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Identification of potential novel hosts and the risk of infection with lymphocytic choriomeningitis virus in humans in Gabon, Central Africa

Yuri Ushijima[a], Haruka Abe[a], Takehiro Ozeki[a,b], Georgelin N. Ondo[c], Marien J.V.M. Mbadinga[c], Rodrigue Bikangui[c], Chimène Nze-Nkogue[d], Etienne F. Akomo-Okoue[d], Ghislain W.E. Ella[d], Lilian B.M. Koumba[d], Branly C.B.B. Nso[d], Rodrigue Mintsa-Nguema[d], Patrice Makouloutou-Nzassi[d], Boris K. Makanga[d], Fred L.M. Nguelet[d], Vahid R. Zadeh[a,b], Shuzo Urata[a,i], Armel V.N. Mbouna[c], Marguerite Massinga-Loembe[e,f], Selidji T. Agnandji[c,g], Bertrand Lell[c,g,h], Jiro Yasuda[a,b,i,*]

[a] Department of Emerging Infectious Diseases, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan
[b] Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
[c] Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon
[d] Institut de Recherche en Ecologie Tropicale, Libreville, Gabon
[e] African Society for Laboratory Medicine, Addis Ababa, Ethiopia
[f] Africa Centres for Disease Control and Prevention, Johannesburg, South Africa
[g] Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany
[h] Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria
[i] National Research Center for the Control and Prevention of Infectious Diseases (CCPID), Nagasaki University, Nagasaki, Japan

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ABSTRACT

Objectives: Lymphocytic choriomeningitis virus (LCMV), a human pathogenic arenavirus, is distributed worldwide. However, no human cases have been reported in Africa. This study aimed to investigate the current situation and potential risks of LCMV infection in Gabon, Central Africa.
Methods: A total of 492 human samples were screened to detect LCMV genome RNA and anti-LCMV IgG antibodies using reverse transcription-quantitative PCR and enzyme-linked immunosorbent assay (ELISA), respectively. ELISA-positive samples were further examined using a neutralization assay. Viral RNAs and antibodies were also analyzed in 326 animal samples, including rodents, shrews, and bushmeat.
Results: While no LCMV RNA was detected in human samples, the overall seroprevalence was 21.5% and was significantly higher in male and adult populations. The neutralization assay identified seven samples with neutralizing activity. LCMV RNA was detected in one species of rodent (*Lophuromys sikapusi*) and a porcupine, and anti-LCMV IgG antibodies were detected in four rodents and three shrews.
Conclusions: This study determined for the first time the seroprevalence of LCMV in Gabon, and revealed that local rodents, shrews, and porcupines in areas surrounding semi-urban cities posed an infection risk. Hence, LCMV infection should be considered a significant public health concern in Africa.

Introduction

Lymphocytic choriomeningitis virus (LCMV) is an enveloped single-stranded RNA virus and a member of the family *Arenaviridae*, which includes highly pathogenic human viruses such as Lassa virus (LASV) and Junin virus (JUNV). Human transmission of arenaviruses usually occurs through direct contact with infected animals, particularly rodents, or by inhalation of airborne viruses through their excreta and other secretions. Although infections with LASV and JUNV are geographically limited by the habitats of their reservoir rodents (*Mastomys natalensis* and *Calomys musculinus*), LCMV circulates among the house mouse (*Mus musculus*), resulting in a global distribution of

* Corresponding author at: Department of Emerging Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki, 852-8523, Japan.
E-mail address: j-yasuda@nagasaki-u.ac.jp (J. Yasuda).

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infection burden (Brisse and Ly, 2019). As reported previously, up to 5% of healthy adults in the US (Riera et al., 2005; Stephensen et al., 1992) and as many as 40% of the residents in a region of Croatia where murine typhus is endemic (Dobec et al., 2006) were shown to be seropositive, with closeness to rodents in daily life leading to a risk of infection.

Compared with LASV and JUNV, which cause hemorrhagic fever, the clinical spectrum of LCMV infection commonly includes asymptomatic cases or mild symptoms in immunocompetent individuals, ranging from a flu-like illness to aseptic meningitis. However, LCMV infection potentially progresses in severity to systemic and fatal central nervous system disease (Bonthius, 2012). Infection during pregnancy results in fetal or neonatal death, as well as hydrocephalus and chorioretinitis in infants (Bonthius et al., 2007). Transmission through organ transplantation results in multiple organ failures (Fischer et al., 2006). Although little is known about the actual incidence rate of LCMV infection as described above, the mortality rate is high (more than 30%) in severe cases (Fischer et al., 2006).

