Lassa Serology in Natural Populations of Rodents and Horizontal Transmission

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Abstract

Lassa virus causes hemorrhagic fever in West Africa. Previously, we demonstrated by PCR screening that only the multimammate mouse, *Mastomys natalensis*, hosts Lassa virus in Guinea. In the present study, we used the same specimen collection from 17 villages in Coastal, Upper, and Forest Guinea to investigate the Lassa virus serology in the rodent population. The aim was to determine the dynamics of antibody development in *M. natalensis* and to detect potential spillover infections in other rodent species. Immunoglobulin G (IgG) antibody screening was performed using the indirect immunofluorescence assay with the Guinean Lassa virus strain Bantou 289 as antigen. The overall seroprevalence was 8% (129/1551) with the following rodents testing positive: 109 *M. natalensis*, seven *Mastomys erythroleucus*, four *Lemniscomys striatus*, four *Praomys daltoni*, three *Mus minutoides*, and two *Praomys rostratus*. Nearly all of them (122/129) originated from Bantou, Tanganya, and Gbetaya, where Lassa virus is highly endemic in *M. natalensis*. The antibody seroprevalence in *M. natalensis* from this high-endemic area (27%; 108/396) depended on the village, habitat, host age, and host abundance. A main positive factor was age; the maximum seroprevalence reached 50% in older animals. Our data fit with a model implicating that most *M. natalensis* rodents become horizontally infected, clear the virus within a period significantly shorter than their life span, and develop antibodies. In addition, the detection of antibodies in other species trapped in the habitats of *M. natalensis* suggests spillover infections.

Key Words: Lassa virus—Antibodies—IgG—Rodents—*Mastomys*—*Praomys*—*Mus*—*Lemniscomys*—Host age—Horizontal transmission—Tropics—West Africa.

Introduction

LASSA FEVER IS A VIRAL HEMORRHAGIC fever caused by Lassa virus (LASV, family Arenaviridae). It was first discovered in 1969 in Nigeria (Buckley et al. 1970, Frame et al. 1970). The disease is endemic in the West African countries of Sierra Leone, Liberia, Guinea, and Nigeria (Fichet-Calvet and Rogers 2009). Human case numbers are estimated to reach 300,000 per year with up to 5000 deaths (McCormick et al. 1987b, McCormick 1999). In Guinea, an epidemiological survey in humans showed the highest seroprevalence in the south, with up to 40% near the border with Sierra Leone (Lukashevich et al. 1993). Acute cases were also recorded in regional hospitals (Bausch et al. 2001).

A spatial survey of small mammals confirmed the multimammate mouse *Mastomys natalensis* as the only reservoir host of LASV in Guinea (Monath et al. 1974, Lecompte et al. 2006). A 2-year longitudinal survey on *M. natalensis* in

Guinea revealed that the reservoir host is more abundant inside than outside of houses, especially during the dry season (Fichet-Calvet et al. 2007). This could explain the higher risk of transmission to humans during this season (McCormick et al. 1987a, Bausch et al. 2001). However, LASV prevalence in the rodent population was two to three times higher in the rainy season (Fichet-Calvet et al. 2007), which could be a consequence of an improved survival of the virus outside the host under wet and relatively cold conditions (Fichet-Calvet and Rogers 2009). This hypothesis implies horizontal transmission by indirect contact with contaminated surfaces. Direct horizontal transmission between *M. natalensis* could also be higher during the rainy season, when males and females are more active in patrolling their home range for mating or breeding. Despite a continuous reproduction in commensal *M. natalensis*, a complete turnover of the population has been observed between the beginning and the end of the rainy season (Fichet-Calvet et al. 2008). This suggests

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a seasonal pattern of fecundity, which could possibly increase contact rates between animals.

Experimental infections done by Walker et al. in 1975 using *M. natalensis* (there is some uncertainty on whether *M. natalensis* or *M. coucha* have been used in the laboratories at this time; Kruppa et al. 1990) show that LASV establishes a chronic infection in neonatal animals and a transient infection in adults. Thus, horizontal transmission of LASV is possible. Horizontal transmission of arenaviruses between rodents has been demonstrated experimentally, for example, in mice infected by lymphocytic choriomeningitis virus (Traub 1936), or in *Calomys callosus* infected with Machupo virus (Webb et al. 1975). Under natural conditions, horizontal transmission appears to occur with Junin virus among *C. musculus* (Mills et al. 1992, 1994) and with Morogoro virus among *M. natalensis* (Borremans et al. 2011).

