhn E a, DeBaca R, St Jeor SC (2000) Role of maternal antibody in natural infection of Peromyscus maniculatus with Sin Nombre virus. J Virol 74:2426–2429. doi: 10.1128/JVI.74.5.2426-2429.2000

Boulinier T, Staszewski V (2008) Maternal transfer of antibodies: raising immuno-ecology issues. Trends Ecol Evol 23:282–288. doi: 10.1016/j.tree.2007.12.006

Boyer N, Réale D, Marmet J, Pisanu B, Chapuis JL (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks Tamias sibiricus. J Anim Ecol 79:538–547. doi: 10.1111/j.1365-2656.2010.01659.x

Bredeck JF (1933) The Story of the Epidemic of Encephalitis in St. Louis. Am J Public Health Nations Health 23:1135–40.

Britton T (2010) Stochastic epidemic models: A survey. Math Biosci 225:24–35. doi: 10.1016/j.mbs.2010.01.006

Buckley SM, Casals J, Downs WG (1970) Isolation and antigenic characterization of lassa virus. Nature 227:174.

Buhnerkempe MG, Prager KC, Strelioff CC, Greig DJ, Laake JL, Melin SR, DeLong RL, Gulland FMD, Lloyd-Smith JO (2017) Detecting signals of chronic shedding to explain pathogen persistence: Leptospira interrogans in California sea lions. J Anim Ecol 86:460–472. doi: 10.1111/1365-2656.12656

Bull JJ, Lauring AS (2014) Theory and Empiricism in Virulence Evolution. PLoS Pathog 10:1–4. doi: 10.1371/journal.ppat.1004387

Burnet, F.M.; Fenner F (1949) The Production of Antibodies, 2nd ed. Macmillan Magazines Ltd, Melbourne, Australia,

Burthe S, Telfer S, Begon M, Bennett M, Smith A, Lambin X (2008) Cowpox virus infection in natural field vole Microtus agrestis populations: Significant negative impacts on survival. J Anim Ecol 77:110–119. doi: 10.1111/j.1365-2656.2007.01302.x

Carere C, Maestripieri D (2013). *Animal Personalities*. *Animal Personalities: Behavior, Physiology, and Evolution* (Vol. 53). University of Chicago Press. doi:10.7208/chicago/9780226922065.001.0001

Carey DE, Kemp GE., Pinneo HAW, Addy RF, Fom ALMD, Stroh G, Henderson BE (1972) Lassa fever epidemiological aspects of the 1970 epidemic, Jos, Nigeria. Trans R Soc Trop Med Hyg 66:402–408.

Carlos J, Torre D, Oldstone M (1992) Selective disruption of growth hormone transcription machinery by viral infection. Proc Natl Acad Sci U S A 89:9939–9943.

Carrat F, Vergu E, Ferguson NM, Lemaitre M, Cauchemez S, Leach S, Valleron A-J (2008) Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol 167:775–85. doi: 10.1093/aje/kwm375

Carstens EB (2012) Part I: Introduction, Ninth Report of the International Committee on Taxonomy of Viruses. Virus Taxon 1–20. doi: 10.1016/B978-0-12-384684-6.00036-7

Cashins SD, Grogan LF, McFadden M, Hunter D, Harlow PS, Berger L, Skerratt LF (2013) Prior Infection Does Not Improve Survival against the Amphibian Disease Chytridiomycosis. PLoS One 8:1–7. doi: 10.1371/journal.pone.0056747

Chantrey J (1999) The epidemiology of cowpox in its reservoir hosts. (Doctoral dissertation, University of Liverpool)

Chantrey J, Meyer H, Baxby D, Begon M, Bown KJ, Hazel SM, Jones T, Montgomery WI, Bennett M (1999) Cowpox: reservoir hosts and geographic range. Epidemiol Infect 122:455–460. doi: 10.1017/S0950268899002423

Charrel RN, Coutard B, Baronti C, Canard B, Nougairede a, Frangeul a, Morin B, Jamal S, Schmidt CL, Hilgenfeld R, Klempa B, de Lamballerie X (2011) Arenaviruses and hantaviruses: from epidemiology and genomics to antivirals. Antiviral Res 90:102–14. doi: 10.1016/j.antiviral.2011.02.009

Childs JE, Peters CJ (1993) Ecology and epidemiology of arenaviruses and their hosts. In: Salvato MS (ed) The Arenaviridae. Springer US, New York, pp 331–384

Choquet R, Lebreton JD, Gimenez O, Reboulet AM, Pradel R (2009) U-CARE: Utilities for performing goodness of fit tests and manipulating CApture-REcapture data. Ecography (Cop) 32:1071–1074. doi: 10.1111/j.1600-0587.2009.05968.x

Clay CA, Lehmer EM, Previtali A, St Jeor S, Dearing MD (2009). Contact heterogeneity in deer mice: implications for Sin Nombre virus transmission. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1660), 1305–12. doi:10.1098/rspb.2008.1693

Coburn BJ, Wagner BG, Blower S (2009) Modeling influenza epidemics and pandemics: Insights into the future of swine flu (H1N1). BMC Med 7:1–8. doi: 10.1186/1741-7015-7-30

Coetzee CG (1975) The biology, behaviour, and ecology of Mastomys natalensis in southern Africa. Bull World Health Organ 52:637–44.

Compton SR, Jacoby RO, Paturzo FX, Smith AL (2004) Persistent Seoul virus infection in Lewis rats. Arch Virol 149:1325–1339. doi: 10.1007/s00705-004-0294-y

Contopoulos-Ioannidis D, Newman-Lindsay S, Chow C, LaBeaud AD (2018) Mother-to-child transmission of Chikungunya virus: A systematic review and meta-analysis. PLoS Negl Trop Dis 12:1–20. doi: 10.1371/journal.pntd.0006510

Cooch EG, Conn PB, Ellner SP, Dobson AP, Pollock KH (2012) Disease dynamics in wild populations: modeling and estimation: a review. J Ornithol 152:485–509. doi: 10.1007/s10336-010-0636-3

Cressler CE, Mcleod D V., Rozins C, Van Den Hoogen J, Day T (2016) The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. Parasitology 143:915–930. doi: 10.1017/S003118201500092X

Cross ML, Buddle BM, Aldwell FE (2007) The potential of oral vaccines for disease control in wildlife species. Vet J 174:472–480. doi: 10.1016/j.tvjl.2006.10.005

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging Infectious Diseases of Wildlife - Threats to Biodiversity and Human Health. Adv Sci 287:443–449. doi: 10.1126/science.287.5452.443

Davis S, Begon M, Bruyn L De, Ageyev VS, Klassovskiy N, Pole SB, H. HV, Stenseth NC, Leirs H (2004) Predictive Thresholds for Plague in Kazakhstan. Science (80-) 304:736–738. doi: 10.1126/science.1095854

Davis S, Fichet-Calvet E, Leirs H (2005) Fluctuating Rodent Populations and Risk to Humans from Rodent-Borne Zoonoses. Vec born and zoon dis 5: 305-313.

de Bellocq JG, Borremans B, Katakweba A, Makundi R, Baird SJE, Becker-Ziaja B, Günther S, Leirs H (2010) Sympatric occurrence of 3 arenaviruses, Tanzania. Emerg Infect Dis 16:692–5. doi: 10.3201/eid1604.091721

de Bellocq JG, Leirs H (2009) Complementary DNA sequences encoding the multimammate rat MHC class II DQ alpha and beta chains and cross-species sequence comparison in rodents. Tissue Antigens 74:233–7. doi: 10.1111/j.1399-0039.2009.01305.x

De La Torre JC, Mallory M, Brot M, Gold L, Koob G, Oldstone MBA, Masliah E (1996) Viral Persistence in Neurons Alters Synaptic Plasticity and Cognitive Functions without Destruction of Brain Cells. Virology 220:508–515. doi: 10.1006/viro.1996.0340

Demartini J, Green D, Monath TP (1975) Lassa virus infection in Mastomys natalensis in Sierra Leone Gross and microscopic findings in infected and uninfected animals. Bull World Heal organ. 52:651–663.

Demby AH, Inapogui A, Kargbo K, Koninga J, Kourouma K, Kanu J, Coulibaly M, Wagoner KD, Ksiazek TG, Peters CJ, Rollin PE, Bausch DG (2001) Lassa Fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. Vector borne zoonotic Dis 1:283–299.

