Received: 21 June 2019 | Accepted: 21 July 2019

DOI: 10.1111/1365-2656.13107

RESEARCH ARTICLE

Journal of Animal Ecology | BRITISH ECOLOGICAL SOCIETY

Density dependence and persistence of Morogoro arenavirus transmission in a fluctuating population of its reservoir host

Joachim Mariën[1] | Benny Borremans[1,2,3] | Christophe Verhaeren[1] | Lucinda Kirkpatrick[1] | Sophie Gryseels[1,4,5] | Joëlle Goüy de Bellocq[6] | Stephan Günther[7] | Christopher A. Sabuni[8] | Apia W. Massawe[8] | Jonas Reijniers[1,9] | Herwig Leirs[1]

[1]Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium; [2]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, California, LA, USA; [3]Interuniversity Institute for Biostatistics and Statistical Bioinformatics (I-BIOSTAT), Hasselt University, Hasselt, Belgium; [4]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA; [5]Clinical and Epidemiological Virology, Rega Institute, KU Leuven, Leuven, Belgium; [6]Institute of Vertebrate Biology, Research Facility Studenec, The Czech Academy of Sciences, Brno, Czech Republic; [7]Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany; [8]PestManagement Centre, Sokoine University of Agriculture, Morogoro, Tanzania and [9]Department of Engineering Management, University of Antwerp, Antwerp, Belgium

Correspondence
Joachim Mariën
Email: Joachim_marien@hotmail.com

Funding information
Antwerp study centre for disease (ASCID), Grant/Award Number: INCO-DEV grant ICA4-CT2002-10050 and GOA BOF FFB3567; German Research Foundation, Grant/Award Number: Focus Programs GU 883/3-1 and GU 883/3-2 from Deut

Handling Editor: Andy Fenton

Abstract

1. A key aim in wildlife disease ecology is to understand how host and parasite characteristics influence parasite transmission and persistence. Variation in host population density can have strong impacts on transmission and outbreaks, and theory predicts particular transmission–density patterns depending on how parasites are transmitted between individuals. Here, we present the results of a study on the dynamics of Morogoro arenavirus in a population of multimammate mice (*Mastomys natalensis*). This widespread African rodent, which is also the reservoir host of Lassa arenavirus in West Africa, is known for its strong seasonal density fluctuations driven by food availability.
2. We investigated to what degree virus transmission changes with host population density and how the virus might be able to persist during periods of low host density.
3. A seven-year capture–mark–recapture study was conducted in Tanzania where rodents were trapped monthly and screened for the presence of antibodies against Morogoro virus. Observed seasonal seroprevalence patterns were compared with those generated by mathematical transmission models to test different hypotheses regarding the degree of density dependence and the role of chronically infected individuals.
4. We observed that Morogoro virus seroprevalence correlates positively with host density with a lag of 1–4 months. Model results suggest that the observed seasonal seroprevalence dynamics can be best explained by a combination of vertical and horizontal transmission and that a small number of animals need to be infected chronically to ensure viral persistence.

5. Transmission dynamics and viral persistence were best explained by the existence of both acutely and chronically infected individuals and by seasonally changing transmission rates. Due to the presence of chronically infected rodents, rodent control is unlikely to be a feasible approach for eliminating arenaviruses such as Lassa virus from *Mastomys* populations.

KEYWORDS

arenavirus, capture-mark-recapture, mathematical modelling, Morogoro virus, multimammate mouse, parasite-host interactions, rodent-borne parasite, transmission dynamics

1 | INTRODUCTION

Ecological factors can exert strong pressures on wildlife populations, often resulting in substantial seasonal or multi-annual density fluctuations (Krebs, 2013; Stenseth et al., 2002; Myers, 2018). These fluctuations can influence the transmission dynamics of many parasites and have been linked to disease outbreaks in both wildlife and humans (Altizer et al., 2006; Davis et al., 2004; 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, Fichet-Calvet, & Leirs, 2005; Lloyd-Smith et al., 2005). This could be partly explained by the short duration of most field studies (mostly less 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 (Begon et al., 2009; Hudson, Rizzoli, Grenfell, Heesterbeek, & Dobson, 2002).

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, Miller, White, Knell, & Boots, 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 time-scale 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, Ksiazek, Peters, & Childs, 1999). This situation is likely when host births are limited to short breeding periods (birth pulses) and when the likelihood of becoming infected increases with age, thus when transmission is (mainly) horizontal (Adler, Pearce-Duvet, & Dearing, 2008). In contrast, such a juvenile dilution effect is not predicted for vertically transmitted parasites, as newborns immediately enter the population as infected and simultaneously increase host density and prevalence (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., 2005). 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 (*Bison bison*; 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 lifelong 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., 2005). 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; Morters et al., 2012; Borremans, Reijniers, Hens, & Leirs, 2017).

Here, we investigate the relationship between population density and transmission characteristics for Morogoro arenavirus (MORV) in a population of its reservoir host, the Natal multimammate mouse (*Mastomys natalensis*). We analysed a seven-year capture–mark–recapture time series and compared the results to simulations of an individual-based (mathematical) model to investigate whether (a) the seasonal *M. natalensis* fluctuations generate seasonal MORV seroprevalence cycles; (b) transmission is affected by host density; (c) a juvenile dilution effect occurs at the end of the breeding season; (d) vertical transmission affects this dilution effect; and (e) chronic carriers are necessary to ensure viral persistence. To further investigate whether chronically infected individuals are indeed present

in natural conditions, we performed a small laboratory experiment in which wild-caught rodents were caged and sampled for 8 weeks.

2 | MATERIALS AND METHODS

2.1 | The MORV-*Mastomys natalensis* model

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 has been studied intensively and the virus is not pathogenic for humans (Borremans et al., 2011; Borremans, Sluydts, Makundi, & Leirs, 2015; Borremans, Vossen, et al., 2015; Gryseels et al., 2017; Günther et al., 2009; Leirs, 1994). The system provides a safe substitute for studying closely related but pathogenic arenaviruses such as Lassa virus (LASV), the aetiologic 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, Borremans, Gryseels, Broecke, et al., 2017). The available data suggest that most animals are acutely infected in natural conditions, shedding up to 30 days after infection (Borremans et al., 2011; Borremans, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015; Mariën, Borremans, Gryseels, Broecke, et al., 2017). Infected animals develop a lifelong antibody response and seem to experience no or only small pathogenic effects (Borremans, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015; Mariën, Borremans, Gryseels, Broecke, et al., 2017; Mariën, Borremans, Gryseels, Soropogui, et al., 2017; Mariën, Kourouma, Magassouba, Leirs, & Fichet-Calvet, 2018; Mariën, Sluydts, et al., 2018).

The distribution of MORV is limited to *M. natalensis* in East Africa, where infection prevalence ranges from 0% to 20% (Borremans et al., 2011; Gryseels et al., 2017; Günther et al., 2009). In this region, *M. natalensis* populations exhibit strong density fluctuations between seasons and years, generally ranging from 20 to 300 individuals per hectare but occasionally reaching outbreak densities of 600 individuals/ha or higher (Leirs, 1994; Sluydts, Davis, Mercelis, & Leirs, 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. *Mastomys natalensis* has a promiscuous mating system and is not territorial or aggressive towards conspecifics (Kennis, Sluydts, Leirs, & Hooft, 2008). Home range overlap is generally high and increases significantly with abundance, suggesting contact rates to be density-dependent, probably nonlinearly (Borremans et al., 2013, 2017, 2016). Given that MORV transmission is most likely 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, Reijniers, Borremans, & Leirs, 2013).