This study aimed at investigating the possible modes of LASV transmission by determining LASV specific in natural populations of *M. natalensis* living in high- and low-endemic zones for LASV. We also examined the serology in other species living in sympatry with *M. natalensis*. In the previous PCR-based study performed in 2002–2005, we did not find LASV-positive species except for *M. natalensis* (Lecompte et al. 2006). However, it is possible that the virus circulated in other species only very transiently, which may be detectable by serology. Finally, we examined the dynamics of LASV infection in *M. natalensis* by investigating the influence of various parameters, such as locality, habitat, season, host sex, age, and abundance on the seroprevalence.

Materials and Methods

Study sites and small mammal sampling

Small mammals were sampled from October, 2002, to October, 2004, in 17 villages located in different vegetation zones in Guinea and Mali (Fig. 1). Trapping was performed using a standardized protocol in houses, cultivations, and forest (see Fichet-Calvet et al. 2007, 2009a,b for a more detailed description of the habitats). After trapping, the animals were necropsied in the field and identified morphologically (weight and body length). Blood, spleen, liver, and biopsies from other organs were collected. The collection was stored at -80°C at the Philipps University in Marburg, Germany. Blood has been used for arenavirus PCR testing (results published in Lecompte et al. 2006).

The rodent species was identified molecularly by sequencing the cytochrome *b* gene. From the field data, demographic parameters were estimated for *M. natalensis*: The abundance as measured by the trapping success for 3 days ($TS = 100 \times n_{\text{trapped individuals}}/n_{\text{traps}}$), as well as fecundity and fertility for both sexes. The age was measured by using eye lens weight (ELW; note that ELW in our study is the weight of both lenses), which was shown to be the best indirect measure of age for mammals (Lord 1959, Martinet 1966; for review see, Morris 1971). Leirs (1994) correlated age with ELW for *M. natalensis* bred in Tanzania as follows: $a = e^{(10.46088 + w)/4.35076}$, where a is age in days, and w is the weight of one lens in milligrams. We used this correlation to estimate the age of *M. natalensis*. However, as the confidence interval (CI) for the age becomes very large at higher ELW values (>100 days), and due to the fact that the formula is derived from a *M. natalensis* population far from our study

FIG. 1. Trapping sites in Guinea and Mali. The 17 villages in which small mammals were trapped from October, 2002, to October, 2004, are marked on the map. The specimens from these sites were tested serologically in the present study. Geographical coordinates: Bamba ($10^{\circ}00'02''\text{N}$; $13^{\circ}53'06''\text{W}$), Bantou ($10^{\circ}03'3''\text{N}$; $10^{\circ}35'14''\text{W}$), Bhoita ($08^{\circ}05'13''\text{N}$; $08^{\circ}54'50''\text{W}$), Franfina ($09^{\circ}38'43''\text{N}$; $08^{\circ}56'44''\text{W}$), Gagai ($11^{\circ}05'1''\text{N}$; $12^{\circ}17'51''\text{W}$), Gania ($10^{\circ}03'58''\text{N}$; $12^{\circ}32'27''\text{W}$), Gbetaya ($09^{\circ}50'27''\text{N}$; $11^{\circ}02'24''\text{W}$), Kaali ($09^{\circ}55'39''\text{N}$; $12^{\circ}42'13''\text{W}$), Khoneya ($10^{\circ}08'53''\text{N}$; $12^{\circ}40'33''\text{W}$), Kodoko ($10^{\circ}29'45''\text{N}$; $09^{\circ}01'04''\text{W}$), Macenta ($08^{\circ}33'46''\text{N}$; $09^{\circ}29'20''\text{W}$), Maikou ($09^{\circ}02'14''\text{N}$; $09^{\circ}01'29''\text{W}$), Massakoroma ($12^{\circ}17'58''\text{N}$; $08^{\circ}46'29''\text{W}$), Sangassou ($08^{\circ}36'49''\text{N}$; $09^{\circ}28'27''\text{W}$), Saourou ($11^{\circ}30'42''\text{N}$; $09^{\circ}00'36''\text{W}$), Tanganya ($10^{\circ}00'02''\text{N}$; $10^{\circ}58'22''\text{W}$), Yafra ($10^{\circ}00'43''\text{N}$; $13^{\circ}40'44''\text{W}$).

site (6000 km), we converted ELW categories to age categories only after statistical analysis. The rainy season was defined as the period from May to October, and the dry season as the period from November to April (for detailed explanations of the different variables, see Fichet-Calvet et al. 2007, 2008).