Demissie S, Mengisitie B (2017) The Impact of El Niño on Diarrheal Disease Incidence : A Systematic Review. 5:446–451. doi: 10.11648/j.sjph.20170506.16

Dietz K (1967) Epidemics and Rumours: A Survey. J R Stat Soc Ser A 130:505. doi: 10.2307/2982521

Dizney L & Dearing MD (2013). The role of behavioural heterogeneity on infection patterns: implications for pathogen transmission. *Animal Behaviour*, *86*(5), 911–916. doi:10.1016/j.anbehav.2013.08.003

Dobay A, Pilo P, Lindholm AK, Origgi F, Bagheri HC, König B (2015) Dynamics of a tularemia outbreak in a closely

monitored free-roaming population of wild house mice. PLoS One 10:1–12. doi: 10.1371/journal.pone.0141103
Dobson AP, Meagher M (1996) The population dynamics of brucellosis in the Yellowstone National Park. Ecology 77:1026–1036.
Donaldson RI (2009) The Lassa ward: one man's fight against one of the world's deadlist diseases. New York, St. Martin's Press.
Doumayrou J, Avellan A, Froissart R, Michalakis Y (2013) An experimental test of the tranmission-virulence trade-off hypothesis in a plant virus. Evolution (N Y) 67:477–486. doi: 10.1111/j.1558-5646.2012.01780.x
Dwyer G, Levin S, Buttel L (1990) A Simulation Model of the Population Dynamics and Evolution of Myxomatosis. Ecol Monogr 60:423–447.
Ebert D, Bull JJ (2003) Challenging the trade-off model for the evolution of virulence: Is virulence management feasible? Trends Microbiol 11:15–20. doi: 10.1016/S0966-842X(02)00003-3
Ewald PW (1983) Host-parasites relations, vectors, and the evolution of disease severity. Annu Rev Ecol Evol Syst 465–485.
Fichet-Calvet E, Lecompte E, Koivogui L, Soropogui B, Doré A, Kourouma F, Sylla O, Daffis S, Koulémou K, Ter Meulen J (2007) Fluctuation of abundance and Lassa virus prevalence in Mastomys natalensis in Guinea, West Africa. Vector Borne Zoonotic Dis 7:119–28. doi: 10.1089/vbz.2006.0520
Fichet-Calvet E, Lecompte E, Koivogui L, Daffis S, ter Meulen J (2008) Reproductive characteristics of Mastomys natalensis and Lassa virus prevalence in Guinea, West Africa. Vector Borne Zoonotic Dis 8:41–8. doi: 10.1089/vbz.2007.0118
Fichet-Calvet E, Rogers DJ (2009) Risk maps of Lassa fever in West Africa. PLoS Negl Trop Dis 3:1–13. doi: 10.1371/journal.pntd.0000388
Fichet-calvet E, Audenaert L, Barrie P, Verheyen E (2009) Diversity , dynamics and reproduction in a community of small mammals in Upper Guinea , with emphasis on pygmy mice ecology. Afr J Ecol 48:600–614.
Fichet-Calvet E, Becker-Ziaja B, Koivogui L, Günther S (2014) Lassa serology in natural populations of rodents and horizontal transmission. Vector Borne Zoonotic Dis 14:665–74. doi: 10.1089/vbz.2013.1484
Fichet-Calvet E (2014) Lassa fever. In Johnson N (ed.) *The role of animals in emerging viral disease* (pp 89-123). Londen: Elseviers.
Fichet-Calvet E, Ölschläger S, Strecker T, Koivogui L, Becker-Ziaja B, Camara AB, Soropogui B, Magassouba N, Günther S (2016) Spatial and temporal evolution of Lassa virus in the natural host population in Upper Guinea. Sci Rep 1–6. doi: 10.1038/srep21977
Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comer JA, Guarner J, Paddock CD, DeMeo DL, Shieh W-J, Erickson BR, Bandy U, DeMaria A, Davis JP, Delmonico FL, Pavlin B, Likos A, Vincent MJ, Sealy TK, Goldsmith CS, Jernigan DB, Rollin PE, Packard MM, Patel M, Rowland C, Helfand RF, Nichol ST, Fishman JA, Ksiazek T, Zaki SR, Team L in TRI (2006) Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med 354:2235–2249. doi: 10.1056/NEJMoa053240
Fisher-Hoch SP, Hutwagner L, Brown B, McCormick JB (2000) Effective Vaccine for Lassa Fever. J Virol 74:6777–6783. doi: 10.1128/JVI.74.15.6777-6783.2000
Fisher-Hoch SP, McCormick JB (2001) Towards a human Lassa fever vaccine. Rev Med Virol 11:331–341.
Fisher-Hoch SP, Tomori O, Nasidi a, Perez-Oronoz GI, Fakile Y, Hutwagner L, McCormick JB (1995) Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. BMJ 311:857–859. doi: 10.1136/bmj.311.7009.857
Foley JE, Foley P, Pedersen NC (1999) The persistence of a SIS disease in a metapopulation. Journal of applied ecology 36: 555–563.
Forbes KM, Sironen T, Plyusnin A (2018) Hantavirus maintenance and transmission in reservoir host populations. Curr Opin Virol 28:1–6. doi: 10.1016/j.coviro.2017.09.003
Frank S (1996) Models of parasite virulence. Q Rev Biol 71:37–78.
Fraser C, Hollingsworth TD, Chapman R, de Wolf F, Hanage WP (2007) Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. Proc Natl Acad Sci U S A 104:17441–174416. doi: 10.1073/pnas.0708559104
Fulhorst CF, Ksiazek TG, Peters CJ, Tesh RB (1999) Experimental infection of the cane mouse Zygodontomys brevicauda (family Muridae) with guanarito virus (Arenaviridae), the etiologic agent of Venezuelan hemorrhagic fever. J Infect Dis 180:966.
Fulhorst CF, Milazzo ML, Bradley RD, Peppers LL (2001) Experimental infection of Neotoma albigula (Muridae) with Whitewater Arroyo virus (Arenaviridae). Am J Trop Med Hyg 65:147–51.
Fulhorst CF, Milazzo ML, Duno G, Salas RA (2002) Experimental infection of the Sigmodon alstoni cotton rat with Caño Delgadito virus, a South American hantavirus. Am J Trop Med Hyg 67:107–11.
Gallagher J, Clifton-Hadley RS (2000) Tuberculosis in badgers; a review of the disease and its significance for other animals. Res Vet Sci 69:203–217. doi: 10.1053/rvsc.2000.0422
Gibb R, Moses LM, Redding DW, Jones KE (2017) Understanding the cryptic nature of Lassa fever in West Africa. Pathog Glob Health 111:276–288. doi: 10.1080/20477724.2017.1369643
Gibson G, Renshaw E (1998) Estimating parameters in stochastic compartmental models using Markov chain methods. Math Med Biol 15:19–40. doi: 10.1093/imammb/15.1.19
Girard MP, Tam JS, Assossou OM, Kieny MP (2010) The 2009 A (H1N1) influenza virus pandemic: A review. Vaccine 28:4895–4902. doi: 10.1016/j.vaccine.2010.05.031
Glass GE, Childs JE, Leduc JW, Cassard SD, Donnenberg a D (1990) Determining Matrilines By Antibody-Response To Exotic Antigens. J Mammal 71:129–138.
Gold LH, Brot MD, Polis I, Schroeder R, Tishon A, de la Torre JC, Oldstone MBA, Koob GF (1994) Behavioral effects of persistent lymphocytic choriomeningitis virus infection in mice. Behav Neural Biol 62:100–109. doi: 10.1016/S0163-1047(05)80031-7

Gómez-Rubio V (2017) ggplot2 - Elegant Graphics for Data Analysis (2nd Edition). J Stat Softw 77:3–5. doi: 10.18637/jss.v077.b02

Gonzalez JP, McCormick JB, Saluzzo JF, Herve JP, Georges AJ, Johnson KM (1983) An arenavirus isolated from wild-caught rodents (Praomys species) in the Central African Republic. Intervirology 19:105–112. doi: 10.15713/ins.mmj.3

Goüy de Bellocq J, Borremans B, Katakweba A, Makundi R, Baird SJE, Becker-Ziaja B, Günther S, Leirs H (2010) Sympatric occurrence of 3 arenaviruses, Tanzania. Emerg Infect Dis 16:692–5. doi: 10.3201/eid1604.091721

Goyens J, Reijniers J, Borremans B, Leirs H (2013) Density thresholds for Mopeia virus invasion and persistence in its host Mastomys natalensis. J Theor Biol 317:55–61. doi: 10.1016/j.jtbi.2012.09.039

Gryseels S, Rieger T, Oestereich L, Cuypers B, Borremans B, Makundi R, Leirs H, Günther S, Goüy de Bellocq J (2015) Gairo virus, a novel arenavirus of the widespread Mastomys natalensis: Genetically divergent, but ecologically similar to Lassa and Morogoro viruses. Virology 476:249–56. doi: 10.1016/j.virol.2014.12.011

Gryseels S (2015) Evolutionary relationships between arenaviruses and their rodent hosts. PhD dissertation at University of Antwerp.