2.2 | Study area and trapping

Between May 2010 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 300 × 100 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.) were placed evenly at 10-m 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). Blood samples were taken with a heparinized microcapillary tube from the retro-orbital sinus and preserved on prepunched filter paper (±15 µl/punch; Serobuvard, LDA 22, Zoopole). 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, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015) and afterwards released at its capture site.

2.3 | 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 −20°C. 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). Most samples were tested only once, but if they were uncertain, they were tested a second time for the final decision.

2.4 | 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 titres falling below the detection threshold of the assay, as *M. natalensis* normally develops a long-term Ab production after infection (Mariën, Borremans,

Gryseels, Broecke, et al., 2017). These samples were considered positive for further analyses, except for very young individuals (body weight at first capture <15 g) that still might have had maternally derived Ab, which are likely to disappear after 3 weeks (Demby et al., 2001; Fichet-Calvet, Becker-Ziaja, Koivogui, & Günther, 2014).

2.5 | 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 (5,000 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). Because antibody titres could fall below the detection limit of the used assay (Mariën, Borremans, Gryseels, Broecke, et al., 2017), we highlight that the given seroprevalences may be slight underestimates. But while this will affect the amplitude of the presented time series, it will not affect the periodicity that we are interested in.

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, Kallio, Niemimaa, Vapalahti, & Henttonen, 2016). GAMs were used because we expected a nonlinear response for the seasonal seroprevalence pattern. Model 1 (GAM1) was the intercept (null) 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 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. This was investigated in order to assess potential differences between years caused by variation in sample storage conditions. Model 4 (GAM4) combined the smoothed seasonal and fixed year effects. Selection of the latter model would suggest a consistent seasonal effect and annual changes in mean seroprevalence. We did not include a model with an interaction between season and year because this 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

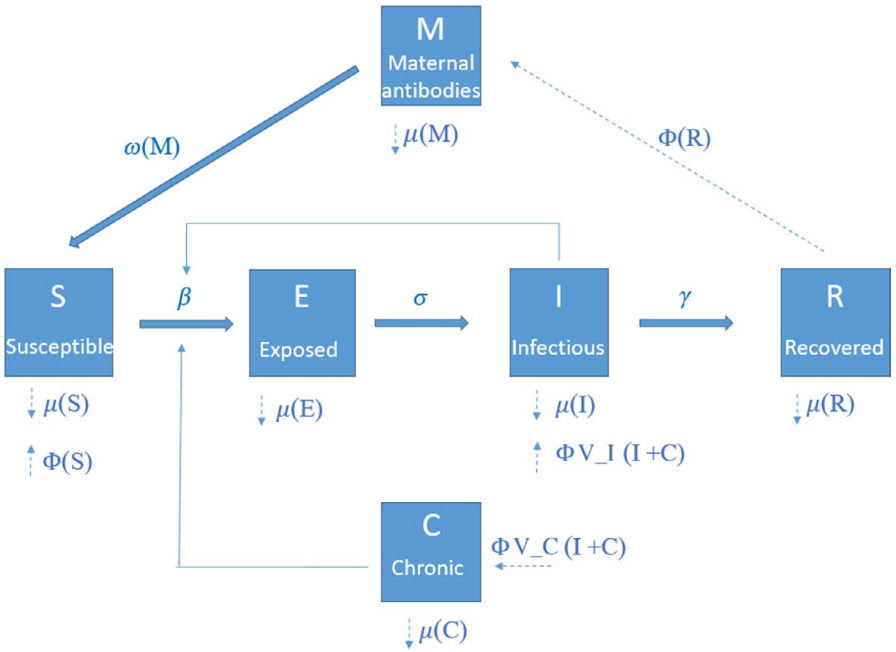

FIGURE 1 Illustration of the individual-based model used to simulate the spread of Morogoro virus in populations of *Mastomys natalensis* in Tanzania. Individual rodents are assigned different states according to infection status: susceptible (S), exposed (E), acutely infectious (I), recovered (R), maternal 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 (with V_I + V_C = 1 in the model with vertical transmission). 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

the R-packages: LUBRIDATE, MGCV, GAMM4, GGPLOT2, SCALES and MVTNORM (Genz et al., 2017; Wickham, 2009, 2017; Wood & Scheipl, 2017).

2.6 | Mathematical modelling

Based on data from this study as well as 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) (Supplementary file: MORV model). The model allowed us to explore whether the presence or absence of vertical transmission 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.

2.6.1 | Demographic component

The main reason we used an IBM was that it allowed us 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 throughout the year (due to a seasonal birth pulse 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 in Leirs, 1994) and abundance (similar to the abundance time series in this study) (Figure S1). In order to ensure that the modelled age distribution resembled the observed distribution, birth and mortality rates changed depending on time of the year and individual age (for details, see Supplementary Information). Since we recently showed that MORV has no or only limited adverse effects on *M. natalensis* (Mariën, Borremans, Gryseels, Soropogui, et al., 2017; Mariën, Kourouma, et al., 2018; Mariën, Sluydts, et al., 2018), the model assumes that the birth and death rates are equal for susceptible and infected animals. We highlight that modelling demography was not the prime goal of this study and that the parameters were optimized only to obtain a realistic age distribution and rodent density, while still being biologically meaningful.

2.6.2 | Transitions

The model assumes that when a susceptible individual (S) becomes acutely infected, it first passes a latency period (E stage) for a fixed period of 6 days (σ^{-1}) during which it is not yet infectious. It then becomes infectious for a fixed period of 21 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, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015). Furthermore, we assume that individuals born to recovered mothers (*R*) will enter the population carrying maternally derived antibodies (Φ*R*) that protect juveniles in this state (M) against infection for a fixed period of 28 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 LASV and MORV in field studies and for LCMV in laboratory experiments (Fichet-Calvet et al., 2014; Mariën, Borremans, Gryseels, Broecke, et al., 2017; Oldstone, 2002).

2.6.3 | Horizontal transmission: density dependence

Horizontal transmission of MORV (i.e. via contacts between individuals, as opposed to vertical transmission from mother to offspring) was modelled as infection rate $\frac{\beta K^q S(I+C)}{N^q}$, following the implementation of Smith et al. (2009). This formulation allows to shape 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 β represents the transmission coefficient, which is depended on the contact rate to a given density and the probability of transmission when an I individual contacts an S (Begon et al., 2002). Given that *M. natalensis* is not territorial and its home range overlap is generally high, we assumed a homogeneously mixed community in which all individuals are also identical with respect to susceptibility and infectivity (Borremans et al., 2013).

2.6.4 | 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 5% 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 (Borremans, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015; Fichet-Calvet et al., 2014; Mariën, Borremans, Gryseels, Broecke, et al., 2017; Walker, Wulff, Lange, & Murphy, 1975). Chronic carriers were assumed to stay in this state permanently 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. In the model with vertical transmission, all offspring born to infected mothers (either I or C) will become infectious (either I or C), thus V_I + V_C = 1.