Serology

Vero cells infected with LASV strain Bantou 289 were spread on immunofluorescence slides, air dried, and fixed with acetone (Wulff and Lange 1975). Two types of slides were produced: (1) Regular slides with infected cells on the two rows, and (2) mixed slides with infected cells on the top row and uninfected cells on the bottom row. Doubtful signals were reanalyzed with mixed slides, with the serum being evaluated simultaneously on both infected and uninfected cells. The serum was diluted 1:20 in phosphate-buffered saline (PBS). If the blood had been fully used for the previous PCR testing (Lecompte et al. 2006), spleen or liver was vigorously washed in $100\ \mu\text{L}$ of PBS and the washing fluid was used for the assay. The diluted serum or organ fluid was incubated with the cells, and bound immunoglobulin G (IgG) was detected with anti-mouse IgG fluorescein isothiocyanate (FITC; Jackson ImmunoResearch). Signals were evaluated with a fluorescence microscope, and the signal intensity was semiquantitatively graded as weak (1), medium (2), or strong (3). The initial testing was performed twice on regular slides on which positive and negative controls were included. The positive control was an anti-LASV nucleoprotein

monoclonal antibody 2F1 (Hufert et al. 1989). All specimens with only one intensity unit in both initial tests, as well as specimens from non-*M. natalensis* species, were retested with mixed slides. Animals were considered positive when the signal was two or three intensity units in the two initial tests (regular slides), or one intensity unit in all three tests (regular plus mixed slides).

Data analysis

In the area with high LASV endemicity in *M. natalensis* as previously determined by PCR screening (Bantou, Gbetaya, or Tanganya), the influence of various variables on the LASV serostatus was analyzed. To this end, a multiple logistic regression with a binary outcome (LASV IgG-positive = 1, LASV IgG-negative = 0). The following independent variables were performed: Village (Bantou, Gbetaya, or Tanganya), habitat (houses, proximal cultivations, or distal cultivations), season (dry season, early, or late rainy season), sex, ELW as surrogate for age, and TS as surrogate for host abundance. Logistic regression analysis was performed through a generalized linear mixed model with logit link function and binomial residual distribution by using R software (CRAN project). The model included the "village" as a random effect, because our primary interest is to test the effects of habitat, season, sex, age, and abundance across different villages, in which local geographical components can influence the prevalence.

Modeling infection transmission

An *M. natalensis* cohort was simulated by a 20 × 20 matrix with 20 age classes from birth to the age of 600 days for 20 animals using Excel software (Microsoft). Infection of an animal by LASV, clearance of the virus, and presence of antibodies were simulated for each animal. The prevalence of vertical transmission in the model was assumed to be 5%; we assumed that another 5% of the offspring are infected shortly after birth, most likely by their mothers as well. Both sum up to 10%, which corresponds to the virus prevalence in pregnant females (Fichet-Calvet et al. 2008). However, because there are no field or experimental data indicating whether vertical transmission occurs at all, vertical transmission and horizontal transmission in the youngest age class are interchangeable without any effects on the model outcome.

The model assumes that animals having maternal antibodies do not become infected early in life for two reasons. First, offspring born to seropositive mothers cannot be infected by their mothers (which are supposedly the source of transmission for animals infected *in utero* or as neonates). Second, experiments in non-human primates demonstrate therapeutic efficacy of convalescent serum containing neutralizing antibodies (Jahrling and Peters 1984). Thus, assuming that *M. natalensis* develops neutralizing antibodies following infection, it is conceivable that the offspring born to these mothers are protected. The model parameters (prevalence of maternal antibodies, incidence of infection, and duration of viremia) were optimized empirically until the prevalences for virus and antibody per ELW class obtained from the simulation best reflected the real data. Experimental data for antibody prevalence are from this study, and data for LASV prevalence in the same locality are from Fichet-Calvet

et al. (2008). Age classes were transformed to ELW classes according to Leirs (1994).

Results

Validation of serological analysis from tissue fluids

To increase the number of animals in the analysis, we tested if the residual serum in the organs could be used for serology. To validate the procedure, 10 spleens and 10 livers from 10 *M. natalensis* that tested positive for LASV IgG in blood were tested in parallel with the organ wash fluid. All organ fluids tested positive, indicating a technical sensitivity of 100% (95% CI 72–100%) of the organ technique compared to the standard technique. Therefore, the method was used to test the serostatus of the animals with missing blood specimens. In total, 1551 animals were tested in this study, of which 1037 were tested with blood, 496 were tested with spleen fluid, and 18 were tested with liver fluid. The organ testing revealed 26 seropositive animals. The difference of seropositivity between organs (5.1%, 26/514) and serum (9.9%, 103/1037) testing is statistically different ($\chi^2 = 10.99$, $p < 0.05$).