Gryseels S, Baird SJE, Borremans B, Makundi R, Leirs H, Goüy de Bellocq J (2017a) When Viruses Don't Go Viral: The Importance of Host Phylogeographic Structure in the Spatial Spread of Arenaviruses. PLoS Pathog 13:1–22. doi: 10.1371/journal.ppat.1006073

Guivier E, Galan M, Chaval Y, Xuéreb A, Ribas Salvador A, Poulle ML, Voutilainen L, Henttonen H, Charbonnel N, Cosson JF (2011) Landscape genetics highlights the role of bank vole metapopulation dynamics in the epidemiology of Puumala hantavirus. Mol Ecol 20:3569–3583. doi: 10.1111/j.1365-294X.2011.05199.x

Günther S, Hoofd G, Charrel R, Röser C, Becker-Ziaja B, Lloyd G, Sabuni C, Verhagen R, van der Groen G, Kennis J, Katakweba A, Machang'u R, Makundi R, Leirs H (2009) Mopeia virus-related arenavirus in natal multimammate mice, Morogoro, Tanzania. Emerg Infect Dis 15:2008–12. doi: 10.3201/eid1512.090864

Günther S, Lenz O (2004) Lassa virus. Crit Rev Clin Lab Sci 41:339–90. doi: 10.1080/10408360490497456

Hallam HJ, Hallam S, Rodriguez SE, Barrett ADT, Beasley DWC, Chua A, Ksiazek TG, Milligan GN, Sathiyamoorthy V, Reece LM (2018) Baseline mapping of Lassa fever virology, epidemiology and vaccine research and development. npj Vaccines 3:11. doi: 10.1038/s41541-018-0049-5

Han BA, Kramer AM, Drake JM (2016) Global Patterns of Zoonotic Disease in Mammals. Trends Parasitol 32:565–577. doi: 10.1016/j.pt.2016.04.007

Han BA, Schmidt JP, Bowden SE, Drake JM (2015) Rodent reservoirs of future zoonotic diseases. Proc Natl Acad Sci 112:7039–7044. doi: 10.1073/pnas.1501598112

Hanski I (1999) Habitat Connectivity, Habitat Continuity, and Metapopulations in Dynamic Landscapes. Oikos 87:209. doi: 10.2307/3546736

Happold DCD (1977) A population study on small rodents in the tropical rain forest of Nigeria. LaTerre la Vie 31:385–458.

Hardestam J, Karlsson M, Falk KI, Olsson GE, Klingström J, Lundkvist Å (2008) Puumala Hantavirus Excretion Kinetics in Bank Voles (Myodes glareolus). Emerg Infect Dis 14:1209–1215. doi: 10.3201/eid1408.080221

Hartley DM, Barker CM, Le Menach A, Niu T, Gaff HD, Reisen WK (2012) Effects of temperature on emergence and seasonality of West Nile virus in California. Am J Trop Med Hyg 86:884–894. doi: 10.4269/ajtmh.2012.11-0342

Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO (2017) Structural basis for antibody-mediated neutralization of Lassa virus. Science (80-) 356:923–928.

Heesterbeek H, Anderson RM, Andreasen V, Bansal S, DeAngelis D, Dye C, Eames KTD, Edmunds WJ, Frost SDW, Funk S, Hollingsworth TD, House T, Isham V, Klepac P, Lessler J, Lloyd-Smith JO, Metcalf CJE, Mollison D, Pellis L, Pulliam JRC, Roberts MG, Viboud C, Arinaminpathy N, Ball F, Bogich T, Gog J, Grenfell B, Lloyd AL, Mclean A, O'Neill P, Pearson C, Riley S, Tomba GS, Trapman P, Wood J (2015) Modeling infectious disease dynamics in the complex landscape of global health. Science (80-). doi: 10.1126/science.aaa4339

Heesterbeek JAP, Dietz K (1996) The concept of R_o in epidemic theory. Stat Neerl 50:89–110. doi: 10.1111/j.1467-9574.1996.tb01482.x

Hens N, Shkedy Z, Aerts M, Faes C, Van Damme P, Beutels P (2012) Modeling infectious disease parameters based on serological and social contact data. A Moders statistical perspective. Springer.

Hess BH (2009) Mouse Ear Tattoo: A Quick and Easy Alternative to Ear Punches and Tags. J Am Assoc Lab Anim Sci 48:587–588.

Hoffmann A, Klingel H (2001) Spatial and temporal patterns in Mastomys cf . natalensis by radiotracking. In: African small mammals, Collection Colloques et seminaires, IRD Edition, Paris. Proceedings of the 8th International African Small Mammal Symposium, Paris 1999..

Holt J, Davis S, Leirs H (2006) A model of Leptospirosis infection in an African rodent to determine risk to humans: Seasonal fluctuations and the impact of rodent control. Acta Trop 99:218–225. doi: 10.1016/j.actatropica.2006.08.003

Hone J (1992) Rate of Increase and Fertility Control. J Appl Ecol 29:695–698.

Hopkins AS, Whitetail-eagle JOE, Corneli AMYL, Person B, Ettestad PJ, Dimenna M, Norstog JON, Creswell J, Khan ALIS, Olson JG, Cavallaro KF, Bryan RT, Cheek JE, Begay B, Hoddenbach GA, Ksiazek TG, Mills JN (2002) Experimental Evaluation of Rodent Exclusion Methods Native American Community in New Mexico. Vector borne zoonotic Dis 2:61–68.

Hotchin JE, Cinits M (1958) Lymphocytic choriomeningitis infection of mice as a model for the study of latent virus infection1. Can J Microbiol 4:149–163.

Hudson PJ, Rizzoli A, Grenfell B, Heesterbeek H, Dobson AP (2002) The ecology of wildlife disease. Hudson PJ (ed.) New York: Oxford university press.

Hufert FT, Liidke W, Sehmitz H (1989) Epitope mapping of the Lassa virus nucleoprotein using monoclonal anti-nucleocapsid antibodies. Arch Virol 106:201–212.

Humphrys S, Lapidge SJ (2008) Delivering and registering species-tailored oral antifertility products: A review. Wildl Res