2.6.5 | Model selection

To investigate the importance of the vertical transmission component and the transmission–density relation in the model, we compared the seroprevalence pattern (infected, recovered or maternal antibodies) of models to the seroprevalence field data by minimizing the AIC, which is here given by $2K - 2\ln[\mathcal{L}(\text{model}|\text{data})]$ with K the number of parameters and $\mathcal{L}$ the likelihood function (Sakamoto, Ishiguro, & Kitagawa, 1986). Models with and without vertical transmission were compared for three values of the transmission–density relation (q = 0, 0.5 and 1). The likelihood function was maximized for each model with respect to the transmission coefficient (β) based on 500 simulations assuming a multivariate normal distribution of the monthly seroprevalence. Simulations were run for 12 years, discarding the initial five years. In order to mimic stochasticity introduced 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.

Although AIC values differed between models (>10 units, see results), the best fitting model did not produce a satisfactory fit to the observed field data (Figure S2). The main reason for this seemed to be that this model predicts a juvenile dilution effect after the breeding season (August–October), which is not observed in the field data. We therefore included an additional model (starting from the model with the best AIC in the previous step) in which the parameter β was allowed to change seasonally (hence, accounting for two extra degrees of freedom/parameters) 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). Inference of the different βs was performed by running 20,000 MCMC chains in which the parameters were updated sequentially using a standard random-walk Metropolis–Hastings algorithm (Gibson & Renshaw, 1998). We assumed uniform priors $U(0, 100)$ for the different βs and tested different starting values in the simulations, to ascertain that they all converged to the same posterior distribution (which is an indication that the simulation result is not merely a local minimum of the AIC).

2.6.6 | Viral persistence and chronic infections

To investigate whether the presence of chronically infected individuals is necessary for MORV persistence, we carried out simulations in which we varied the percentage of chronically infected individuals in the population from 0% to 10% by steps of 0.5 (and adjusted the transmission coefficients to arrive at the same seasonal seroprevalence maximum) using the best fitting model with season-specific β. Simulations (n = 500) were run for each V_C at different populations sizes [from 5 ha to 30 ha fields by steps of 2.5], as the likelihood of persistence increases 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 at the end of the dry season (November) and 30 individuals/ha at the end of the rainy season (May). Transmission in a model run was considered to be persistent if the virus did not go extinct within ten years (Bartlett, 1957). The proportion of 'persistent simulations' was used as a measure of persistence probability.

2.7 | Cage experiment

In order to investigate whether chronic infections indeed occur in natural conditions (as was suggested by the modelling results), we performed a small field experiment in which we caged wild *M. natalensis* for 8 weeks, sampling weekly. From 6 July to 8 August 2016, 270 *M. natalensis* were captured using the same protocol as described for the long-term experiment. Animals were housed in pairs in cages (28 × 11.5 × 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 letting the animal chew on a small filter paper slip for a few seconds. Animals were kept in a plastic bag until they urinated (max. 5 min). Urine was preserved on filter paper, which was dried and stored as described above. Maximally 8 weeks after capture, the mice were euthanized using chloroform, humanely killed by cervical dislocation and dissected. Kidneys, liver and spleen were stored in RNAlater.

Weekly blood samples of all captured mice were screened for antibodies against MORV as described above. For all captured mice, viral RNA was extracted from kidney biopsies using the NucleoSpin RNA II kit (Macherey-Nagel) following manufacturer instructions. As kidney samples usually result in a higher RNA yield than filter paper samples (simply because there is more RNA material), we assumed that when kidney samples were negative filter paper samples would also be negative. Therefore, we only screened blood, urine and saliva samples for mice that had MORV RNA-positive kidneys. For the filter paper samples, viral RNA was extracted using the QIAmp vRNA Mini Kit (Qiagen). RT–PCR was done on the L-segment using MoroL3359-forward and MoroL3753-reverse primers following the protocol described in Günther et al. (2009) and Vieth et al. (2007). All amplicons were confirmed by Sanger sequencing in both directions at the Vlaams Instituut voor Biotechnologie.

2.8 | 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 & 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.

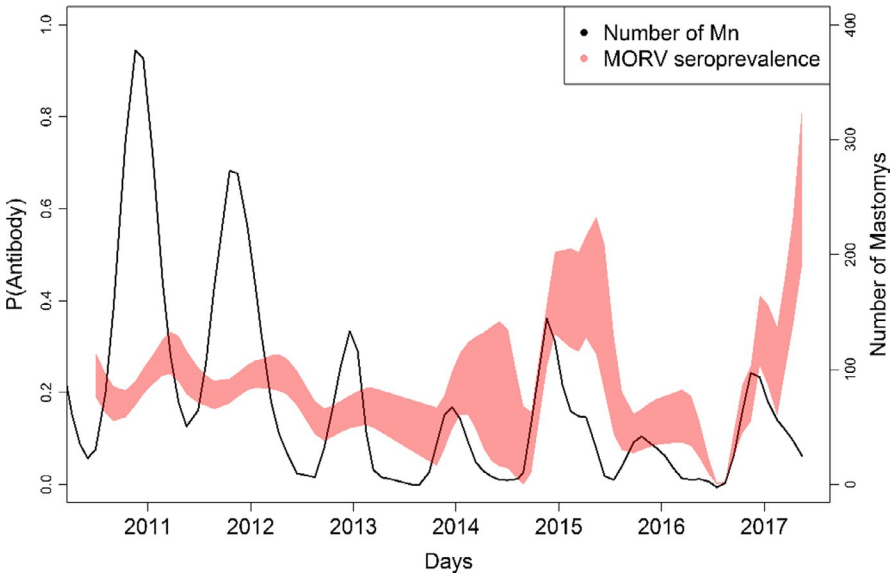

FIGURE 2 Time series (using cyclic-cubic regression splines) of *Mastomys natalensis* abundance and MORV seroprevalence in function of time. The black line represents the monthly number of *Mastomys natalensis* captured each month (black). The envelope represents the 95% confidence interval (CI) on the seroprevalence estimation

3 | RESULTS

3.1 | Seasonality and seroprevalence

There were clear seasonal cycles for both rodent abundance and seroprevalence (Figure 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%; Figure 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 (Figure 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, $p < .0001$). The best fitting model also suggests that the overall seroprevalence differs significantly between years (*df* = 5, chisq = 93.06, $p < .0001$).