Serology in the small mammal community

Of the 1551 animals, 129 tested positive for IgG antibodies against LASV: 109 *M. natalensis*, seven *Mastomys erythroleucus*, four *Lemniscomys striatus*, four *Praomys daltoni*, three *Mus minutoides*, and two *Praomys rostratus* (Table 1). They were mainly found in the three villages Bantou, Gbetaya, and Tanganya (122/129, 94%, 95% CI 89–97%), where a high level of LASV infection in *M. natalensis* has been previously found by PCR testing. Seropositive non-*M. natalensis* species were also more frequent in these three villages (LASV high-endemic villages in Table 2), compared to the villages Bamba, Gania, and Kaali at the coast of Guinea (LASV low-endemic villages in Table 2). At the village level, most of the seropositive non-*M. natalensis* rodents (14/20, 70%, 95% CI 48–85%) were trapped in habitats near houses, such as cultivations or fallow lands (Table 3). In Bantou and Tanganya, four of them were trapped together with a LASV-positive *M. natalensis* on the same line. The other ones were trapped at a distance between 50 and 250 meters from a LASV-positive *M. natalensis* (Table 3).

Serology in *M. natalensis*

The overall seroprevalence in *M. natalensis* was 18% (109/596, 95% CI 15–21%). With one exception, all seropositive animals were found in the LASV high-endemic villages. The remaining seropositive *M. natalensis* was found in Saourou in Upper Guinea, close to Mali. The prevalence in the high-endemic villages was 27% (108/396, 95% CI 23–32%), specifically 21% (43/201, 95% CI 16–28%) in Bantou, 12% (5/40, 95% CI 5–27%) in Gbetaya, and 39% (60/155, 95% CI 41–47%) in Tanganya. Eleven of the 109 seropositive animals were also positive by PCR (10%, 95% CI 6–18%). They were distributed in each age class, including four very young with an ELW of 10.1, 10.6, 10.8, and 11.1 mg, which correspond to 35–40 days.

Statistical analysis was performed with the data from the 396 *M. natalensis* from the LASV high-endemic villages Bantou, Gbetaya, and Tanganya. The logistic regression

TABLE 1. DETECTION OF LASV-SPECIFIC OR CROSS-REACTING IMMUNOGLOBULIN G ANTIBODIES IN SMALL MAMMALS FROM GUINEA AND MALI BY IMMUNOFLOUORESCENCE ASSAY (POSITIVE/RESTED)

Species	Village ^a																	Total
	Bam	Ban	Bho	Fra	Gag	Gan	Gbe	Kaa	Kho	Kod	Mac	Mai	Mas	San	Sao	Tan	Yaf	
<i>Arvicanthis ansorgei</i>		0/1											0/1					0/2
<i>Cricetomys gambianus</i>					0/1												0/1	0/2
<i>Crocidura buettikoferi</i>			0/1	0/2		0/2			0/4	0/1	0/4			0/4		0/2		0/20
<i>Crocidura crossei</i>		0/1														0/1		0/2
<i>Crocidura lamottei</i>		0/2														0/2		0/4
<i>Crocidura olivieri</i>			0/2		0/3	0/4												0/9
<i>Crocidura theresae</i>		0/2													0/1	0/1		0/3
<i>Gerbilliscus guineae</i>		0/5		0/2		0/10								0/2	0/2	0/2		0/21
<i>Hylomyscus sinus</i>						0/1								0/4				0/5
<i>Lemniscomys bellieri/zebra</i>									0/2				0/8					0/10
<i>Lemniscomys linulus</i>		0/2														0/3		0/5
<i>Lemniscomys striatus</i>	1/2	2/18		0/1		1/5		0/1								0/5		4/32
<i>Lophuromys sikapusi</i>			0/8	0/8		0/20		0/1			0/7	0/2		0/3				0/49
<i>Mastomys erythroleucus</i>	1/34	2/26			0/4	1/105	0/1	2/36	0/4	0/1			0/20		0/4	1/14	0/2	7/251
<i>Mastomys natalensis</i>		43/201	0/24	0/70	0/11		5/40		0/6	0/9			0/39	0/14	1/33	60/155		109/596
<i>Mus musculus</i>																		0/6
<i>Mus baoulei</i>									0/1							0/2		0/3
<i>Mus mattheyi</i>		0/44			0/2	0/7	0/1									0/39		0/93
<i>Mus minutoides</i>		1/14		0/2	0/2	0/5	0/8		0/1	0/1						2/8		3/40
<i>Mus musculoides</i>									0/5									0/5
<i>Mus setulosus</i>			0/8	0/4			0/4			0/5	0/1			0/6				0/28
<i>Praomys daltoni</i>		1/40			0/11	0/1		0/5		0/1		0/1	0/9		0/17	3/27		4/112
<i>Praomys rostratus</i>	0/1	2/32	0/7	0/17	0/9	0/20	0/8		0/4	0/10		0/9	0/17	0/11		0/18		2/163
<i>Rattus rattus</i>	0/20				0/23			0/5									0/24	0/72
<i>Taterillus gracilis</i>													0/5					0/5
<i>Uranomys ruddi</i>						0/9										0/4		0/13
Total	2/57	51/388	0/50	0/106	0/43	2/212	5/62	2/48	0/14	0/34	0/13	0/17	0/99	0/42	1/56	66/283	0/27	129/1551