35:578–585. doi: 10.1071/WR07145
Ishii A, Thomas Y, Moonga L, Nakamura I, Ohnuma A, Hang'ombe B, Takada A, Mweene A, Sawa H (2011) Novel arenavirus, Zambia. Emerg Infect Dis 17:1921–4. doi: 10.3201/eid1710.101452
Jacob J, Jones D, Singleton G (2002) Retention of the bait marker Rhodamine B in wild house mice. Wildl Res 29:159–164.
Jamieson DJ, Kourtis AP, Bell M, Rasmussen SA (2006) Lymphocytic choriomeningitis virus: An emerging obstetric pathogen? Am J Obstet Gynecol 194:1532–1536. doi: 10.1016/j.ajog.2005.11.040
Johnson DS, Laake JL, Melin SR, DeLong RL (2016) Multivariate state hidden Markov models for mark-recapture data. Stat Sci 31:233–244. doi: 10.1214/15-STS542
Jones KE, Patel NG, Levy M a, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990–3. doi: 10.1038/nature06536
Julliard R, Leirs H, Stenseth NC, Yoccoz NG, Prévot-Julliard AC, Verhagen R, Verheyen W (1999) Survival-variation within and between functional categories of the African multimammate rat. J Anim Ecol 68:550–561. doi: 10.1046/j.1365-2656.1999.00304.x
Kallio ER, Begon M, Henttonen H, Koskela E, Mappes T, Vaheri A, Vapalahti O (2010) Hantavirus infections in fluctuating host populations: the role of maternal antibodies. Proc Biol Sci 277:3783–3791. doi: 10.1098/rspb.2010.1022
Kallio ER, Helle H, Koskela E, Mappes T, Vapalahti O (2015) Age-related effects of chronic hantavirus infection on female host fecundity. J Anim Ecol 84:1264–1272. doi: 10.1111/1365-2656.12387
Kallio ER, Henttonen H, Koskela E, Lundkvist A, Mappes T, Vapalahti O (2013) Maternal antibodies contribute to sex-based difference in hantavirus transmission dynamics. Biol Lett 9:20130887. doi: 10.1098/rsbl.2013.0887
Kallio ER, Klingström J, Gustafsson E, Manni T, Vaheri A, Henttonen H, Vapalahti O, Lundkvist Å (2006) Prolonged survival of Puumala hantavirus outside the host: Evidence for indirect transmission via the environment. J Gen Virol 87:2127–2134. doi: 10.1099/vir.0.81643-0
Kallio ER, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Mappes T, Koskela E (2011) Endemic Hantavirus Infection Impairs the Winter Survival of Its Rodent Host. Ecol Soc Am 88:1911–1916.
Kamya MR, Gasasira AF, Yeka A, Bakyaita N, Nsobya SL, Francis D, Havlir D (2006). Effect of HIV-1 Infection on Antimalarial Treatment Outcomes in Uganda: A Population-Based Study. *The Journal of Infectious Diseases*, *193*(1), 9–15. doi:10.3390/bs8050051
Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, Aldrich S, Harrington T, Formenty P, Loh EH, MacHalaba CC, Thomas MJ, Heymann DL (2012) Ecology of zoonoses: Natural and unnatural histories. Lancet 380:1936–1945. doi: 10.1016/S0140-6736(12)61678-X
Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, MeKearse M, Moir R, Wilson A, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.
Keeling MJ, Rohani P (2008). Modeling infectious disease in humans and animals. Princeton university press, New Jersey.
Kennis J, Sluydts V, Leirs H, van Hooft WFP (2008) Polyandry and polygyny in an African rodent pest species, Mastomys natalensis. Mammalia 72:150–160. doi: 10.1515/MAMM.2008.025
Kerber R, Rieger T, Busch C, Flatz L, Pinschewer DD, Kümmerer BM, Günther S (2011) Cross-species analysis of the replication complex of Old World arenaviruses reveals two nucleoprotein sites involved in L protein function. J Virol 85:12518–28. doi: 10.1128/JVI.05091-11
Kermack WO, McKendrick (1927) A Contribution to the Mathematical Theory of Epidemics. Proc R Soc A Math Phys Eng Sci 115:700–721. doi: 10.1098/rspa.1927.0118
Kernéis S, Koivogui L, Magassouba N, Koulemou K, Lewis R, Aplogan A, Grais RF, Guerin PJ, Fichet-Calvet E (2009) Prevalence and risk factors of Lassa seropositivity in inhabitants of the forest region of Guinea: a cross-sectional study. PLoS Negl Trop Dis 3:e548. doi: 10.1371/journal.pntd.0000548
Kerr PJ (2012) Myxomatosis in Australia and Europe: A model for emerging infectious diseases. Antiviral Res 93:387–415. doi: 10.1016/j.antiviral.2012.01.009
King AA, Shrestha S, Harvill ET, Bjørnstad ON (2009) Evolution of Acute Infections and the Invasion-Persistence Trade-Off. Am Nat 173:446–455. doi: 10.1086/597217
Kingdon J, Happold D, Butynski T, Hoffmann M, Happold M, Kalina J (2013) The mammals of Africa. Volume III Rodents, hares and rabbits. Bloomsbury Publishing, Londen, United Kingdom
Knell RJ (2004) Syphilis in renaissance Europe: rapid evolution of an introduced sexually transmitted disease? Proc Biol Sci 271 Suppl:S174-6. doi: 10.1098/rsbl.2003.0131
Kollipara A, George C, Hanger J, Loader J, Polkinghorne A, Beagley K, Timms P (2012) Vaccination of healthy and diseased koalas (Phascolarctos cinereus) with a Chlamydia pecorum multi-subunit vaccine: Evaluation of immunity and pathology. Vaccine 30:1875–1885. doi: 10.1016/j.vaccine.2011.12.125
Kouadio L, Nowak K, Akoua-Koffi C, Weiss S, Allali BK, Witkowski PT, Krüger DH, Couacy-Hymann E, Leendertz FH, Allali BK (2015) Lassa virus in multimammate rats, Côte d'Ivoire, 2013. Emerg Infect Dis 21:1481–1483. doi: 10.3201/eid2108.150312
Krebs JC (2013) Population fluctuations in rodents. Chicago: University of Chicago press.
Kreuder Johnson C, Hitchens PL, Smiley Evans T, Goldstein T, Thomas K, Clements A, Joly DO, Wolfe ND, Daszak P, Karesh WB, Mazet JK (2015) Spillover and pandemic properties of zoonotic viruses with high host plasticity. Sci Rep 5:1–8. doi: 10.1038/srep14830
Kronmann KC, Nimo-paintsil S, Obiri-danso K, Ampofo W, Fichet-calvet E (2013) Arenaviruses Detected in Pygmy Mice, Ghana. Emerg Infect Dis 19:1832–1835.
Ksiazek TG, Erdman D, Goldsmith C, Anderson L (2003) A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. Nejm 348:339–354. doi: 10.1056/NEJMoa1411087
Kunz S, Rojek JM, Roberts AJ, Mcgavern DB, Oldstone MBA, Carlos De La Torre J (2006) Altered Central Nervous System