3.2 | Mathematical modelling

3.2.1 | Transmission

The mathematical model with the lowest AIC value included a vertical and density-dependent horizontal (q = 0) transmission

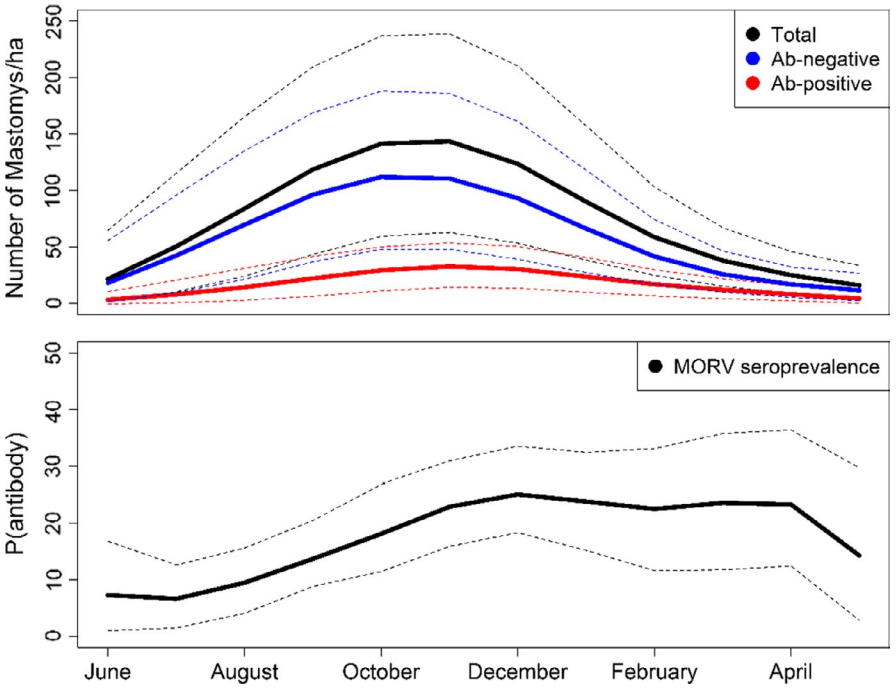

FIGURE 3 Seasonal component of *Mastomys natalensis* abundance (upper) and MORV seroprevalence (lower) time series. The bold lines represent means and dotted lines 95% confidence intervals

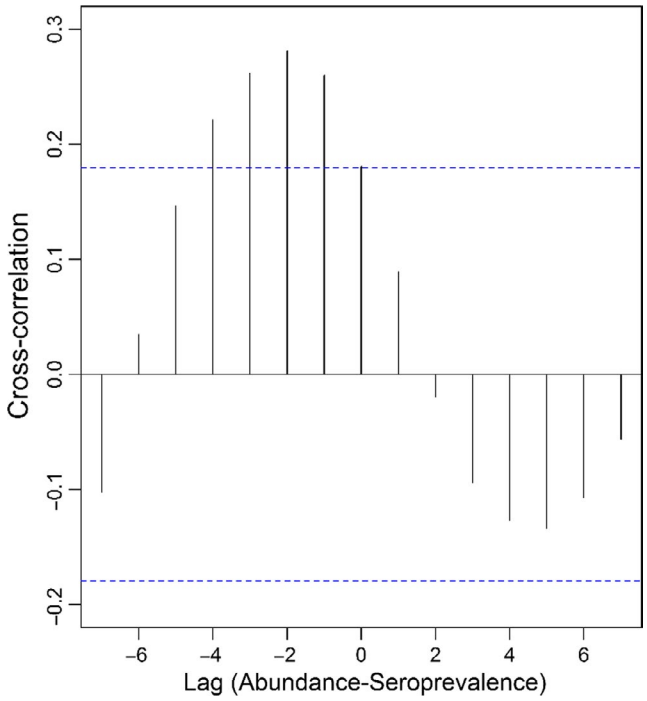

FIGURE 4 Cross-correlation analysis between *Mastomys natalensis* density (number of uniquely captured individuals per month/ha) and MORV seroprevalence. The blue dotted lines represent 95% confidence intervals which indicate when a correlation at a given time lag might be considered significant

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

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

component (Model 3: AIC = 1,092; Table 2). This model was 142 AIC units removed from the second best fitting model that also included a vertical transmission component and in which transmission followed a saturating power function (q = 0.5; model 5: AIC = 1,234). These results suggest that the seasonal variation in seroprevalence can be best explained by a combination of vertical and horizontal transmission (with q = 0). Importantly, a season-specific β (for three different periods) drastically improved the model fit (model 7: AIC = 536), as it did not exhibit the juvenile dilution effect that was observed in all other models but not in the field data (Figure S1) as a result of an increased β during and after the breeding season. For example, assuming vertical and horizontal (q = 0) transmission with 1% chronic carriers, the best model fit

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	Par	AIC
M1	Frequency (q = 1)	Yes	15	1,456
M2	Frequency (q = 1)	No	14	1,908
M3	**Density (q = 0)**	**Yes**	**16**	**1,092**
M4	Density (q = 0)	No	15	1,510
M5	Freq-Dens (q = 0.5)	Yes	17	1,234
M6	Freq-Dens (q = 0.5)	No	16	1,642
M7	**Seasonal beta (q = 0)**	**Yes**	**18**	**536**

Note: The first six models contain a fixed β. The last model contains a seasonal β for three periods: β_1 = January–April (low-density period); β_2 = April–July (low density and breeding period); β_3 = August–December (high-density period). Bold values indicate the best fitting models based on AIC values.
Abbreviations: AIC, Akaike information criterion; Par, number of identifiable parameters.

was obtained with a β that is almost five times higher during the breeding season and almost three times higher during the peak density period: $\beta_{\text{January–April}}$ = 1.0 (95% Credible Interval: 0.1–2.6), $\beta_{\text{April–July}}$ = 4.7 (95% CI: 2.8–7.2) and $\beta_{\text{August–December}}$ = 2.7 (95% CI: 1.4–3.9).

3.2.2 | Chronic infections

As expected, the probability that infections persist over ten years increased with population size and the proportion of chronically infected animals (Figure 5). Interestingly, we found that MORV can never persist when there are no chronically infected animals (even at an area corresponding to 30 ha). At least a few chronic carriers (2.5%–10%) are needed in the population to ensure viral persistence throughout the low host density period.

3.3 | Cage experiment

Of the 270 captured *M. natalensis*, 42 (15%) were antibody positive during the 8-week captivity, and seven (3%) tested positive for MORV RNA in the kidneys. Six mice with RNA-positive kidneys were antibody positive at least once. Two of the mice with RNA-positive kidneys showed signs of chronic infections with continuous presence of viral RNA in excretions for 8 weeks (Figures S4 and S5), one of the mice showed signs of chronic infection with virus retreated into the organs (Figure S6), while the other four mice only showed signs of recent infection (viral RNA in blood during the last week only). This suggests that at least 8% (3/38) of infected animals became a chronic carrier or had MORV RNA in the body for a time period of at least 8 weeks.

