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Original Contribution

Arenavirus Dynamics in Experimentally and Naturally Infected Rodents

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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.

Keywords: Arenavirus, Morogoro virus, *Mastomys natalensis*, Challenge experiment, Multimammate mice, Time of infection

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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 dynam-

ics, 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; Hardestam et al. 2008; Carrat 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 and Beran 1992; Schmid-Hempel and 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. 2015a). 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. 2015a). 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 100 m by 100 m 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 10-m 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 ($\pm$15 µL/punch) (Borremans 2014). Animals were indi-

vidually marked using toe clipping (Borremans et al. 2015b), 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 twofold dilution series, starting with a minimum dilution of 1:10 (Borremans et al. 2015a). 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 recaptured 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 20 g (=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 2016) 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 confidence 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

Table 1. Number of *M. natalensis* Recaptured During Multiple Trapping Sessions.

Times captured	Total	Number of individuals			
		Antibody and MORV RNA negative	Antibody positive[a]	MORV RNA positive[b]	Antibody and 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.

those resulting from random data, where observation data (Ab, vRNA) are randomized 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, and 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 × two weeks and one × 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 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% confidence 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).

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

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 and 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.
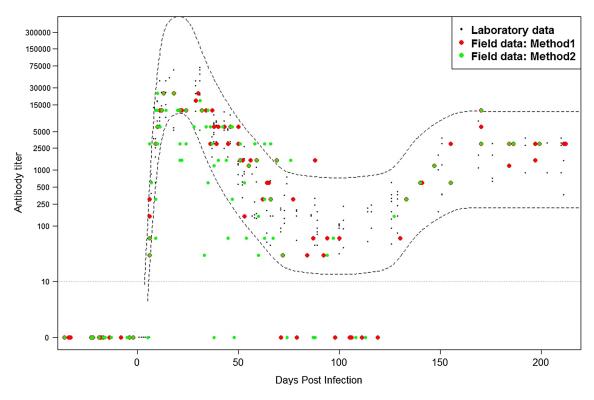

Figure 1. Temporal Ab dynamics of naturally and experimentally MORV-infected *M. natalensis*. Small black dots represent Ab levels from experimentally infected rodents in the laboratory [data derived from (Borremans et al. 2015a)], and *red* and *green dots* represent Ab titers from naturally infected 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 (Color figure online).
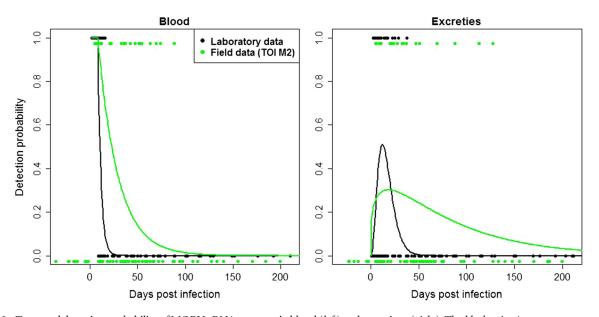
Figure 2. Temporal detection probability of MORV vRNA presence in blood (*left*) and excretions (*right*). The *black points/curve* represent raw data (*points*) and the proportion of samples (*curve*) that were vRNA-positive in laboratory conditions [i.e. vRNA presence probability; data derived from (Borremans et al. 2015a)]. 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 (Color figure online).

Abs (Fig. S29), and overall lower Ab titers in naturally infected animals (Fig. S1).

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 randomized data were much too likely. Results of method 3 were therefore not further discussed in this paper.

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 randomized 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 and Fenner 1949; Weigand and Hotchin 1961). However, when analysed later using more sensitive im-
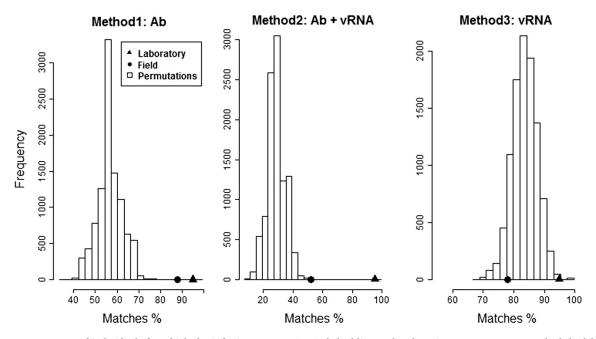
Figure. 3. Percentages of individuals for which the infection patterns in viral shedding and/or host immune response matched the laboratory data. Random permutations of field data (*bars*) were compared with real field data (*dots*) and laboratory data (triangles, by default 95%) for the three different TOI methods: Ab titers (method 1), Ab titers and vRNA in blood and excretions (method 2), and vRNA in blood and excretions (method 3).

mune assays, Abs did appear to be present, but at low titers (Oldstone and 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. 2015a).

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 and 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 (Figs. 1, 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 is most convincingly shown 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-week 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. 2015a; 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 and 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 19 g) 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 20 g). Here, maternal Abs might explain the presence and rapid decrease in 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- and 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 titers 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.

ACKNOWLEDGEMENTS

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST The authors declare no conflict of interest.

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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