Under poor sanitary conditions, such as in some African countries, people are at high risk of exposure to infected house mice. High birth rates (The World Bank, 2021) can also increase the frequency of contact with mice during pregnancy. Although advanced healthcare procedures, such as transplantation, are still challenging to perform in Africa, weakened immunity due to nutritional insufficiency or immunocompromised status can pose a risk of serious LCMV infection. However, LCMV infection has been neglected, especially in Africa. To date, except for two publications (el Karamany and Imam, 1991; Nadine et al., 2015), neither surveillance studies nor human cases of LCMV in Africa have been reported.

We conducted a surveillance study to investigate the current status and history of LCMV infection in Gabon, Central Africa. A total of 492 serum samples collected from febrile patients and healthy individuals were screened for LCMV. A total of 327 samples collected from animals, including rodents, shrews, and bushmeat, were also investigated. Although circulating LCMV was not observed in humans, the detection of neutralizing antibodies and epidemiological characteristics by our study showed, for the first time, LCMV seroprevalence in Gabon and Africa. Analyses using animal samples suggested that African rodents, shrews, and porcupines could be associated with the transmission of the virus in the country.

Materials and methods

Human samples

A total of 492 serum/plasma samples were used: 351 serum samples were collected from febrile patients (≥37.5 °C body temperature) who visited the Albert Schweitzer Hospital (HAS) between January 2015 and June 2017; and 141 plasma samples were collected from healthy African individuals who visited the Centre de Recherches Médicales de Lambaréné (CERMEL) between November 2014 and January 2017. HAS and CERMEL are both located in Lambaréné, Gabon (Figure 1). For this study, the age of the participants was restricted to ≥1 year. Demographic information (age and sex) for the recruited participants was also collected.

Animal samples

A total of 326 animal samples were used: 152 rodents and 129 shrews were caught using Sherman and Tomahawk traps in a forest near a suburban area and around human dwellings in Lambaréné, and 45 samples of fresh bushmeat (wildlife) were collected between May 2019 and March 2020 in bushmeat markets, where legally hunted bushmeat was sold. Captured rodents and shrews were euthanized with ether or ketamine, and tissues and serum/plasma samples were collected. All procedures on live animals were conducted in compliance with the applicable institutional and national guidelines for the use and handling of animals. All samples were stored at −80 °C until further analysis.

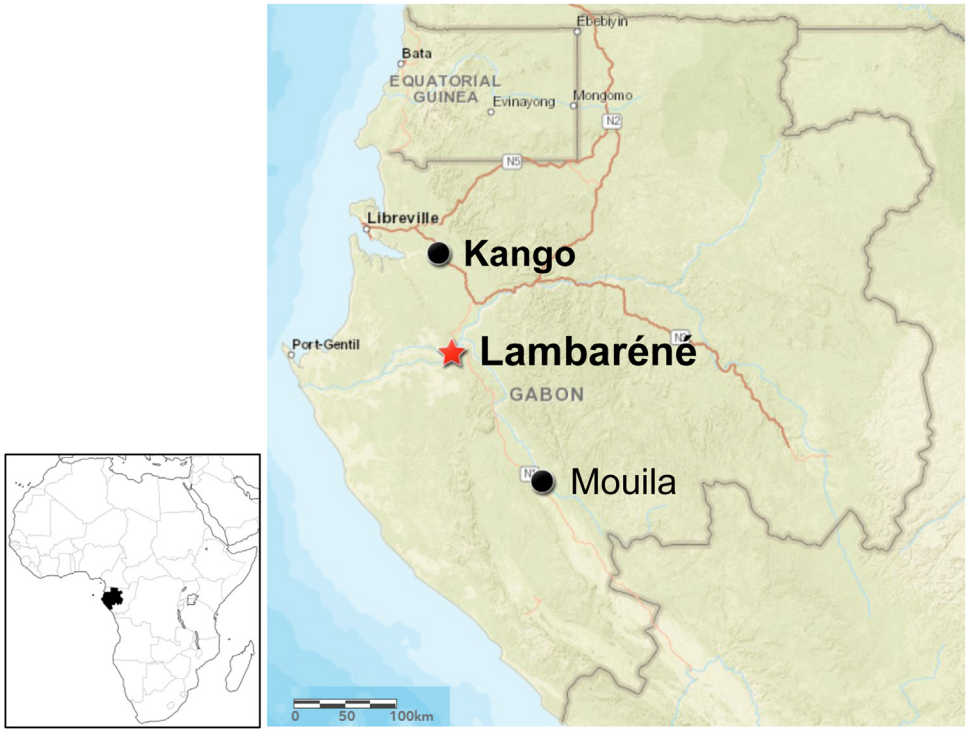

Figure 1. Location of Gabon in Africa and names of the cities described in the text. The study site, Lambaréné, is indicated by a red star. The map was created by ArcGISPro (ESRI Japan, Tokyo, Japan).