^aSpecimens originated from the villages Bamba (Bam), Bantou (Ban), Bhoita (Bho), Franfina (Fra), Gagat (Gag), Gania (Gan), Gbetaya (Gbe), Kaali (Kaa), Khoneya (Kho), Kodoko (Kod), Macenta (Mac), Maikou (Mai), Massakoroma (Mas), Sangassou (San), Saourou (Sao), Tanganya (Tan), Yafraya (Yaf).

LASV, Lassa virus.

TABLE 2. LASV SEROPREVALENCE IN NON-*MASTOMYS NATALENSIS* SPECIES IN VILLAGES WITH HIGH AND LOW ENDEMICITY OF LASV IN *M. NATALENSIS*

Species	LASV high-endemic villages	LASV low-endemic villages	Two-tailed Fisher exact test
<i>M. minutoides</i>	10% (3/30)	0% (0/10)	NS
<i>L. striatus</i>	9% (2/23)	22% (2/9)	NS
<i>P. daltoni</i>	6% (4/67)	0% (0/45)	NS
<i>M. erythroleucus</i>	7% (3/41)	2% (4/210)	NS
<i>P. rostratus</i>	3% (2/58)	0% (0/105)	NS
Total	6.4% (14/219)	1.6% (6/379)	$p=0.004$

LASV, Lassa virus; NS, not significant; p , p value of the statistical test.

analysis identified several variables that are statistically significantly associated with the serostatus: Village ($p < 0.05$, $\chi^2 = 14.7$, degrees of freedom [df] = 2), habitat ($p < 0.05$, $\chi^2 = 7.0$, df = 2), and age ($p < 0.05$, $\chi^2 = 30.1$, df = 1). Locality Tanganya (odds ratio [OR] = 1.72, 95% CI 1.63–1.82), proximal cultivation (OR = 2.18, 95% CI 1.00–3.82), and high age (OR = 1.11, $p < 0.05$) correlated with a significant higher serostatus. Season ($p > 0.05$, $\chi^2 = 2.152$, df = 2), sex ($p > 0.05$, $\chi^2 = 0.241$, df = 1), and TS ($p > 0.05$, $\chi^2 = 1.902$, df = 1) had no effect. The TS was entered in a second logistic model without habitat because of a strong correlation between these two variables. The seroprevalence in Tanganya ranged from 26% to 41%, depending on the season, whereas in Bantou, it ranged from 7% to 30%, and was 12% in Gbetaya (Fig. 2). This antibody prevalence was combined with the LASV RNA prevalence (63/396, 16%, 95% CI 13–20%) to estimate the overall infection rate in the rodent

TABLE 3. DATA ON LAND USE, TRAPPING LINE, THE NUMBER OF LASSA VIRUS-POSITIVE *M. NATALENSIS* CAUGHT ON THE NEAREST LINE (*M. NAT. LASV*⁺), DISTANCE TO THE NEAREST LASV-POSITIVE *M. NATALENSIS* (METERS TO NEAREST), DISTANCE TO HOUSE (METERS TO HOUSES), AND TRAPPING SEASON FOR THE 20 SEROPOSITIVE NON-*M. NATALENSIS* SPECIES