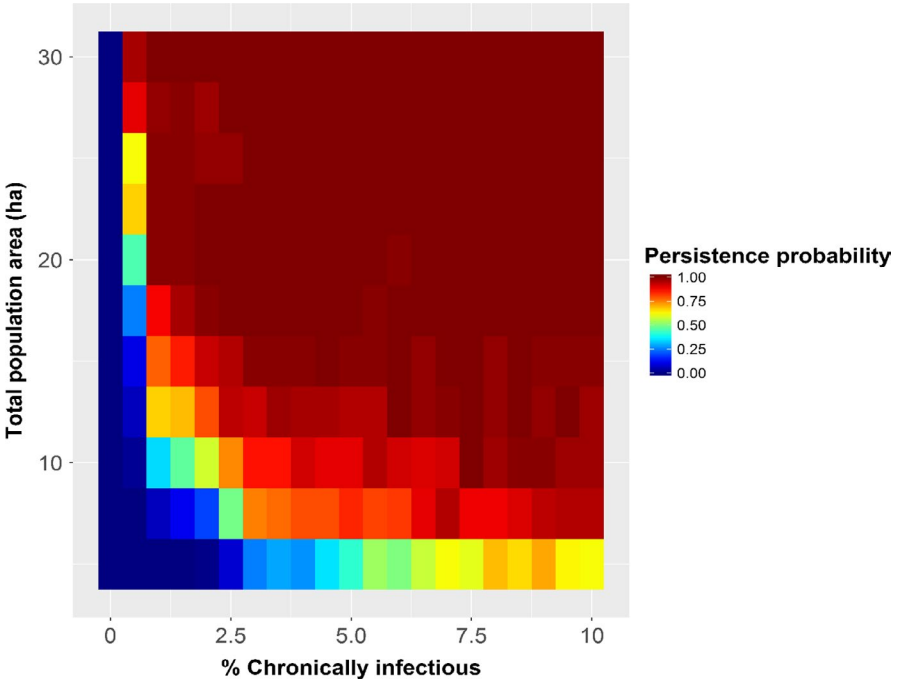

FIGURE 5 Persistence probabilities for Morogoro virus in populations of *Mastomys natalensis* as a function of the total population area (in hectares) and the proportion of animals that becomes chronically infectious in the mathematical model (model 7)

4 | 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 this is exactly what would be expected based on the transmission and demographic characteristics, past studies on similar systems have struggled to find such positive correlations (Davis et al., 2005). Begon et al. (2009) argued that this scarcity of significant correlations might be due to the short time periods of most studies (insufficient data to observe significant patterns), as in their long-term time series they did observe clear positive correlations between cowpox virus prevalence and density of field voles (*Microtus agrestis*). The results of our long-term study add to this rare evidence for the predicted positive correlations between parasite prevalence and host density.

The mathematical model suggests that the seasonal seroprevalence pattern is best explained by a density-dependent transmission component ($q = 0$). Density-dependent transmission was already expected for *M. natalensis*, as it is a non-territorial generalist species of which the contact rate is likely to increase with density (Borremans et al., 2013). We do emphasize, however, that it remains difficult to infer the exact transmission–density function based solely on serology data and that controlled field experiments are necessary for more definite answers. For example, an enclosure experiment found that contact rates of *M. natalensis* increase with density in a sigmoid pattern, suggesting that MORV transmission might be a nonlinear function of density (Borremans et al., 2016). Another enclosure experiment found that Sin Nombre hantavirus transmission increases with deer mouse (*Peromyscus maniculatus*) density and that this effect could change between seasons (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 had expected to see 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, but soon afterwards (August–November) seroprevalence increased steeply. This rapid increase was unexpected, given the high rate at which juveniles, which are presumed to be seronegative, entered the population. The short time lag between density and seroprevalence (1–4 months) suggests that transmission rates are high during this period. One possible explanation for this is that the proportion of vertically infected juveniles is high, which is supported by the mathematical models. This possibility is also supported by the relatively high seroprevalence (15%) of very young individuals (<20 g; Figure S3), suggesting high levels of vertical transmission or maternally derived antibodies. Vertical transmission and maternal antibodies are also suggested for LASV transmission in *M. natalensis* and Junin arenavirus in *Calomys musculinus*, while they have been directly observed in the laboratory for LCMV in *Mus musculus* (Demby et al., 2001; Oldstone, 2002; Vitullo & Merani, 1988). Another possibility that may explain the lack of a clear dilution effect is a seasonal change in the transmission coefficient β, which was also supported by the model simulations. 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, Larson, & Dominik, 1984).

Evidence from experimental infections and natural observations suggests that acute infections with virus shedding during a few weeks and lifelong immunity are standard among arenavirus infections in adult natural hosts (Borremans, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015; Demby et al., 2001; Fichet-Calvet et al., 2014; Fulhorst, Milazzo, Bradley, & Peppers, 2001; Gonzalez et al., 1983; Milazzo & Fulhorst, 2012). Furthermore, given that MORV transmission is not frequency-dependent, an interesting question is how the virus could survive the low-density periods of its host. The mathematical model suggests that a small proportion of chronically infectious animals (2.5%–10% of the total infected population) is likely to be crucial for virus persistence, as these individuals could maintain the transmission chain during the critical low-density periods (especially in combination with increased rates of vertical transmission). Interestingly, when monitoring wild-caught *M. natalensis* in the laboratory for 8 weeks, about 8% of MORV infected animals had a long-term infection, which is consistent with the prediction of the mathematical models. Chronic infections have also been observed after inoculation of newborn hosts in laboratory conditions and have been suggested by other field studies in natural conditions (Borremans, Sluydts, et al., 2015; Borremans, Vossen, et al., 2015; Fichet-Calvet et al., 2014; Mariën, Borremans, Gryseels, Broecke, et al., 2017; Oldstone, 2002; Walker et al., 1975). It is conceivable that some animals develop latent chronic infections, where the virus resides in organs and is reactivated under stressful conditions (Plowright et al., 2016). Viral reactivation might for example 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 vertical transmission and chronic infections, other factors that could increase the persistence probability of MORV during the low host density periods remain to be investigated. For example, indirect transmission (due to prolonged survival outside the host) has found to be important for the persistence of several rodent-borne viruses, such as Puumala hantavirus in populations of bank voles (*M. glareolus*) (Forbes, Sironen, & Plyusnin, 2018). Although survival outside the host is arguably important to explain intraspecies transmission for many arenaviruses (e.g. LASV is most likely transmitted from rodents to humans by the environment; Bonwitt et al., 2017), empirical data to support a free-living stage are rare. The only available data come from laboratory experiments where LASV was deposited on solid surfaces in the dark and aerosolized, (>50% inactivation at 60 hr) (Sagripanti, Rom, & Holland, 2010; Stephenson et al., 1984). While these data suggest low survival outside the host, the situation in the natural environment can differ substantially (e.g. UV radiation or humidity is known to severely affect the biological half-life of RNA viruses) (Lytle & Sagripanti, 2005).

Another mechanism that could in theory allow virus persistence during low-density periods is metapopulation dynamics, if connected subpopulations fluctuate asynchronously in time or space (Foley, Foley, & Pedersen, 1999; Guivier et al., 2011; Hanski, 1999). To the best of our knowledge, metapopulation dynamics have never been investigated for hosts of arenaviruses but could be examined by looking at the spatiotemporal phylodynamics of these viruses (Rambaut et al., 2009). Such a genetic study was recently performed for Puumala hantavirus in populations of bank voles in Germany, where genetic analyses suggested strong geographical structuring at a highly localized scale and where strains were observed to persist throughout multiple years, which led to the conclusion that transmission between neighbouring host populations was unlikely to contribute to the persistence of this virus (Weber de Melo et al., 2015).