Gene Expression Caused by Congenitally Acquired Persistent Infection with Lymphocytic Choriomeningitis Virus. J Virol 80:9082–9092. doi: 10.1128/JVI.00795-06
Laake J, Johnson D, Conn P, Laake MJ (2016) Package " marked ." CRAN 3–92.
Labuschagne L, Swanepoel LH, Taylor PJ, Belmain SR, Keith M (2016) Are avian predators effective biological control agents for rodent pest management in agricultural systems? Biol Control 101:94–102. doi: 10.1016/j.biocontrol.2016.07.003
Lalis A, Evin A, Janier M, Koivogui L, Denys C (2015) Host evolution in Mastomys natalensis (Rodentia: Muridae): an integrative approach using geometric morphometrics and genetics. Integr Zool 10:505–514. doi: 10.1111/1749-4877.12164
Lauring AS, Andino R (2010) Quasispecies theory and the behavior of RNA viruses. PLoS Pathog 6:1–8. doi: 10.1371/journal.ppat.1001005
Lecompte E, Fichet-calvet E, Daffis S, Koulémou K, Sylla O, Kourouma F, Doré A, Soropogui B, Aniskin V, Allali B, Kan SK, Lalis A, Koivogui L, Günther S, Denys C, Meulen J (2006) Lassa Fever , West Africa.
Leirs H, Stuyck J, Verhagen R, Verheyen W (1990) Seasonal variation in growth of Mastomys natalensis (Rodentia: Muridae) in Morogoro, Tanzania. Afr J Ecol 28:298–306. doi: 10.1111/j.1365-2028.1990.tb01164.x
Leirs H, Verheyen W, Michiels M, Verhagen R, Stuyck J (1990) The relation between rainfall and the breeding season of mastomys natalensis (smith, 1834) in morogoro, tanzania. Ann la société R Zool Belgique 119:59–64.
Leirs H (1994) Population Ecology of Mastomys Natalensis (Smith, 1834). Implications for Rodent Control in Africa, Brussels: Belgian Administration for Development Cooperation
Leirs H, Verhagen R, Sabuni C, Mwanjambe P, Verheyen W (1997) Spatial patterns in Mastomys natalensis in Tanzania (Rodentia, Muridae). Belgian J Zool 127:29–38.
Lloyd-Smith JO, Cross PC, Briggs CJ, Daugherty M, Getz WM, Latto J, Sanchez MS, Smith AB, Swei A (2005) Should we expect population thresholds for wildlife disease? Trends Ecol Evol 20:511–519. doi: 10.1016/j.tree.2005.07.004
Lo Iacono G, Cunningham AA, Fichet-Calvet E, Garry RF, Grant DS, Khan SH, Leach M, Moses LM, Schieffelin JS, Shaffer JG, Webb CT, Wood JLN (2015) Using Modelling to Disentangle the Relative Contributions of Zoonotic and Anthroponotic Transmission: The Case of Lassa Fever. PLoS Negl Trop Dis. doi: 10.1371/journal.pntd.0003398
Lord RD (1959) Wildlife Society The Lens as an Indicator of Age in Cottontail Rabbits. J Wildl Manage 23:358–360.
Luis AD, Douglass RJ, Hudson PJ, Mills JN, Bjørnstad ON (2012) Sin Nombre hantavirus decreases survival of male deer mice. Oecologia 169:431–439. doi: 10.1007/s00442-011-2219-2
Lukashevich IS, Clegg JCS, Sidibe K (1993) Lassa virus activity in Guinea: Distribution of human antiviral antibody defined using enzyme-linked immunosorbent assay with recombinant antigen. J Med Virol 40:210–217. doi: 10.1002/jmv.1890400308
Lukashevich IS, Maryankova R, Vladyko AS, Nashkevich N, Koleda S, Djavani M, Horejsh D, Voitenok NN, Salvato MS (1999) Lassa and Mopeia virus replication in human monocytes/macrophages and in endothelial cells: different effects on IL-8 and TNF-alpha gene expression. J Med Virol 59:552–560.
Lytle CD, Sagripanti J-L (2005) Predicted inactivation of viruses of relevance to biodefense by solar radiation. J Virol 79:14244–52. doi: 10.1128/JVI.79.22.14244-14252.2005
Buchmeier GM, Welsh RM, Futko Ambao FJ (1978) The Virology a n d lmmunobiology o f Lymphocytic C horiomen ing itis Virus Infection. Adv Immunol 30:275–331.
Maes P, Alkhovsky S V., Bào Y, Kuhn JH (2018) Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. Arch Virol 163:2295–2310. doi: 10.1007/s00705-018-3843-5
Mahlaba TAM, Monadjem A, McCleery R, Belmain SR (2017) Domestic cats and dogs create a landscape of fear for pest rodents around rural homesteads. PLoS One 12:1–9. doi: 10.1371/journal.pone.0171593
Marchandeau S, Chaval Y, Goff E Le (2000) The abundance of wild European rabbits Oryctolagus cuniculus and high immunity level over three years following the arrival of rabbit haemorrhagic disease Prolonged decline in the abundance of wild European rabbits Oryctolagus cuniculus and high immu. Wildlife Biol 6:141–147.
Mariën J, Borremans B, Gryseels S, Broecke B Vanden, Becker-Ziaja B, Makundi R, Massawe A, Reijniers J, Leirs H (2017a) Arenavirus Dynamics in Experimentally and Naturally Infected Rodents. Ecohealth 14:463–473. doi: 10.1007/s10393-017-1256-7
Mariën J, Borremans B, Gryseels S, Soropogui B, De Bruyn L, Bongo GN, Becker-Ziaja B, de Bellocq JG, Günther S, Magassouba N, Leirs H, Fichet-Calvet E (2017b) No measurable adverse effects of Lassa, Morogoro and Gairo arenaviruses on their rodent reservoir host in natural conditions. Parasit Vectors 10:210. doi: 10.1186/s13071-017-2146-0
Mariën J, Kourouma F, Magassouba N, Leirs H, Fichet-Calvet E (2018a) Movement Patterns of Small Rodents in Lassa Fever-Endemic Villages in Guinea. Ecohealth 15: 348-359. doi: 10.1007/s10393-018-1331-8
Mariën J, Sluydts V, Borremans B, Gryseels S, Vanden Broecke B, Sabuni CA, Katakweba AAS, Mulungu LS, Günther S, de Bellocq JG, Massawe AW, Leirs H (2018b) Arenavirus infection correlates with lower survival of its natural rodent host in a long-term capture-mark-recapture study. Parasit Vectors 11:90. doi: 10.1186/s13071-018-2674-2
Martin LB, Weil ZM, Nelson RJ (2008) Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philos Trans R Soc Lond B Biol Sci 363:321–39. doi: 10.1098/rstb.2007.2142
Massawe AW, Makundi RH, Zhang Z, Mhamphi G, Liu M, Li HJ, Belmain SR (2018) Effect of synthetic hormones on reproduction in Mastomys natalensis. J Pest Sci 91:157–168. doi: 10.1007/s10340-017-0894-4
Massawe AW, Mulungu LS, H MR, Eiseb SJ, Kirsten F, Mahlaba T, Malebane P, Maltitz E Von, Monadjem A, Taylor P, Tutjavi V, Belmain SR (2011) Spatial and temporal population dynamics of rodents in three geographically different regions in Africa : Implication for ecologically-based. African Zool 46:393–405.
Matloubian M, Concepcion RJ, Ahmed R (1994) CD4 + T Cells Are Required To Sustain CD8 + Cytotoxic T-Cell Responses during Chronic Viral Infection. J Virol 68:8056–8063.