Ethics statement

This study was approved by the Institutional Review Boards of CERMEL and Nagasaki University (approval numbers CEI-007 and 170921177, respectively). Written informed consent was obtained from all the participants or their parents. In Gabon, permission to collect animal samples in the environment was granted by the Centre National de la Recherche Scientifique et Technologique (CENAREST).

Viral RNA extraction and detection by reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

For human samples, viral RNA was extracted from 140 μL of each serum sample collected from febrile patients using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), as described previously (Abe et al., 2020a). For animal samples, 30 mg of kidney tissue was shredded with scissors, 600 μL ice-cold PBS was added, and the tissue was homogenized using a pestle. Total RNA was extracted from the supernatant of the homogenate using the PureLink RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. RT-qPCR was performed in a 20 μL reaction volume using a One-Step PrimeScript III RT-qPCR Mix (Takara Bio, Shiga, Japan), as described previously (Abe et al., 2020b). Primers and probes specific for LCMV were designed using previously reported sequences (Cordey et al., 2011). RT-qPCR assays were performed using a StepOnePlus instrument (Thermo Fisher Scientific, Waltham, MA, USA) under the following conditions: 5 min at 52 °C, 10 s at 95 °C, 50 cycles of 5 s at 95 °C, and 35 s at 58 °C. Data collected from the RT-qPCR assays were analyzed using the system's software. RT-qPCR assays were performed in duplicate, and samples reaching threshold cycle (Ct) values under 40 were set as positive.

Identification of animal species

Rodent, shrew, and bushmeat species collected in this study were identified by analyzing the nucleotide sequence of the cytochrome b gene (*cytb*). DNA was extracted from the supernatants of the kidney homogenates that were used for the RNA extraction using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. To amplify the *cytb* fragment, universal primers for rodents, shrews, and bushmeat were used (Ishii et al., 2012; Parson et al., 2000). The PCR was performed under the following conditions: 30 cycles each of 10 s at 98 °C, 15 s at 51 °C, and 1 min at 68 °C. The amplified bands at the expected sizes were further analyzed using gene sequencing.

Enzyme-linked immunosorbent assay (ELISA)

All human and animal serum/plasma samples were tested for anti-LCMV IgG antibodies using an in-house indirect ELISA. LCMV GPC (Armstrong strain) was expressed in human embryonic kidney 293T cells transfected with the pCAGGS-based expression plasmid for LCMV GPC (Urata et al., 2011). After 24 h post-transfection, the cells were lysed in a buffer containing 20 mM Tris (pH 8.0), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and cOmplete (Merck KGaA, Darmstadt, Germany), followed by overnight incubation at 4 °C. The cell lysates were centrifuged at 20 000 ×*g* for 10 min at 4 °C, and the supernatants were stored in aliquots at −30 °C. Whole-cell lysates from mock-transfected 293T cells were used as background controls. MaxiSorp ELISA plates (Thermo Fisher Scientific, Waltham, MA, USA) were coated with 200 ng of cell lysate in 100 μL phosphate-buffered saline (PBS) per well and incubated overnight at 4 °C. The wells were then blocked with 100 μL of 5% skimmed milk in PBS containing 0.05% Tween 20 (PBS-T) for 1 h at 23–25 °C. After incubation, the wells were washed thrice with PBS-T. Human or animal serum/plasma samples were diluted 1:100 in PBS-T containing 2% skimmed milk and added to obtain the final volume of 100 μL per well. The plates were incubated for 1 h at 37 °C and washed as described above. After washing, 100 μL of 1:20 000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody (Bethyl Laboratories, Montgomery, TX, USA) was added to each well and incubated for 1 h at 23–25 °C. For animal samples, the corresponding HRP-conjugated secondary antibodies (1:20 000 dilution) were used for each animal species: goat antimouse IgG (Merck KGaA) for rodents, recombinant protein A/G (Thermo Fisher Scientific) for shrews and African palm civets, goat antirat IgG (Merck KGaA) for African brush-tailed porcupines, rabbit antibovine IgG (Merck KGaA) for blue duikers, and rabbit antimonkey IgG (Merck KGaA) for moustached guenons. After washing five times, 100 μL of 3,3',5,5'-tetramethylbenzidine substrate (Thermo Fisher Scientific) was added to the wells, and the plates were incubated for 10 min. The reaction was stopped by adding 100 μL of 0.2 M sulfuric acid, and the optical density (OD) was measured at 450 nm using a FilterMax F5 microplate reader (Molecular Devices, San Jose, CA, USA) or a Multiskan FC microplate reader (Thermo Fisher Scientific). ELISA was performed in duplicate, and the samples demonstrating OD values higher than 0.4 were considered positive. The cut-off value was determined by calculating the mean value plus 3× standard deviation of the ODs of sera collected from LCMV-unexposed donors (LCMV-negative samples). The reactivity of the ELISA antigen was confirmed with anti-LCMV GP2 rabbit serum as a positive control. Anti-LCMV GP2 polyclonal antibodies were generated by the immunization of rabbit using a synthetic peptide corresponding to the cytoplasmic tail of LCMV GP2 (CGAFKVPGVKTVWKRR) (MBL, Nagoya, Japan). The OD value in a 100-fold dilution of antiserum was 1.747. A dilution of more than 3200-fold produced an OD value of less than 0.4.