Region	Village	Species	Land use	Trapping line	<i>M. nat.</i> LASV+	meters to nearest	meters to houses	Session
Boffa	Bamba	<i>Lemniscomys striatus</i>	Dist. savanah	Grassland savannah				Oct 2002
		<i>Mastomys erythroleucus</i>	Dist. savanah	Grassland savannah/forest				Oct 2002
Kindia	Gania	<i>Lemniscomys striatus</i>	Prox. cultiv.	Fallowland				May 2004
		<i>Mastomys erythroleucus</i>	Prox. cultiv.	Fallowland				May 2004
	Kaali	<i>Mastomys erythroleucus</i>	Prox. cultiv.	Fallowland				Oct 2003
		<i>Mastomys erythroleucus</i>	Prox. cultiv.	Fallowland				Oct 2003
Faranah	Bantou	<i>Mastomys erythroleucus</i>	Prox. cultiv.	Maize (line 18)	2 (line 18)	0	150	Oct 2003
		<i>Mastomys erythroleucus</i>	Prox. cultiv.	Fallowland (line 06)	1 (line 05)	50–100	70	May 2004
		<i>Mus minutoides</i>	Dist. cultiv.	Sweet potatoes (line 05)	1 (line 02)	150–200	800	May 2003
		<i>Praomys daltoni</i>	Houses	Houses (line 01)	7 (line 01)	0	0	Oct 2004
		<i>Praomys rostratus</i>	Prox. cultiv.	Maize (line 11)	3 (line 10)	50–100	50	Oct 2003
		<i>Praomys rostratus</i>	Prox. cultiv.	Maize (line 12)	1 (line 13)	50–100	50	Oct 2003
		<i>Lemniscomys striatus</i>	Prox. cultiv.	Maize (line 13)	1 (line 13)	0	50	Oct 2003
		<i>Lemniscomys striatus</i>	Prox. cultiv.	Maize (line 11)	1 (line 10)	50–100	50	Oct 2004
	Tanganya	<i>Mastomys erythroleucus</i>	Dist. cultiv.	Cassava (line 08)	1 (line 09)	150–200	400	Jan 2004
		<i>Mus minutoides</i>	Prox. cultiv.	Marsh/fallowland (line 04)	4 (line 01)	70	70	Jan 2004
		<i>Mus minutoides</i>	Prox. cultiv.	Marsh/forest (line 05)	4 (line 01)	150	150	Jan 2004
		<i>Praomys daltoni</i>	Houses	Houses (line 01)	7 (line 01)	0	0	Oct 2003
		<i>Praomys daltoni</i>	Prox. cultiv.	Orchard (line 07)	4 (line 01)	250	250	Jan 2004
		<i>Praomys daltoni</i>	Prox. forest	Wooded fallowland (line 10)	2 (line 01)	130	130	Oct 2004

The geographical coordinates of each trapping line were recorded for each starting (trap 1) and ending point (trap 20) of the line. We used Google Earth to locate the tapping lines and calculate the distance between the line with the seropositive animal from the nearest line containing one LASV-positive *M. natalensis*. Similarly, we evaluated the distance of the seropositive animal from the peripheral houses of the village. These distances remain approximate because each line was 100 meters long. The date of satellite image is 2006.

M. nat., natalensis; prox., proximal; dist., distal; cultiv., cultivations.

FIG. 2. Prevalence of Lassa virus (LASV)-specific antibodies and LASV prevalence in *M. natalensis* from high-endemic areas ($n=396$) depending on trapping session and village (Gb., Gbetaya). Sample size (N), number of seropositives, and LASV positives and double positives (sero+LASV) are given below the graphs for each trapping session. The numbers of LASV positive animals are from Fichet-Calvet et al. (2007). Error bars represent 95% confidence interval (CI).

population (the 11 double positives were counted only once). The total infection rate was quite high, with a maximum of 59% during May in Tanganya, and a mean of 43% (171/396, 95% CI 38–48%) in the three villages (Fig. 2). Grouping the seroprevalence according to habitat reveals a higher seroprevalence in proximal cultivations (54/130 41%, 95% CI 33–50%), then into the houses (52/259, 20%, 95% CI 15–25%). The number of animals captured in distal cultivations was too small for a reliable prevalence calculation (2/7, 28%, 95% CI 5–70%). The correlation between seroprevalence and ELW, as a surrogate for age of the host, is shown in Figure 3. Antibodies were already found in very young animals; four had ELW values of 7.3, 9.4, 9.6, and 9.9 mg, corresponding to

26–33 days old. A slight decrease in the seroprevalence from young to juveniles followed by an increase from juvenile to adult age was observed.

Discussion

The small mammal community

Six of the 26 small mammal species analyzed in this study (five shrews, 21 rodents) were LASV antibody positive: *M. natalensis*, *L. striatus*, *M. minutoides*, *P. daltoni*, *M. erythroleucus*, and *P. rostratus*. *M. natalensis* was expected to be seropositive; however, five additional species showed signs of arenavirus infection as well. As none of these animals was positive by arenavirus PCR (with a pan-Old World arenavirus test developed by Vieth et al. 2007), we assume they had only a transient infection. We cannot prove they had been infected with LASV due to serological cross-reactivity between arenavirus species. For example, a previously described *M. minutoides* from Tanganya village was infected both by a new arenavirus (Kodoko virus; Lecompte et al. 2007) and seropositive in a LASV-based serological test. However, because the LASV seroprevalence in non-*M. natalensis* animals in the current study was four times higher in the LASV high-endemic compared to the low-endemic villages (Table 2), at least some of them might have been infected by LASV. In addition, the seropositive animals were mainly found in the proximal cultivations, the same habitat that was associated with a high LASV seroprevalence in *M. natalensis*. We sometimes caught these species on the same trapping lines, sometimes on lines near 50–200 meters at most from a PCR-positive *M. natalensis*.