May R, Anderson R (1978) Regulation and Stability of Host-Parasite Population Interactions : II . Destabilizing Processes. J Anim Ecol 47:249–267.
McCallum H (2016) Models for managing wildlife disease. Parasitology 143:805–820. doi: 10.1017/S0031182015000980
McCallum H, Barlow N, Hone J (2001) How should pathogen transmission be modelled? Trends Ecol Evol 16:295–300.
Mccormick JB, Webb PA, Krebs JW, Johnson KM, Smith ES, Africa W (1987) A Prospective Study of the Epidemiology and Ecology of Lassa Fever carried out primarily in the eastern province of Sierra. J Infect Dis 155:437–444.
McCormick JB (1999) Lassa Fever, Emergence, Berlin Heidelberg: Elsevier
Mdangi M, Mulungu LS, Massawe AW, Eiseb SJ, Tutjavi V, Kirsten F, Mahlaba T, Malebane P, von Maltitz E, Monadjem A, Dlamini N, Makundi RH, Belmain SR (2013) Assessment of rodent damage to stored maize (*Zea mays* L.) on smallholder farms in Tanzania. Int J Pest Manag 59:55–62. doi: 10.1080/09670874.2012.744495
Medina RA (2018) 1918 Influenza Virus: 100 Years on, Are We Prepared Against the Next Influenza Pandemic? Nat Rev Microbiol 16:61–62. doi: 10.1038/nrmicro.2017.174
Meerburg BG, Singleton GR, Kijlstra A (2009) Rodent-borne diseases and their risks for public health. Crit Rev Microbiol 35:221-70. DOI: 10.1080/10408410902989837.
Mendoza EJ, Warner B, Kobinger G, Nicholas H, Safronetz OD (2018) Baited vaccines : A strategy to mitigate rodent - borne viral zoonoses in humans. 1–17. doi: 10.1111/zph.12487
Messinger SM, Ostling A (2009) The Consequences of Spatial Structure for the Evolution of Pathogen Transmission Rate and Virulence. Am Nat 174:441–454. doi: 10.1086/605375
Milazzo ML, Fulhorst CF (2012) Duration of Catarina Virus Infection in the Southern Plains Woodrat (*Neotoma micropus*). Vector-Borne Zoonotic Dis 12:321–324. doi: 10.1089/vbz.2011.0852
Mills JN (2005) Infectious Diseases from Nature: Mechanisms of Viral Emergence and Persistence. Arch Virol 19:45–47. doi: 10.1007/3-211-29981-5
Mills JN, Childs JE, Ksiazek TG, Peters CJ, Velleca WM (1995) Methods for trapping and sampling small mammals for virologic testing. Atlanta: Centers for disease control and prevention,
Mills JN, Ksiazek TG, Peters CJ, Childs JE (1999) Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. Emerg Infect Dis 5:135–42. doi: 10.3201/eid0501.990116
Mills SC, Grapputo A, Jokinen I, Koskela E, Mappes T, Poikonen T (2010) Fitness trade-offs mediated by immunosuppression costs in a small mammal. Evolution 64:166–79. doi: 10.1111/j.1558-5646.2009.00820.x
Mims CA (1970) Observations on Mice Infected Congenitally or Neonatally with Lymphocytic Choriomeningitis (LC31) Virns. Arch ges Virusforsch 74:67–74.
Mims C a (1981) Vertical transmission of viruses. Microbiol Rev 45:267–86.
Mlyashimbi ECM, Mariën J, Kimaro DN, Tarimo AJP, Isabirye M, Makundi RH, Massawe AW, Mdangi ME, Kifumba D, Nakiyemba A, Leirs H, Belmain SR, Mulungu LS (2018) Relationships between seasonal changes in diet of Multimammate rat (Mastomys natalensis) and its breeding patterns in semi-arid areas in Tanzania. Cogent Food Agric. doi: 10.1080/23311932.2018.1507509
Mohr K, Leirs H, Katakweba A, Machang R (2007) Monitoring rodents movements with a biomarker around introduction and feeding foci in an urban environment in Tanzania. African Zool 42:294–298.
Monadjem A, Mahlaba T, Dlamnini N, Eiseb S, Belmain S, Mulungu L, Massawe AW, Makundi RH, Mohr K, Taylor PJ (2011) Impact of crop cycle on movement patterns of pest rodent species between fi elds and houses in Africa. Wildl Res 38:603–609.
Monadjem A, Perrin M (1998) The effect of supplementary food on the home range of the multimammate mouse *Mastomys natalensis*. South African J Wildl Res 28:1–3.
Monath TP (1987) Lassa Fever: New Issues Raised by Field Studies in West Africa. J Infect Dis 155:433–436. doi: 10.1093/infdis/155.3.433
Monath TP, Newhouse VF, Kemp GE, Setzer HW CA (1974) Lassa Virus Isolation from Mastomys natalensis Rodents during an Epidemic in Sierra Leone. Science (80-) 185:263–265.
Morris P (1972) A review of mammalian age determination methods. Mamm Rev 2:69–104.
Morse SS, Mazet JAK, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, Daszak P (2012) Prediction and prevention of the next pandemic zoonosis. Lancet 380:1956–1965. doi: 10.1016/S0140-6736(12)61684-5
Morters MK, Restif O, Hampson K, Cleaveland S, Wood JLN, Conlan AJK (2012) Evidence-based control of canine rabies: A critical review of population density reduction. J Anim Ecol 82:6–14. doi: 10.1111/j.1365-2656.2012.02033.x
Morters MK, Restif O, Hampson K, Cleaveland S, Wood JLN, Conlan AJK (2013) Evidence-based control of canine rabies: a critical review of population density reduction. J Anim Ecol 82:6–14. doi: 10.1111/j.1365-2656.2012.02033.x
Mulungu LS (2017) Control of rodent pests in maize cultivation: the case of Africa. 317–337. doi: 10.19103/AS.2016.0002.18
Mulungu LS, Borremans B, Ngowo V, Mdangi ME, Katakweba AS, Tesha P, Mrosso FP, Mchomvu M, Kilonzo BS (2015) Comparative study of movement patterns of Mastomys natalensis in irrigated rice and fallow fields in eastern Tanzania. Afr J Ecol 53:1–7. doi: 10.1111/aje.12233
Murphy, F. A., Winn, W. C., Walker, D. H., Flemister, M. R. & Whitfield SG (1976) Early lymphoreticular viral tropism and antigen persistence Tamiami virus infection in the cotton rat. Lab Invest 34:125–140.
Mwanjabe PS, Sirima FB, Lusingu J (2002) Crop losses due to outbreaks of Mastomys natalensis (Smith, 1834) Muridae, Rodentia, in the Lindi Region of Tanzania. Int Biodeterior Biodegradation 49:133–137. doi: 10.1016/S0964-8305(01)00113-5
Nagaraj T, Vasanth JP, Desai A, Kamat A, Madhusudana SN, Ravi V (2006) Ante mortem diagnosis of human rabies using saliva samples: Comparison of real time and conventional RT-PCR techniques. J Clin Virol 36:17–23. doi: 10.1016/j.jcv.2006.01.009

Neerinckx SB, Peterson AT, Gulinck H, Deckers J, Leirs H (2008) Geographic distribution and ecological niche of plague in sub-Saharan Africa. Int J Health Geogr 7:54. doi: 10.1186/1476-072X-7-54

Olayemi A, Cadar D, Magassouba N, Obadare A, Kourouma F, Oyeyiola A, Fasogbon S, Igbokwe J, Rieger T, Bockholt S, Jérôme H, Schmidt-Chanasit J, Garigliany M, Lorenzen S, Igbahenah F, Fichet J-N, Ortsega D, Omilabu S, Günther S, Fichet-Calvet E (2016a) New Hosts of The Lassa Virus. Sci Rep 6:25280. doi: 10.1038/srep25280

Olayemi A, Obadare A, Oyeyiola A, Igbokwe J, Fasogbon A, Igbahenah F, Ortsega D, Asogun D, Umeh P, Pahlman M, Becker-ziaja B, Günther S, Fichet-calvet E (2016b) Arenavirus diversity and phylogeography of Mastomys natalensis rodents, Nigeria. Emerg Infect Dis 22:694–697. doi: http://dx.doi.org/10.3201/eid2204.150155

Olayemi A, Obadare A, Oyeyiola A, Fasogbon S, Igbokwe J, Igbahenah F, Ortsega D, Günther S, Verheyen E, Fichet-Calvet E (2017) Small mammal diversity and dynamics within Nigeria, with emphasis on reservoirs of the lassa virus. Syst Biodivers 2000:1–10. doi: 10.1080/14772000.2017.1358220

Olayemi A, Obadare A, Oyeyiola A, Igbokwe J, Fasogbon A, Igbahenah F, Ortsega D, Asogun D, Umeh P, Vakkai I, Abejegah C, Pahlman M, Becker-Ziaja B, Günther S, Fichet-Calvet E (2016c) Arenavirus diversity and phylogeography of mastomys natalensis Rodents, Nigeria. Emerg Infect Dis 22:694–697. doi: 10.3201/eid2204.150155

Olayemi A, Oyeyiola A, Obadare A, Igbokwe J, Adesina AS, Onwe F, Ukwaja KN, Ajayi NA, Rieger T, Günther S, Fichet-Calvet E (2018) Widespread arenavirus occurrence and seroprevalence in small mammals, Nigeria. Parasites and Vectors 11:1–9. doi: 10.1186/s13071-018-2991-5

Oldstone, M.B.; Dixon FJ (1967) Lymphocytic choriomeningitis: Production of antibody by "tolerant" infected mice. Science (80-) 1193–1195.

Oldstone M (2002a) Biology and pathogenesis of lymphocytic choriomeningitis virus infection. Springer-Verlag, Berlin Heidelberg

Oldstone M, Dixon F (1968) Susceptibility of different mouse strains to lymphocytic choriomeningitis virus. J Immunol 100:355–357.

Oldstone M, Sinha Y, Blount P, Tishon A, Rodriguez M, von Wedel R, Lampert P (1982) Virus-Induced Alterations in Homeostasis: Alterations in. Science (80-) 218:6.

Oldstone M, Southern P, Rodriquez M, Lampert P (1984) Virus persists in beta cells of islets of Langerhans and is associated with chemical manifestations of diabetes. Science (80-) 224:1440–1443. doi: 10.1126/science.6203172

Olschläger S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, Asogun D, Ehichioya D, Omilabu S, Günther S (2010) Improved detection of Lassa virus by reverse transcription-PCR targeting the 5' region of S RNA. J Clin Microbiol 48:2009–13. doi: 10.1128/JCM.02351-09

Pastoret P, Brochier B (1996) The development and use of a vaccinia-rabies recombinant oral vaccine for the control of wildlife rabies; a link between Jenner and Pasteur. Epidemiol Infect 116:235. doi: 10.1017/S0950268800052535

Patassi AA, Landoh DE, Mebiny-Essoh Tchalla A, Halatoko WA, Assane H, Saka B, Naba MA, Yaya I, Edou KA, Tamekloe TA, Banla AK, Davi KM, Manga M, Kassankogno Y, Salmon-Ceron D (2017) Emergence of Lassa Fever Disease in Northern Togo: Report of Two Cases in Oti District in 2016. Case Rep Infect Dis 2017:1–5. doi: 10.1155/2017/8242313

Peel AJ, Pulliam JRC, Luis AD, Plowright RK, Shea TJO, Hayman DTS, Wood JLN, Webb CT, Restif O, Rk P, Tj OS, Dts H, Jln W, Ct W, The RO (2014) The effect of seasonal birth pulses on pathogen persistence in wild mammal populations.