Neutralization assay

Pseudotyped vesicular stomatitis viruses (VSVs) bearing LCMV GPC (LCMpv) and a luciferase reporter gene as an artificial viral genome, and capable of a single round of replication, was prepared as follows. Human embryonic kidney 293T cells were grown to 80% confluence on collagen-coated tissue culture plates and then transfected with LCMV GPC-expressing plasmids. After 24 h post-transfection, the cells were infected with G-complemented (*G) VSV△G/Luc (*G-VSV△G/Luc), in which the VSV-G gene was replaced with the luciferase gene and pseudotyped with the G protein (Tani et al., 2010) at a multiplicity of infection (MOI) of 0.2. The cells were absorbed for 1 h with *G-VSV△G/Luc and then washed five times with PBS. After 24 h incubation in Dulbecco's modified Eagle's medium (Merck KGaA) containing 2% fetal bovine serum, the culture supernatants containing LCMpv with the luciferase reporter gene were centrifuged to remove cell debris and stored in aliquots at −80 °C until further use. To determine the pseudovirus titer, monolayers of 8×10^5 Vero76 cells per well in a white-walled 96-well plate were infected with serial dilutions of the virus stock. The relative light unit (RLU) of luciferase activity in the cultures was determined using the Steady-Glo Luciferase Assay Kit (Promega, Madison, WI, USA) 24 h after inoculation. Luminescence was measured using a FilterMax F5 microplate reader (Molecular Devices).

The presence of neutralizing antibodies in the ELISA-positive samples was examined using a neutralization assay. The plasma samples were heat-inactivated at 56 °C for 30 min and serially diluted from 1:10 to 1:160. Diluted plasma samples were mixed with an equal volume of LCMpv containing 4000–5000 RLU, followed by incubation at 37 °C for 90 min for a virus–antibody

Table 1
Results of RT-qPCR in febrile patients and ELISA for anti-LCMV IgG antibodies in Gabonese residents.

Characteristics	Total No.	Prevalence, *n* (%)	OR	*p*-value
RT-qPCR	281	0 (0)		
ELISA	492	106 (21.5)		
Sex				
Male	221	66 (29.9)	1.67	0.008
Female	157	28 (17.8)	–	–
Unknown	114	12 (10.5)		
Age				
Adult (≥18)	233	84 (36.1)	5.23	<0.0001
Children (1–17)	145	10 (6.9)	–	–
Unknown	114	12 (10.5)		
Years old				
1–10	118	10 (8.5)	0.31	0.0212
11–20	59	10 (16.9)	0.62	0.3513
21–30	111	44 (39.6)	1.45	0.3398
31–40	46	17 (37.0)	1.36	0.5851
41–50	22	7 (31.8)	1.17	>0.9999
≥51	22	6 (27.3)	–	–

OR: odds ratio.

neutralization reaction. The virus and serum/plasma mixture were inoculated in the Vero76 monolayer in a white-wall 96-well plate (Thermo Fisher Scientific) at 37 °C for 90 min. After 24 h post-infection, the infectivity of LCMpv was assessed by examining the luciferase activity. Cells were lysed, and luciferase activity was measured using the Dual-Glo Luciferase Assay System (Promega). The neutralization assay was performed in triplicate. Samples were considered neutralization-positive when the luciferase activity was reduced to less than 50% in a 10-fold diluted sample compared with that in cells infected with LCMpv without the samples. The cytotoxic effect was confirmed in each sample using CellTiter-Glo (Promega). Pseudotyped VSV bearing Lassa virus GPC (Urata et al., 2011) was included as a specificity control for the assay. The positive control available for LASV was reconstituted at 62.5 μg/mL LASV neutralizing antibody 37.7H (Absolute Antibody, Boston, MA, USA) in the negative sample.