These results investigated at two spatial scales, regional (higher seropositivity in high endemic zone) and local (spatial proximity with viral positive *M. natalensis*), point in the direction of a spillover infection rather than cross-reacting antibodies. *M. natalensis* may spread the virus when urinating (Walker et al. 1975). Other species sharing the same habitat and searching for food could be infected by moving, scratching, or digging contaminated surfaces. On the other hand, we have no proof that the anti-mouse IgG conjugate,

FIG. 3. Lassa virus (LASV) seroprevalence in *M. natalensis* from high-endemic area ($n=396$) depending on the eye lens weight (ELW) as a surrogate for the age of the animals. The prevalence was calculated for the ELW ranges (5–10 mg, 10–15 mg, 15–20 mg, 20–25 mg, 25–30 mg, and 30–38 mg) corresponding the approximate age classes <35 days, 35–60 days, 60–110 days, 110–190 days, 190–340 days, and 340–600 days, respectively. Age classes were adapted from Leirs (1994). Sample size (N), number of seropositives, and LASV positives and double positives (sero+LASV) are given below the graphs for each age class. Error bars represent 95% confidence interval (CI).

which was used for the detection of the rodent IgG in the immunofluorescence assay, detects equally well the IgG of all rodent species. Therefore, it is possible that we underestimated the true arenavirus seroprevalence in particular in species that are only distantly related to *Mus musculus*. Moreover, the lower seropositivity in the organ fluids than in sera indicates an underestimation of the overall seroprevalence.

The LASV/arenavirus seropositive *M. erythroleucus* and *L. striatus* in Coastal Guinea, where there is no LASV reservoir, are more surprising. A likely explanation is the presence of another arenavirus that we missed in our trapping. In fact, we used Sherman traps on the ground, which are inadequate to catch climbing or diurnal species. These results are comparable to those obtained in Côte d'Ivoire, where some *M. natalensis*, *M. minotoides*, and *Lophuromys sikapusi* were found arenavirus seropositive in localities that are not endemic for LASV (Coulibaly N'Golo et al. 2011). Similarly, an arenavirus antibody screening in Tanzania revealed positive *Arvicanthis* sp. and *Lemniscomys* sp. in localities free of *M. natalensis* infected with Morogoro virus, which otherwise is widely distributed in Tanzania (Günther et al. 2009). Therefore, we assume that the antibody screening detected the circulation of arenaviruses that remain to be discovered.

LASV infection in *M. natalensis*

The mean seroprevalence in *M. natalensis* in the three LASV high-endemic villages was 27%, which is comparable to the 20% observed by Demby et al. (2001) in Guinea. The seroprevalence is also comparable to that of another African arenavirus, namely Morogoro virus in *M. natalensis*, with 17% in 1985–1989 (Günther et al. 2009) and 12% in 2008 (Gouy de Bellocq et al. 2010). In our study, the summation of virus and antibody prevalence demonstrates a high LASV infection rate (43%) in the rodent population. Again, this is somewhat more than in Demby's study (29%). Our sampling was done five times per village, including four times during the rainy season when the LASV prevalence was higher, whereas Demby et al. did only once per village, and mainly in the dry season when the LASV prevalence was lower. This may explain this difference. The high virus prevalence in the rodent population could explain the high human seroprevalence of up to 40% in the area (Klempa et al. 2013). A similar pattern was observed in Sierra Leone in two villages, Konia and Palima, where a high seroprevalence of 33% and 36% in rodents, was associated with high seroprevalence of 37% and 52% in humans (McCormick et al. 1987b). The other five villages investigated in this previous study had lower seroprevalence in both rodents and humans.

The larger proportion of seropositive animals (61%, 97/160) compared to LASV-positive animals (39%, 63/160) also suggests that the infection was often transient, with almost two-thirds of *M. natalensis* clearing the virus from the blood after infection. Indeed, these seronegative animals can continue to excrete the virus intermittently in their urines for at least 103 days after infection (Walker et al. 1975). The individuals that were LASV-positive, but seronegative, could have been transiently or chronically infected. Unless individuals are followed up longitudinally, it is not possible to distinguish between acute or chronic infections. The detection of antibodies in very young animals (26–33 days) sug-

gests that these antibodies have been transferred *in utero* from the mother to the fetus or in early life to the suckling mice. Such a transfer of antibodies to the offspring has been demonstrated in an experimental mouse model (Jimenez de Oya et al. 2011).