Plowright RK, Peel AJ, Streicker DG, Gilbert AT, McCallum H, Wood J, Baker ML, Restif O (2016) Transmission or Within-Host Dynamics Driving Pulses of Zoonotic Viruses in Reservoir–Host Populations. PLoS Negl Trop Dis 10:1–21. doi: 10.1371/journal.pntd.0004796

Plowright RK, Parrish CR, McCallum H, Hudson PJ, Ko AI, Graham AL, Lloyd-Smith JO (2017) Pathways to zoonotic spillover. Nat Rev Microbiol 15:502–510. doi: 10.1038/nrmicro.2017.45

Pollock KH, Nichols JD, Brownie C, Hines JE (1990) Statistical Inference for Capture-Recapture Experiments. Wildl Monogr 3–97. doi: 10.2307/3830560

Potapov A, Merrill E, Lewis MA (2012) Wildlife disease elimination and density dependence. Proc R Soc B Biol Sci 279:3139–3145. doi: 10.1098/rspb.2012.0520

Pradel R, Sanz-Aguilar A (2012) Modeling trap-awareness and related phenomena in capture-recapture studies. PLoS One. doi: 10.1371/journal.pone.0032666

Pradel R, Wintrebert CMA, Gimenez O, Biometrics S, Mar N, Pradel R, Wintrebert CMA, Gimenez O (2003) A Proposal for a Goodness-of-Fit Test to the Arnason-Schwarz Multisite Capture-Recapture Model Published by : International 59:43–53.

R Core Team (2016) R: a language and environment for statistical 688 computing., R Foundati. Vienna

Radoshitzky SR, Bào Y, Buchmeier MJ, Charrel RN, Clawson AN, Clegg CS, DeRisi JL, Emonet S, Gonzalez JP, Kuhn JH, Lukashevich IS, Peters CJ, Romanowski V, Salvato MS, Stenglein MD, de la Torre JC arlo. (2015) Past, present, and future of arenavirus taxonomy. Arch Virol 160:1851–1874. doi: 10.1007/s00705-015-2418-y

Rahelinirina S, Duplantier J, Ratsimba M, Ratovonjato J, Ramilijaona O, Papillon Y, Rahalison L (2009) Assessment of Rhodamine B for labelling the plague reservoir Rattus rattus in Madagascar. Afr J Ecol 48:662–666.

Rambaut A, Pybus OG, Nelson MI, Viboud C, Jeffery K, Holmes EC (2009) The genomic and epidemiological dynamics of human influenza A virus. Nature 453:615–619.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291–318. doi:10.1111/j.1469-185X.2007.00010.x

Redding DW, Moses LM, Cunningham AA, Wood J, Jones KE (2016) Environmental-mechanistic modelling of the impact of global change on human zoonotic disease emergence: a case study of Lassa fever. Methods Ecol Evol 7:646–655.

Rieger T, Merkler D, Günther S (2013) Infection of type I interferon receptor-deficient mice with various old world arenaviruses: a model for studying virulence and host species barriers. PLoS One 8:e72290.
Roberts L (2018) Nigeria hit by unprecedented Lassa fever outbreak. Science (80-) 359:1201–1202. doi: 10.1126/science.359.6381.1201
Rossi C, Jenik P (1996) Immunological identification of Tacaribe virus proteins. 203–211.
Ryder JJ, Miller MR, White A, Knell RJ, Boots M (2007) Host-parasite population dynamics under combined frequency- and density-dependent transmission. Oikos 116:2017–2026. doi: 10.1111/j.2007.0030-1299.15863.x
Safronetz D, Lopez JE, Sogoba N, Traore' SF, Raffel SJ, Fischer ER, Ebihara H, Branco L, Garry RF, Schwan TG, Feldmann H (2010) Detection of lassa virus, mali. Emerg Infect Dis 16:1123–1126. doi: 10.3201/eid1607.100146
Sagripanti JL, Rom AM, Holland LE (2010) Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. Arch Virol 155:2035–2039. doi: 10.1007/s00705-010-0791-0
Sauvage F, Langlais M, Yoccoz NG, Pontier D (2003) Modelling hantavirus in fluctuating populations of bank voles: the role of indirect transmission on virus persistence. J Anim Ecol 72:1–13. doi: 10.1046/j.1365-2656.2003.00675.x
Schmid-Hempel P, Ebert D (2003) On the evolutionary ecology of specific immune defence. Trends Ecol Evol 18:27–32. doi: 10.1016/S0169-5347(02)00013-7
Sikes RS, Gannon WL (2007) Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammal 88:809–823. doi: 10.1644/10-MAMM-F-355.1
Singleton GR, Leirs H, Hinds L a, Zhang ZB (1999) Ecologically-based management of rodent pests-re-evaluating our approach to an old problem. Canberra
Singleton GR, Brown PR, Jacob J, Aplin KP, Sudarmaju (2007) Unwanted and unintended effects of culling: A case for ecologically-based rodent management. Integr Zool 2:247–259. doi: 10.1111/j.1749-4877.2007.00067.x
Singleton G, Belmain S, Brown P, Hardy B (2010) Rodent Outbreaks : Ecology and Impacts. Los Baños (Philippines): International Rice Research Institute.
Slade NA, Russell LA (1998) Distances as Indices to Movements and Home-Range Size From Trapping Records of Small Mammals. J Mammal 79:346–351. doi: 10.2307/1382871
Sluydts V, Crespin L, Davis S, Lima M, Leirs H (2007) Survival and maturation rates of the African rodent, Mastomys natalensis: density-dependence and rainfall. Integr Zool 2:220–32. doi: 10.1111/j.1749-4877.2007.00065.x
Sluydts V, Davis S, Mercelis S, Leirs H (2009) Comparison of multimammate mouse (Mastomys natalensis) demography in monoculture and mosaic agricultural habitat: Implications for pest management. Crop Prot 28:647–654. doi: 10.1016/j.cropro.2009.03.018
Smith GC, Cheeseman CL (2002) A mathematical model for the control of diseases in wildlife populations: Culling, vaccination and fertility control. Ecol Modell 150:45–53. doi: 10.1016/S0304-3800(01)00471-9
Smith MJ, Telfer S, Kallio ER, Burthe S, Cook AR, Lambin X, Begon M (2009) Host-pathogen time series data in wildlife support a transmission function between density and frequency dependence. Proc Natl Acad Sci 106:7905–9.
Stenseth NC, Leirs H, Mercelis S, Mwanjabe P (2010) Comparing strategies for controlling an African pest rodent : an empirically based theoretical study. 38:1020–1031.
Stenseth NC, Mysterud A, Ottersen G, Hurrel JW, Chan K-S, Lima M (2002) Ecological effects of climate fluctuations. Ecol Climatol 297:1292–1296. doi: 10.1126/science.1071281
Stephenson E, Larson E, JW (1984) Effect of Environmental Factors on Aerosol-Induced Lassa Virus Infection. J Med Virol 14:295–303.
Straumanis JP, Angeles L, Perez IA (2018) Mother-to-Child Transmission of Hepatitis G Department of Pediatrics Division of Pediatric Critical Care Department of Pediatrics Division of Laboratory Research Department of Pediatrics Kunitada Shimotohno , PhD Division of Pediatric Pulmonology Departme.
Swanepoel LH, Swanepoel CM, Brown PR, Eiseb SJ, Goodman SM, Keith M, Kirsten F, Leirs H, Mahlaba TAM, Makundi RH, Malebane P, Von Maltitz EF, Massawe AW, Monadjem A, Mulungu LS, Singleton GR, Taylor PJ, Soarimalala V, Belmain SR (2017) A systematic review of rodent pest research in Afro-Malagasy small-holder farming systems: Are we asking the right questions? PLoS One. doi: 10.1371/journal.pone.0174554
Tang JW, Tang JW (2009) The effect of environmental parameters on the survival of airborne infectious agents The effect of environmental parameters on the survival of airborne infectious agents. J R Soc interface 5:1001–1007.
Taylor PJ, Arnitzen L, Hayter M, Iles M, Frean J, Belmain S (2008) Understanding and managing sanitary risks due to rodent zoonoses in an African city: beyond the Boston Model. Integr Zool 3:38–50. doi: 10.1111/j.1749-4877.2008.00072.x
Taylor PJ, Downs S, Monadjem A, Eiseb SJ, Mulungu LS, Massawe AW, Mahlaba TA, Kirsten F, Von Maltitz E, Malebane P, Makundi RH, Lamb J, Belmain SR (2012) Experimental treatment-control studies of ecologically based rodent management in Africa: Balancing conservation and pest management. Wildl Res 39:51–61. doi: 10.1071/WR11111
Telfer S, Bennett M, Bown K, Cavanagh R, Crespin L, Hazel S, Jones T, Begon M (2002) The effects of cowpox virus on survival in natural rodent populations : increases and decreases. J Anim Ecol 71:558–568.
Telfer, S., Birtles, R., Bennett, M., Lambin, X., Paterson, S., & Begon, M. (2008). Parasite interactions in natural populations: Insights from longitudinal data. *Parasitology*, *135*(7), 767–781. doi:10.1017/S0031182008000395
Tersago K, Crespin L, Verhagen R, Leirs H (2012) Impact of Puumala virus infection on maturation and survival in bank voles: a capture-mark-recapture analysis. J Wildl Dis 48:148–156.
Thomas F, Adamo S, Moore J (2005) Parasitic manipulation: Where are we and where should we go? Behav Processes 68:185–199. doi: 10.1016/j.beproc.2004.06.010
Traub E (1936a) An Epidemic in a Mouse Colony Due To the Virus of Acute Lymphocytic Choriomeningitis. J Exp Med 63:533–548. doi: 10.1084/jem.63.4.533
Traub E (1936b) Persistence of lymphocytic choriomeningitis virus in immune animals and its relation to immunity. J Exp Med 63:847–862.
Traub E (1936c) The epidemiology of Lymphocytic Choriomeningitis in white mice. J Exp Med 64:183–200.