Statistical analysis

Statistical analyses were performed for human samples for which demographic information was available. The chi-square test was used to assess the influence of demographic characteristics on the prevalence of antibodies. Differences were considered statistically significant at $p < 0.05$. To assess anti-LCMV antibodies in animal samples, Smirnov–Grubbs rejection tests, which are widely used to detect significantly higher or lower values (i.e. outliers) that do not belong to the population consisting of all other values in the data set, were used as described previously (Nidom et al., 2012), because negative controls were unavailable. All statistical analyses were performed using GraphPad Prism version 7 (GraphPad Software, San Diego, CA, USA).

Results

LCMV RNA detection in the clinical specimens obtained from febrile patients

Out of 351 serum samples collected from febrile patients (body temperature >37.5 °C), RNA was successfully extracted from 281 serum samples owing to limited volumes. The demographic information collected from these 281 febrile patients was as follows: ratio of females to males 1.25; age range 1–82 years; mean age 13.51 ± 15.38 years

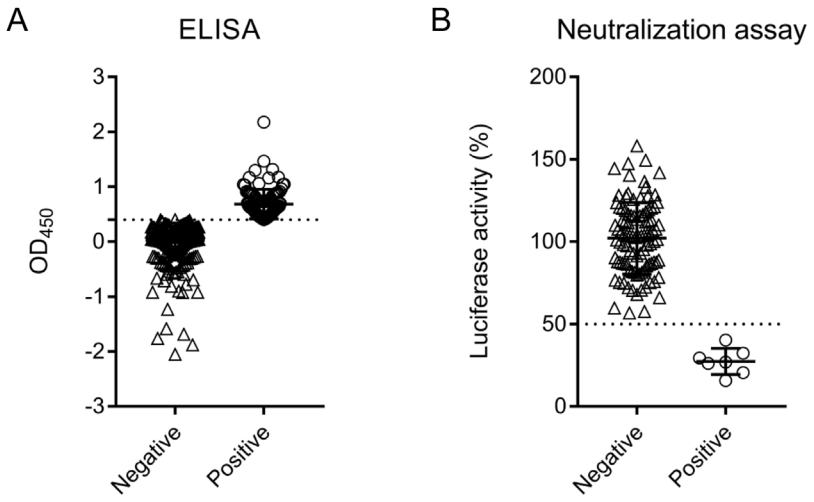

Figure 2. Serological assays of 492 human samples in Gabon, for the detection of IgG antibodies specific to LCMV. (A) Results of LCMV GPC-based ELISA. (B) Neutralization assay performed with 1:10 diluted samples using LCMV GPC pseudotyped VSV.