Overall, the results in this study provide 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 were artificially reduced (e.g. by rodent elimination). This could then lower the force of infection to humans, as there would be fewer rodents as well as a lower prevalence within the rodent population (Davis et al., 2005). However, rodent control is unlikely to completely eliminate LASV from the rodent population because complete removal of rodents is practically impossible (Mariën, Borremans, Kourouma, et al., 2019), and the survival of only a few chronically infected animals would be sufficient for viral persistence. Given the presence of chronic carriers and the high breeding and recolonization capacity of *M. natalensis* (Mariën, Kourouma, et al., 2018; Mariën, Sluydts, et al., 2018), 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.

5 | 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 showed that density-dependent horizontal transmission 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 during periods of low host density. An interesting next step would be to investigate how climate affects MORV transmission, as effects can be expected on both the survival probability of the virus and the host. For example, an extended rainy season is known to result in larger numbers of *M. natalensis*, and drier conditions may increase indirect transmission (Sluydts et al., 2009; Stephenson et al., 1984). 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

We thank the staff (in particular Khalid Kibwana, Geofrey Sabuni and Shabani Lutea) at the Pest Management Centre in Morogoro (Tanzania) for their excellent support in the field. We are grateful to Nathalie Van Houtte of the University of Antwerp and Beate Becker-Ziaja of the Bernhard-Nocht- Institute for Tropical Medicine for laboratory technical support. We also thank the students Simon Reynaert, Bertold Mariën and Julia Port for their help with the IFA analyses.

AUTHORS' CONTRIBUTIONS

Conceived the study: J.M., B.B. and H.L. Wrote the paper: J.M. and B.B. Performed the experiments: J.M., B.B., C.V., S.Gr., J.G.-B. Performed the analyses: J.M., B.B., J.R. and L.K. Supervised field and laboratory work: J.G.-B., C.A.S., S.Gü., A.W.M. and H.L. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.0g22962 (Mariën, Borremans, Verhaeren, et al., 2019).

ORCID

Joachim Mariën https://orcid.org/0000-0001-7023-445X
Lucinda Kirkpatrick https://orcid.org/0000-0002-0161-2469
Herwig Leirs https://orcid.org/0000-0002-7612-5024

REFERENCES

Adler, F. R., Pearce-Duvet, J. M. C., & Dearing, M. D. (2008). How host population dynamics translate into time-lagged prevalence: An investigation of Sin Nombre virus in deer mice. *Bulletin of Mathematical Biology*, *70*(1), 236–252. https://doi.org/10.1007/s11538-007-9251-8

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., & Rohani, P. (2006). Seasonality and the dynamics of infectious diseases. *Ecology Letters*, *9*(4), 467–484. https://doi.org/10.1111/j.1461-0248.2005.00879.x

Anderson, R., & May, R. (1979). Population biology of infectious diseases: Part I. *Nature*, *280*, 361–367. https://doi.org/10.1038/280361a0

Anderson, R. M., & May, R. M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *291*(1054), 451–524. https://doi.org/10.1098/rstb.1981.0005

Antonovics, J., Wilson, A. J., Forbes, M. R., Hauffe, H. C., Kallio, E. R., Leggett, H. C., … Webster, J. P. (2017). The evolution of transmission mode. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1719), 20160083. https://doi.org/10.1098/rstb.2016.0083

Bagamian, K. H., Towner, J. S., Kuenzi, A. J., Douglass, R. J., Rollin, P. E., Waller, L. A., & Mills, J. N. (2012). Transmission ecology of Sin Nombre Hantavirus in naturally infected North American deermouse populations in outdoor enclosures. *PLoS ONE*, *7*(10), 1–10. https://doi.org/10.1371/journal.pone.0047731

Bartlett, M. S. (1957). Measles periodicity and community size. *Journal of Royal Statistical Society*, *120*(1), 48–70. https://doi.org/10.2307/2342553

Begon, M., Bennett, M., Bowers, R. G., French, N. P., Hazel, S. M., & Turner, J. (2002). A clarification of transmission terms in host-microparasite models: Numbers, densities and areas. *Epidemiology and Infection*, *129*(1), 147–153. https://doi.org/10.1017/S0950268802007148

Begon, M., Hazel, S. M., Telfer, S., Bown, K., Carslake, D., Cavanagh, R., … Cavanagh, R. (2003). Rodents, cowpox virus and islands: densities, numbers and thresholds. *Journal of Animal Ecology*, *72*(2), 343–355.

Begon, M., Telfer, S., Smith, M. J., Burthe, S., Paterson, S., & Lambin, X. (2009). Seasonal host dynamics drive the timing of recurrent epidemics in a wildlife population. *Proceedings of the Royal Society B-Biological Sciences*, *276*(January), 1603–1610. https://doi.org/10.1098/rspb.2008.1732

Bonwitt, J., Sáez, A. M., Lamin, J., Ansumana, R., Dawson, M., Buanie, J., … Brown, H. (2017). At home with mastomys and rattus: Human-rodent interactions and potential for primary transmission of lassa virus in domestic spaces. *American Journal of Tropical Medicine and Hygiene*, *96*(4), 935–943. https://doi.org/10.4269/ajtmh.16-0675

Borremans, B. (2014). Ammonium improves elution of fixed dried blood spots without affecting immunofluorescence assay quality. *Tropical Medicine & International Health: TM & IH*, *19*(4), 413–416. https://doi.org/10.1111/tmi.12259

Borremans, B., Hughes, N. K., Reijniers, J., Sluydts, V., Katakweba, A. A. S., Mulungu, L. S., … Leirs, H. (2013). Happily together forever: Temporal variation in spatial patterns and complete lack of territoriality in a promiscuous rodent. *Population Ecology*, *56*, 109–118. https://doi.org/10.1007/s10144-013-0393-2

Borremans, B., Leirs, H., Gryseels, S., Günther, S., Makundi, R., & Gouÿ de Bellocq, J. (2011). Presence of Mopeia virus, an African arenavirus, related to biotope and individual rodent host characteristics: Implications for virus transmission. *Vector Borne and Zoonotic Diseases*, *11*, 1125–1131. https://doi.org/10.1089/vbz.2010.0010

Borremans, B., Reijniers, J., Hens, N., & Leirs, H. (2017). The shape of the contact–density function matters when modelling parasite transmission in fluctuating populations. *Royal Society Open Science*, *4*(11), 171308. https://doi.org/10.1098/rsos.171308

Borremans, B., Reijniers, J., Hughes, N. K., Godfrey, S. S., Gryseels, S., Makundi, R. H., & Leirs, H. (2016). Nonlinear scaling of foraging contacts with rodent population density. *Oikos*, *126*(6), 792–800. https://doi.org/10.1111/oik.03623.

Borremans, B., Sluydts, V., Makundi, R. H., & Leirs, H. (2015). Evaluation of short-, mid- and long-term effects of toe clipping on a wild rodent. *Wildlife Research*, *42*(1985), 143–148.

Borremans, B., Vossen, R., Becker-Ziaja, B., Gryseels, S., Hughes, N., Van Gestel, M., … Leirs, H. (2015). Shedding dynamics of Morogoro virus, an African arenavirus closely related to Lassa virus, in its natural reservoir host *Mastomys natalensis*. *Nature Publishing Group*, *5*(1), 1–8. https://doi.org/10.1038/srep10445

Davis, S., Begon, M., Bruyn, L. D., Ageyev, V. S., Klassovskiy, N. L., Pole, S. B., … Leirs, H. (2004). Predictive thresholds for plague in Kazakhstan. *Science*, *304*(5671), 736–738.