To estimate the parameters of infection, we set up two simple simulations to model the dynamics of LASV spread in the rodent population and the development of antibodies (Fig. 4). From the models, which reflect the experimental data (antibody and LASV prevalence, depending on ELW as shown in Fig. 3 and Fichet-Calvet et al. 2008, respectively) quite well, the following estimates can be inferred: Both model 1 and model 2 predict that most infections are due to horizontal transmission and the incidence of infection is 5% per month in young animals and drops in older animals. The prevalence of maternal antibodies was estimated at 20%, which is somewhat less than the antibody prevalence as determined in this study (30–40%) for the age range of pregnant females (120–360 days; Fichet-Calvet et al. 2008). Model 1 assumes that the duration of viremia does not depend on age and predicts a period of viremia of about 3 months for all age classes. The more complex model 2 describes chronic infections with long-term viremia (up to lifelong) after vertical or horizontal transmission in newborns in conjunction with acute infections with short-term viremia (about 1 month) in horizontally infected older animals. The majority (65%) of the population becomes acutely infected compared to 10% chronic infections. However, the prevalence of chronic infections is about two-fold higher than that of acute infections irrespective of age. Thus, the virus would be spread in the host population mainly by the small fraction of animals that become chronically infected *in utero* or shortly after birth.

Taking into account the data from experimental infections in *Mastomys* (Walker et al. 1975), model 2 is more likely to reflect reality than model 1. Which of the models reflect reality cannot be decided, because both explain the data equally well. Both models do not consider the small fraction of LASV- and antibody-positive animals, which represented 3% of the population, but 17% of the viremic animals. Double positivity was also observed in natural populations infected with Morogoro virus in Tanzania at a level of 10% (Borremans et al. 2011).

The logistic regression analysis disclosed that the risk of a positive serostatus is higher for animals from Tanganya, animals trapped in the proximal cultivations, and in older animals. The age effect is explained by the simulation model: The older the animals get, the more are infected. How the virus is transmitted horizontally in the population is not yet clear. It may be through direct transmission by nosing, allogrooming, sexual contact, or indirect transmission by contact with contaminated surfaces.

In conclusion, this study suggests that various species of the rodent community are infected by LASV in localities with high LASV prevalence in the *M. natalensis* population, although infection with another (so far undetected) arenavirus cannot be excluded. It is tempting to speculate that such a way of transmission might be the initial event in the process of host-switching during evolution (Coulibaly N'Golo et al. 2011, Irwin et al. 2012). This ecological study also indicates that horizontal transmission is the major method of LASV transmission in the rodent population. Many animals clear the virus under natural conditions rather than carry the virus for life.

Parameters of model 1

Vertical transmission: 5% (virus prevalence in pregnant females is about 10%)
 Horizontal transmission: 95%
 Incidence of infection: 5% per 30 d for age <240 d
 5% per 60 d for age >240 d
 Duration of viremia: 90 d for all ages
 Maternal antibodies: 20% in newborns and loss within 120 d
 (pregnant females are mostly 120-340 d old, seroprevalence at this age is 30-40%)

Parameters of model 2

Vertical transmission: 5% (virus prevalence in pregnant females is about 10%)
 Horizontal transmission: 95%
 Incidence of infection: 5% per 30 d for age <240 d
 5% per 60 d for age >240 d
 Duration of viremia: 300-600 d for newborns and age <30 d
 30 d for age >30d
 Maternal antibodies: 20% in newborns and loss within 120 d
 (pregnant females are mostly 120-340 d old, seroprevalence at this age is 30-40%)

FIG. 4. Two models for the dynamics of Lassa virus (LASV) spread in the *M. natalensis* population and the development of antibodies. An animal cohort is from birth to the age of 600 days. Infection of an animal by LASV is indicated by “V” in grey matrix fields, clearance of the virus and presence of antibodies by “a”. Animal #1 (marked with *) is infected vertically; all other animals are infected after birth. Maternal antibodies transferred before birth are indicated with animals #17-20 (“a” in grey matrix fields). Prevalence of antibodies and LASV according to age classes and the corresponding ELW classes are shown below the matrix (prevalence is the absolute frequency of “V” or “a” divided by the number of animals). Age classes were adapted from Leirs (1994). The prevalences for virus and antibody per ELW class obtained from the simulation are shown together with the real data in the graph below the matrix. Data for antibody prevalence are from Fig. 3, and data for LASV prevalence in the same locality are from Fichet-Calvet et al. (2008). Model 2 is more complex, as it assumes a different duration of viremia depending on age. The final model parameters are given below the graph.

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Author Disclosure Statement

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