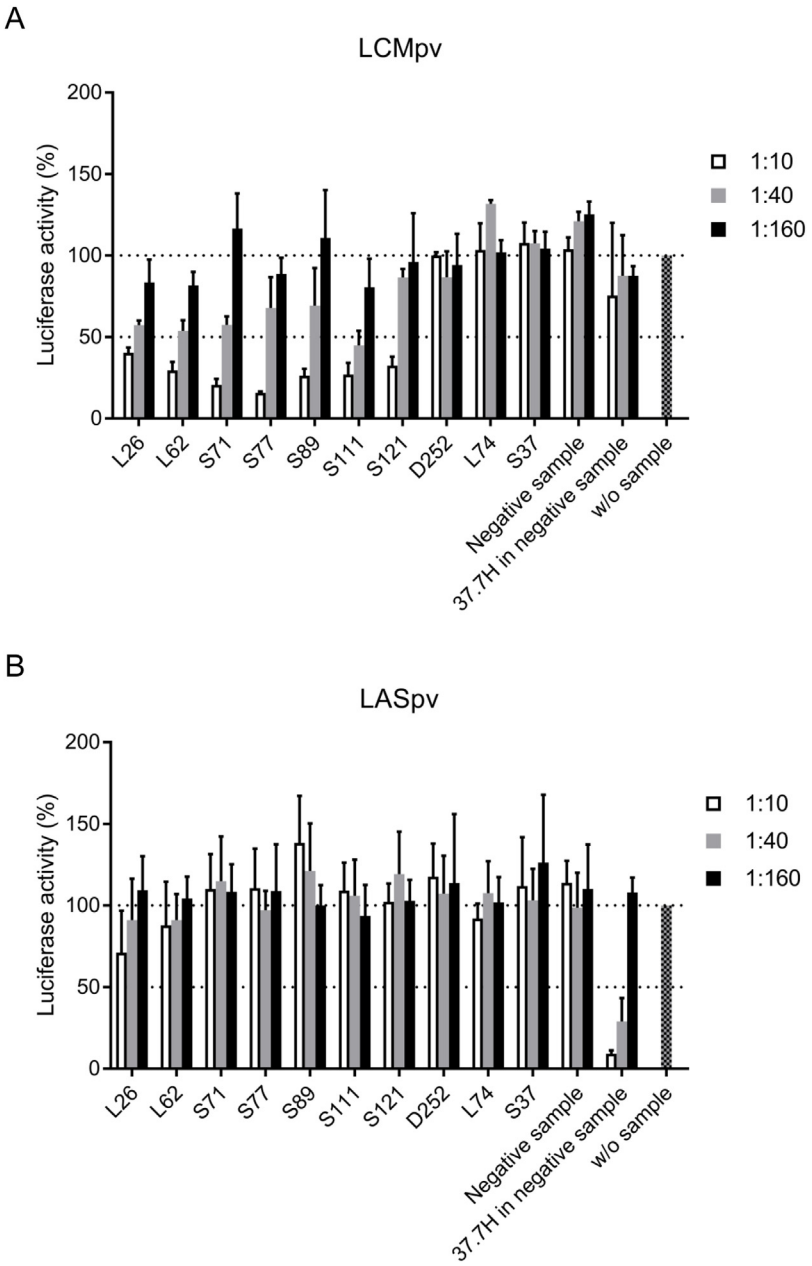

Figure 3. Neutralization assays for (A) LCMV and (B) LASV. Confirmatory neutralization results for the detection of neutralizing antibodies specific to LCMV in seven samples (L26, L62, S71, S77, S89, S111, and S121), three randomly selected neutralization-negative samples (D252, L74, and S37), one LCMV-negative sample, and cells infected without samples as a standard, as well as a positive control for LASV that was reconstituted using 62.5 μg/mL LASV-neutralizing antibody 37.7H in the negative sample, respectively.

(±SD); the dataset consisted of 47 adults (≥18 years of age), 133 children (<17 years of age), and 101 samples with no information (Table 1). The samples were screened for LCMV using RT-qPCR, as previously described (Cordey et al., 2011); no LCMV was detected (Table 1).

Detection of anti-LCMV IgG antibodies in Gabonese residents

To investigate past LCMV infections, 492 serum/plasma samples collected from Gabonese residents, including both febrile patients and healthy individuals, were tested for anti-LCMV IgG antibodies. The demographic information obtained from these 492 Gabonese residents was as follows: female to male ratio 0.71, age range 1–82 years, mean age 21.98 ± 16.7 years (±SD); the dataset consisted of 233 adults (≥18 years of age), 145 children (<17 years of age), and 114 samples with no information (Table 1). All samples were initially analyzed using an in-house ELISA (Figure 2A). The overall prevalence of anti-LCMV antibodies among the 492 Gabonese residents was 21.5% (106/492). When comparing the prevalence according to group variables (sex and age), it was observed that the prevalence in male and adult populations was significantly higher than that in female and child populations (Table 1). Among the different age groups, the peak was observed in those aged 21–30 years, decreasing thereafter in the older age group. These findings indicated that adult males and individuals aged 21–30 bear the highest risk of LCMV infection in Gabon.

The presence of neutralizing antibodies in the positive samples determined by ELISA was further examined with a neutralization assay using the pseudovirus LCMpv (Figure 2B). In this assay, the reduction of virus infectivity by neutralizing antibodies was indicated by a reduction in luciferase activity. A clear reduction in infectivity was observed in seven samples (L26, L62, S71, S77, S89, S111, S121) for LCMpv (Figure 3A), but not for LASpv (Figure

Table 2
Species of animal investigated in this study, and results of RT-qPCR and ELISA assays.

Species	Total No.	RT-qPCR[a]	ELISA[b] (%)
Overall	326	2 (0.61)	7 (2.25)
Rodents			
Mus minutoides	41	0	3
Mus musculus	3	0	0
Praomys missonei	65	0	1
Lophuromys sikapusi	6	1 (Ct, 28)	0
Lemniscomys striatus	3	0	0
Lophuromys sabuni	1	0	0
Hybomys univittatus	5	0	0
Oenomys hypoxanthus	6	0	0
Stochomys longicaudatus	6	0	0
Rattus rattus	16	0	0
Total	152	1 (0.66)	4 (2.72)
Shrews			
Crocidura goliath	129	0	3 (2.52)
Bushmeat			
Porcupine (*Atherurus africanus*)	18	1 (Ct, 39)	0
Blue duiker (*Philantomba monticola*)	16	0	0
Giant rat (*Cricetomys emini*)	1	0	0
African palm civet (*Nandinia binotata*)	4	0	0
Moustached guenon (*Cercopithecus cephus*)	6	0	0
Total	45	1	0

[a] CT values were indicated in RT-qPCR positive samples.
[b] Sera from five rodents and 10 shrews were not available for the test.