Davis, S., Fichet-Calvet, E., & Leirs, H. (2005). Fluctuating rodent populations and risk to humans from rodent-borne zoonoses. *Vector Borne and Zoonotic Diseases*, *5*(4), 305–314. https://doi.org/10.1089/vbz.2005.5.305

Demby, A. H., Inapogui, A., Kargbo, K., Koninga, J., Kourouma, K., Kanu, J., … Bausch, D. G. (2001). Lassa fever in guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne and Zoonotic Diseases*, *1*, 283–299. https://doi.org/10.1089/153036601601600259 12

Dobson, A. P., & Meagher, M. (1996). The population dynamics of brucellosis in the Yellowstone National Park. *Ecology*, *77*(4), 1026–1036. https://doi.org/10.2307/2265573

Fichet-Calvet, E., Becker-Ziaja, B., Koivogui, L., & Günther, S. (2014). Lassa serology in natural populations of rodents and horizontal transmission. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)*, *14*(9), 665–674. https://doi.org/10.1089/vbz.2013.1484

Foley, J. E., Foley, P., & Pedersen, N. C. (1999). The persistence of a SIS disease in a metapopulation. *Journal of Applied Ecology*, *36*(4), 555–563. https://doi.org/10.1046/j.1365-2664.1999.00427.x.

Forbes, K. M., Sironen, T., & Plyusnin, A. (2018). Hantavirus maintenance and transmission in reservoir host populations. *Current Opinion in Virology*, *28*, 1–6. https://doi.org/10.1016/j.coviro.2017.09.003

Fulhorst, C. F., Milazzo, M. L., Bradley, R. D., & Peppers, L. L. (2001). Experimental infection of *Neotoma albigula* (Muridae) with Whitewater Arroyo virus (Arenaviridae). *The American Journal of Tropical Medicine and Hygiene*, *65*(2), 147–151. https://doi.org/10.4269/ajtmh.2001.65.147

Genz, A., Bretz, F., Miwa, T., Mi, X., Leisch, F., & Fabian Scheipl, T. H. (2017). *mvtnorm: Multivariate normal and t distributions*. Retrieved from http://cran.r-project.org/package=mvtnorm

Gibson, G., & Renshaw, E. (1998). Estimating parameters in stochastic compartmental models using Markov chain methods. *Mathematical Medicine and Biology*, *15*(1), 19–40. https://doi.org/10.1093/imammb/15.1.19

Gonzalez, J. P., McCormick, J. B., Saluzzo, J. F., Herve, J. P., Georges, A. J., & Johnson, K. M. (1983). An arenavirus isolated from wild-caught rodents (Praomys species) in the Central African Republic. *Intervirology*, *19*, 105–112. https://doi.org/10.15713/ins.mmj.3

Goyens, J., Reijniers, J., Borremans, B., & Leirs, H. (2013). Density thresholds for Mopeia virus invasion and persistence in its host *Mastomys natalensis*. *Journal of Theoretical Biology*, *317*, 55–61. https://doi.org/10.1016/j.jtbi.2012.09.039

Gryseels, S., Baird, S. J. E., Borremans, B., Makundi, R., & Leirs, H. & Goüy de Bellocq, J. (2017). When viruses don't go viral: The importance of host phylogeographic structure in the spatial spread of arenaviruses. *PLoS Path*, *13*(1), e1006073. https://doi.org/10.1371/journal.ppat.1006073

Guivier, E., Galan, M., Chaval, Y., Xuéreb, A., Ribas salvador, A., Poulle, M.-L., … Cosson, J. F. (2011). Landscape genetics highlights the role of bank vole metapopulation dynamics in the epidemiology of Puumala hantavirus. *Molecular Ecology*, *20*(17), 3569–3583. https://doi.org/10.1111/j.1365-294X.2011.05199.x

Günther, S., Hoofd, G., Charrel, R., Röser, C., Becker-Ziaja, B., Lloyd, G., … Leirs, H. (2009). Mopeia virus-related arenavirus in natal multimammate mice, Morogoro, Tanzania. *Emerging Infectious Diseases*, *15*(12), 2008–2012. https://doi.org/10.3201/eid1512.090864

Hanski, I. (1999). Habitat connectivity, habitat continuity, and metapopulations in dynamic landscapes. *Oikos*, *87*(2), 209. https://doi.org/10.2307/3546736

Hartley, D. M., Barker, C. M., Le Menach, A., Niu, T., Gaff, H. D., & Reisen, W. K. (2012). Effects of temperature on emergence and seasonality of West Nile virus in California. *American Journal of Tropical Medicine and Hygiene*, *86*(5), 884–894. https://doi.org/10.4269/ajtmh.2012.11-0342

Hudson, P. J., Rizzoli, A., Grenfell, B., Heesterbeek, H., & Dobson, A. P. (2002). In P. J. Hudson (Ed.), *The ecology of wildlife disease*. New York: Oxford University Press.

Kennis, J., Sluydts, V., Leirs, H., & van Hooft, W. F. P. (2008). Polyandry and polygyny in an African rodent pest species, *Mastomys natalensis*. *Mammalia*, *72*, 150–160. https://doi.org/10.1515/MAMM.2008.025

Krebs, C. J. (2013). *Population Fluctuations in Rodents* (pp. 306). Chicago: The University of Chicago Press.

Leirs, H. (1994). *Population ecology of* Mastomys natalensis *(Smith, 1834). Implications for rodent control in Africa*. Brussels: A. Belgian Administration for Development Cooperation.

Lloyd-Smith, J. O., Cross, P. C., Briggs, C. J., Daugherty, M., Getz, W. M., Latto, J., … Swei, A. (2005). Should we expect population thresholds for wildlife disease? *Trends in Ecology and Evolution*, *20*(9), 511–519. https://doi.org/10.1016/j.tree.2005.07.004

Lytle, C. D., & Sagripanti, J.-L. (2005). Predicted inactivation of viruses of relevance to biodefense by solar radiation. *Journal of Virology*, *79*(22), 14244–14252. https://doi.org/10.1128/JVI.79.22.14244-14252.2005

Mariën, J., Borremans, B., Gryseels, S., Broecke, B. V., Becker-Ziaja, B., Makundi, R., … Leirs, H. (2017). Arenavirus dynamics in experimentally and naturally infected rodents. *EcoHealth*, *14*(3), 463–473. https://doi.org/10.1007/s10393-017-1256-7

Mariën, J., Borremans, B., Gryseels, S., Soropogui, B., De Bruyn, L., Bongo, G. N., … Fichet-Calvet, E. (2017). No measurable adverse effects of Lassa, Morogoro and Gairo arenaviruses on their rodent reservoir host in natural conditions. *Parasites & Vectors*, *10*(1), 210. https://doi.org/10.1186/s13071-017-2146-0

Mariën, J., Borremans, B., Kourouma, F., Baforday, J., Rieger, T., Günther, S., … Fichet-Calvet, E. (2019). Evaluation of rodent control to fight Lassa fever based on field data and mathematical modelling. *Emerging Microbes & Infections*, *8*(1), 640–649. https://doi.org/10.1080/22221751.2019.1605846