VanDen Broecke B, Borremans B, Marien J, Makundi RH, Massawe AW, Leirs H, Hughes NK (2017) Does exploratory behavior or activity in a wild mouse explain susceptibility to virus infection ? Curr Zool 53:1–15.
Van den Panhuyzen S (2018) Direct and indirect horizontal transmission of Morogoro arenavirrus and the vertical transmission of maternal antibodies in *Mastomsys natalensis*. Master thesis dissertation at University of Antwerp.
Veenstra AJF (1958) The behaviour of the multimammate mouse, Rattus (Mastomys) natalensis (A. Smith). Anim Behav 6:195–206.
Vennemal H, Godeke G, Rossen JWA, Voorhout WF, Horzinek MC, Opstelten DE, Rottier PJM (1996) Nucleocapsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. 15:2020–2028.
Verhagen R, Leirs H, Tkachenko E, van der Groen G (1986) Ecological and epidemiological data on Hantavirus in bank vole populations in Belgium. Arch Virol 91:193–205.
Vieth S, Drosten C, Lenz O, Vincent M, Omilabu S, Hass M, Becker-Ziaja B, ter Meulen J, Nichol ST, Schmitz H, Günther S (2007) RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans R Soc Trop Med Hyg 101:1253–64. doi: 10.1016/j.trstmh.2005.03.018
Vitulla D, Merani M (1988) Is vertical transmission sufficient to maintain Junin virus maintenance in nature? J Gen Virol 69:1437–1440.
Vitullo A, Hodara V, Merani MS (1987) Effect of persistent infection with Junin virus on growth and reproduction of its natural reservoir, Calomys musculinus. Am J Trop Med Hyg 37:663–669.
Vitullo A, Merani M (1990) Vertical transmission of junin virus in experimentally infected adult Calomys musculinus. Intervirology 31:339–344.
Voutilainen L, Kallio ER, Niemimaa J, Vapalahti O, Henttonen H (2016) Temporal dynamics of Puumala hantavirus infection in cyclic populations of bank voles. Nat Publ Gr 1–14. doi: 10.1038/srep21323
Voutilainen L, Sironen T, Tonteri E, Tuiskunen Bäck A, Razzauti M, Karlsson M, Wahlström M, Niemimaa J, Henttonen H, Lundkvist Å, Tuiskunen Back A, Wahlstrom M, Lundkvist A (2015) Life-Long Shedding of Puumala Hantavirus in Wild Bank Voles (Myodes glareolus). J Gen Virol 96:1238–1247. doi: 10.1099/vir.0.000076
Walker, D. H. FAM (1987) Pathology and Pathogenesis of Arenavirus Infections. Curr Top Microbiol Immunol 133:89–113.
Walker DH, Wulff H, Lange J V, Murphy FA (1975) Comparative pathology of Lassa virus infection in monkeys, guinea-pigs, and Mastomys natalensis. Bull World Health Organ 52:523–34.
Webb P, Justines G, Johnson K (1975) Infection of wild and laboratory animals with Machupo and Latino viruses. Bull World Health Organ 52:493–9.
Weber de Melo V, Sheikh Ali H, Freise J, Kühnert D, Essbauer S, Mertens M, Wanka KM, Drewes S, Ulrich RG, Heckel G (2015) Spatiotemporal dynamics of Puumala hantavirus associated with its rodent host, Myodes glareolus. Evol Appl 8:545–559. doi: 10.1111/eva.12263
Webster JP, Brunton CFA, Macdonald DW (1994) Effect of Toxoplasma gondii upon neophobic behaviour in wild brown rats, Rattus norvegicus. Parasitology 109:37. doi: 10.1017/S003118200007774X
Weigand H, Hotchin J (1961) Studies of lymphocytic choriomeningitis in mice. J Immunol 86:401.
Weigand H, Hotchin J, Benson (1963) The pathogenesis of lymphocytic choriomeningitis in mice: the effects of different inoculation routes and the footpad response. J Immunol 61:460–468.
White, G.C. and Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird study 46 supplement, 120–138.
Whitmer SLM, Strecker T, Cadar D, Dienes HP, Faber K, Patel K, Brown SM, …, Ströher U, Günther S (2018) New lineage of lassa virus, Togo, 2016. Emerg Infect Dis 24:599–602. doi: 10.3201/eid2403.171905
Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York
Wolfe ND, Dunavan CP, Diamond J (2007) Origins of major human infectious diseases. Nature 447:279–283. doi: 10.1038/nature05775
Wood S (2017) Smoothing parameter and model selection for general smooth models (with discussion). J Am Stat Assoc 111:1548:1575.
Wood S, Scheipl F (2017) gamm4: Generalized Additive Mixed Models using "mgcv" and "lme4."
Woods GM, Kreiss A, Belov K, Siddle H V., Obendorf DL, Muller HK (2007) The immune response of the Tasmanian devil (Sarcophilus harrisii) and devil facial tumour disease. Ecohealth 4:338–345. doi: 10.1007/s10393-007-0117-1
Woolhouse M, Gaunt E (2007) Ecological origins of novel human pathogens. Crit Rev Microbiol 33:231–242. doi: 10.1080/10408410701647560
World Health Organization (2016) Lassa fever. [online]. In: Available http//www.who.int/csr/disease/lassafever/en/ [Accessed 13 Jun. 2016].
Wulff H, Lange J (1975) Indirect immunofluorescence for the diagnosis of Lassa fever infection. Bull World Heal organ 52:429–436.
Wulff H, McIntosh BM, Hamner DB, Johnson KM (1977) Isolation of an arenavirus closely related to Lassa virus from Mastomys natalensis in south-east Africa. Bull World Health Organ 55:441–444.
Yanagihara R, Amyx HL, Gajdusek DC (1985) Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (Clethrionomys glareolus). J Virol 55:34–8.
Yates TL, Mills JN, Parmentier CA, Ksiazk TG, Permentier RR, Vande Castle JR, CALISHER CH, Nichol ST, Abbot KD, Young JC, Morrison ML, Beaty BJ, Dunnum JL, Baker RJ, Salazar-bravo J, Peters CJ (2002) The Ecology and Evolutionary History of an Emergent Disease: Hantavirus Pulmonary Syndrome. Bioscience 52:989. doi: 10.1641/0006-3568(2002)052[0989:TEAEHO]2.0.CO;2
Zapata JC, Salvato MS (2013) Arenavirus variations due to host-specific adaptation. Viruses 5:241–78.
Zhou X, Ramachandran S, Mann M, Popkin DL (2012) Role of lymphocytic choriomeningitis virus (LCMV) in understanding viral immunology: Past, present and future. Viruses 4:2650–2669.
Ziskind B, Halioua B (2007) La tuberculose en ancienne Égypte. Rev mal respir 24:1277–1283.