3B), indicating that these samples contained neutralizing antibodies specific to LCMV. Only L26 showed a slight reduction in LASpv, which can probably be attributed to cross-reactivity. Demographic information was available for all neutralization-positive samples, except for one (S77). All positive samples were collected from four male and two female adult participants (mean age 32.2 years; 95% CI, 21.6–42.8 years). No correlation was observed between the ELISA OD values and neutralizing activities.

Investigation of LCMV infections in animals

To examine the possibility of LCMV transmission from wild animals to humans in Gabon, small mammals, including rodents (widely known as major hosts of arenaviruses) were captured between May 2019 and March 2020, and investigated for the presence of LCMV. Since bushmeat is widely consumed in Gabon (Bachand et al., 2015), bushmeat samples were also included for the investigation. As shown in Table 2, 152 rodents and 129 shrews were captured in a forest near a suburban area and around human dwellings in Lambaréné, and 45 fresh bushmeat samples were collected from bushmeat markets between Kango and Mouila (Figure 1). RNA extracted from 326 kidney tissue samples collected from these animals was screened for LCMV by RT-qPCR, using the same method as that employed for human samples. The virus was detected in a rodent, *Lophuromys sikapusi*, and a porcupine, *Atherurus africanus* (Table 2). Serum/plasma samples were screened by ELISA for the detection of anti-LCMV IgG antibodies. Serum/plasma samples from 10 shrews and five rodents were not available for testing. The results showed that four house mice (three *Mus minutoides* and one *Praomys missonei*), widely found in sub-Saharan Africa, and three shrews (*Crocidura goliath*) were positive for anti-LCMV antibodies (Table 2). No significant difference in seroprevalence was observed between rodents (4/147, 2.72%) and shrews (3/119, 2.52%). Anti-LCMV IgG antibodies were not detected in any bushmeat samples. These results suggest that contact with not only rodents, but also shrews and porcupines, is a risk factor for LCMV infection in Gabon.

Discussion

To date, most epidemiological studies on LCMV have been conducted in Europe, the USA, and Asia (Blasdell et al., 2016; Kallio-Kokko et al., 2006; Knust et al., 2011; Knust et al., 2014; Leibler et al., 2016; Takagi et al., 2012). As the first surveillance study for LCMV infection in Central Africa, we investigated LCMV infections in both humans and wild animals (rodents, shrews, and bushmeat) by detecting viral genomes and IgG antibodies. Although no LCMV RNA was detected in febrile patients in this study, the overall seroprevalence shown by ELISA was 21.5% (106/492); 6.6% (7/106) of the population comprising positive samples determined with ELISA were confirmed to possess neutralizing antibodies specific to LCMV. This suggests that a relatively large population of Gabonese residents has experienced unrecognized LCMV infections.

The seroprevalence of LCMV infection is not uniformly distributed worldwide. Compared with previous surveillance studies, the seroprevalence of LCMV in Gabon (21.5%) was higher than that in the USA (approximately 5%) (Riera et al., 2005; Stephensen et al., 1992) and Spain (1.7%) (Lledó et al., 2003), but was lower than that in Croatia (36%) (Dobec et al., 2006) and in Slovakia (37.5%) (Reiserová et al., 1999). The high risk of LCMV infection observed among adult male populations in Gabon was similar to that observed in Argentina, for example, as described in some previous studies (e.g. Riera et al., 2005). However, the ELISA results reported here should be considered preliminary because their performance could not be validated owing to the lack of reference samples with predetermined antibody status information. Although only seven samples (7/106, 6.6%) showed anti-LCMV IgG antibodies with neutralizing activity, detection of neutralizing antibodies provided the first evidence of its infection in humans in Gabon.

The house mouse, *Mus musculus*, is known as the main reservoir of LCMV (Gonzalez et al., 2007); however, the genomes and antibodies have also been detected in other rodent species (Blasdell et al., 2008; el Karamany and Imam, 1991; Laakkonen et al., 2006; Ledesma et al., 2009; Lledó et al., 2003). In addition, a recent study reported that LCMV could be isolated from ticks (Zhang et al., 2018). A novel arenavirus (Wenzhou virus) was also

identified in rats and shrews (Li et al., 2015), indicating that LCMV potentially has a broader host range extending beyond rodents. Our study examined two newly identified rodent species—the rusty-bellied brush-furred rat (*Lophuromys sikapusi*) and the African brush-tailed porcupine (*Atherurus africanus*)—as potential hosts of LCMV. The serological analysis also provided the first evidence of LCMV or LCMV-like virus infecting African pigmy mice (*M. minutoides*), African soft-furred mice (*P. missonei*), and Goliath shrews (*Crocidura goliath*).