Mariën, J., Borremans, B., Verhaeren, C., Lucinda, K., & Sophie, G., Goüy de Bellocq, J., … Leirs, H. (2019). Data from: Density dependence and persistence of *Morogoro arenavirus* transmission in a fluctuating population of its reservoir host. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.0g22962

Mariën, J., Kourouma, F., Magassouba, N., Leirs, H., & Fichet-Calvet, E. (2018). Movement patterns of small rodents in Lassa fever-endemic villages in Guinea. *EcoHealth*, *15*(2), 348–359. https://doi.org/10.1007/s10393-018-1331-8

Mariën, J., Sluydts, V., Borremans, B., Gryseels, S., Vanden Broecke, B., Sabuni, C. A., … Leirs, H. (2018). Arenavirus infection correlates with lower survival of its natural rodent host in a long-term capture-mark-recapture study. *Parasites & Vectors*, *11*(1), 90. https://doi.org/10.1186/s13071-018-2674-2

Milazzo, M. L., & Fulhorst, C. F. (2012). Duration of catarina virus infection in the southern plains woodrat (*Neotoma micropus*). *Vector-Borne and Zoonotic Diseases*, *12*(4), 321–324. https://doi.org/10.1089/vbz.2011.0852

Mills, J. N., Ksiazek, T. G., Peters, C. J., & Childs, J. E. (1999). Long-term studies of hantavirus reservoir populations in the southwestern United States: A synthesis. *Emerging Infectious Diseases*, *5*(1), 135–142. https://doi.org/10.3201/eid0501.990116

Monath, T. P. (1987). Lassa fever: New issues raised by field studies in West Africa. *Journal of Infectious Diseases*, *155*, 433–436. https://doi.org/10.1093/infdis/155.3.433

Morters, M. K., Restif, O., Hampson, K., Cleaveland, S., Wood, J. L. N., & Conlan, A. J. K. (2012). Evidence-based control of canine rabies: A critical review of population density reduction. *Journal of Animal Ecology*, *82*(1), 6–14. https://doi.org/10.1111/j.1365-2656.2012.02033.x

Myers, J. H. (2018). Population cycles: generalities, exceptions and remaining mysteries. *Proceedings of the Royal Society B*, *285*. http://doi.org/10.1098/rspb.2017.2841

Oldstone, M. (2002). Biology and pathogenesis of lymphocytic choriomeningitis virus infection. In A. Clarke (Ed.). Berlin, Heidelberg, Germany: Springer-Verlag.

Peel, A. J., Pulliam, J. R. C., Luis, A. D., Plowright, R. K., Shea, T. J. O., Hayman, D. T. S., … Restif, O. (2014). The effect of seasonal birth pulses on pathogen persistence in wild mammal populations. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1786), 20132962. https://doi.org/10.1098/rspb.2013.2962

Plowright, R. K., Peel, A. J., Streicker, D. G., Gilbert, A. T., McCallum, H., Wood, J., … Restif, O. (2016). Transmission or within-host dynamics driving pulses of zoonotic viruses in reservoir-host populations. *PLoS*

Neglected Tropical Diseases, *10*(8), 1–21. https://doi.org/10.1371/journal.pntd.0004796

Rambaut, A., Pybus, O. G., Nelson, M. I., Viboud, C., Jeffery, K., & Holmes, E. C. (2009). The genomic andepidemiological dynamics of human influenza A virus. *Nature*, *453*, 615–619.

Ryder, J. J., Miller, M. R., White, A., Knell, R. J., & Boots, M. (2007). Host-parasite population dynamics under combined frequency- and density-dependent transmission. *Oikos*, *116*(12), 2017–2026. https://doi.org/10.1111/j.2007.0030-1299.15863.x

Sagripanti, J. L., Rom, A. M., & Holland, L. E. (2010). Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. *Archives of Virology*, *155*(12), 2035–2039. https://doi.org/10.1007/s00705-010-0791-0

Sakamoto, Y., Ishiguro, M., & Kitagawa, G. (1986). *Akaike information criterion statistics*. Tokyo, Japan: KTK Scientific Publishers.

Sikes, R. S., & Gannon, W. L. (2007). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, *88*(3), 809–823. https://doi.org/10.1644/10-MAMM-F-355.1

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 Protection*, *28*(8), 647–654. https://doi.org/10.1016/j.cropro.2009.03.018

Smith, M. J., Telfer, S., Kallio, E. R., Burthe, S., Cook, A. R., Lambin, X., & Begon, M. (2009). Host-pathogen time series data in wildlife support a transmission function between density and frequency dependence. *Proceedings of the National Academy of Sciences*, *106*(19), 7905–7909. https://doi.org/10.1073/pnas.0809145106

Stenseth, N. C., Mysterud, A., Ottersen, G., Hurrel, J. W., Chan, K.-S., & Lima, M. (2002). Ecological effects of climate fluctuations. *Ecology and Climatology*, *297*(August), 1292–1296. https://doi.org/10.1126/science.1071281

Stephenson, E., Larson, E., & Dominik, J. W. (1984). Effect of environmental factors on aerosol-induced Lassa virus infection. *Journal of Medical Virology*, *14*, 295–303. https://doi.org/10.1002/jmv.1890140402

Vieth, S., Drosten, C., Lenz, O., Vincent, M., Omilabu, S., Hass, M., … Günther, S. (2007). RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *101*, 1253–1264. https://doi.org/10.1016/j.trstmh.2005.03.018

Vitullo, A., & Merani, M. (1988). Is vertical transmission sufficient to maintain Junin virus in nature? *The Journal of General Virology*, *69*, 1437–1440. https://doi.org/10.1099/0022-1317-69-6-1437

Voutilainen, L., Kallio, E. R., Niemimaa, J., Vapalahti, O., & Henttonen, H. (2016). Temporal dynamics of Puumala hantavirus infection in cyclic populations of bank voles. *Scientific Reports*, *6*(1), 1–14. https://doi.org/10.1038/srep21323

Walker, D. H., Wulff, H., Lange, J. V., & Murphy, F. A. (1975). Comparative pathology of Lassa virus infection in monkeys, guinea-pigs, and *Mastomys natalensis*. *Bulletin of the World Health Organization*, *52*, 523–534.

Weber de Melo, V., Sheikh Ali, H., Freise, J., Kühnert, D., Essbauer, S., Mertens, M., … Heckel, G. (2015). Spatiotemporal dynamics of Puumala hantavirus associated with its rodent host, Myodes glareolus. *Evol Appl*, *8*, 545–559. https://doi.org/ 10.1111/eva.12263

Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer-Verlag.

Wickham, H. (2017). *scales: Scale functions for visualization*. Retrieved from https://cran.r-project.org/package=scales

Wood, S., & Scheipl, F. (2017). *gamm4: Generalized additive mixed models using "mgcv" and "lme4"*, 4.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Mariën J, Borremans B, Verhaeren C, et al. Density dependence and persistence of Morogoro arenavirus transmission in a fluctuating population of its reservoir host. *J Anim Ecol*. 2019;00:1–13. https://doi.org/10.1111/1365-2656.13107