N et al., who detected LCMV for the first time in Africa, previously hypothesized that the virus was introduced from the USA into Gabon, together with *M. musculus domesticus* (Nadine et al., 2015). However, since the origin of LCMV strains emerged around 6742–700 years ago (Albariño et al., 2010; Forni et al., 2018), it still remained to be determined how an ancestral LCMV reached the New World before long-distance travel was discovered. The findings of our study suggest that LCMV-infected African rodent/shrew species and porcupines might demonstrate alternative possibilities; LCMV was originally harbored in local animals and circulated with adaptation/evolution among corresponding species. To clarify this possibility, extensive fieldwork is required to investigate a variety of samples and characterize the virus's transmission and evolution.

Finally, our study demonstrated a high risk of LCMV infection in Gabon and other African countries. However, it is difficult to diagnose and recognize LCMV infections without specific symptoms in low-resource clinical settings. Therefore, personal prevention of LCMV infections by avoiding contact with wildlife such as rodents, shrews, and bushmeat would be significantly effective. Meanwhile, the development of diagnostic methods and strategies for addressing public health concerns regarding LCMV infections should be considered in Africa.

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Conflicts of interest

None declare.

Ethical approval

This study was approved by the Institutional Review Boards of CERMEL and Nagasaki University (approval numbers CEI-007 and 170921177, respectively). Written informed consent was obtained from all the participants or their parents.

Author contributions

YU: study design, sample collection, data collection, data analysis, writing; HA: sample collection, data collection, effective discussion; TO: sample collection, data collection; GNO: data collection; MJVMM: data collection; RB: study assistance; CNN: study assistance, sample collection, data analysis; EFAO: sample collection; GWEE: sample collection; LBMK: sample collection; BCBBN: sample collection; RMN: sample collection; PMN: sample collection; BKM: sample collection; FLMN: sample collection; VRZ: data collection; SU: material preparation; AVNM: study assistance; MML: study assistance; STA: study assistance; BL: study assistance; JY: study design, sample collection, writing. All authors have read and approved the final manuscript.

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References

Abe H, Ushijima Y, Loembe MM, Bikangui R, Nguema-Ondo G, Mpingabo PI, et al. Re-emergence of dengue virus serotype 3 infections in Gabon in 2016–2017, and evidence for the risk of repeated dengue virus infections. Int J Infect Dis 2020a;91:129–36.

Abe H, Ushijima Y, Bikangui R, Ondo GN, Zadeh VR, Pemba CM, et al. First evidence for continuous circulation of hepatitis A virus subgenotype IIA in Central Africa. J Viral Hepat 2020b;27(11):1234–42.

Albariño CG, Palacios G, Khristova ML, Erickson BR, Carroll SA, Comer JA, et al. High diversity and ancient common ancestry of lymphocytic choriomeningitis virus. Emerg Infect Dis 2010;16(7):1093–100.

Bachand Nicholas, Arsenault Julie, Ravel André. Urban household meat consumption patterns in Gabon, Central Africa, with a focus on bushmeat. Hum Dimens Wildl 2015;20(2):147–58.

Blasdell KR, Becker SD, Hurst J, Begon M, Bennett M. Host range and genetic diversity of arenaviruses in rodents, United Kingdom. Emerg Infect Dis 2008;14 (9):1455–8.

Blasdell KR, Duong V, Eloit M, Chretien F, Ly S, Hul V, et al. Evidence of human infection by a new mammarenavirus endemic to Southeastern Asia. Elife 2016;5:.

Bonthius DJ. Lymphocytic choriomeningitis virus: an underrecognized cause of neurologic disease in the fetus, child, and adult. Semin Pediatr Neurol 2012;19 (3):89–95.

Bonthius DJ, Wright R, Tseng B, Barton L, Marco E, Karacay B, et al. Congenital lymphocytic choriomeningitis virus infection: spectrum of disease. Ann Neurol 2007;62(4):347–55.

Brisse ME, Ly H. Hemorrhagic fever-causing arenaviruses: lethal pathogens and potent immune suppressors. Front Immunol 2019;10:372.

Cordey S, Sahli R, Moraz ML, Estrade C, Morandi L, Cherpillod P, et al. Analytical validation of a lymphocytic choriomeningitis virus real-time RT-PCR assay. J Virol Methods 2011;177(1):118–22.

Dobec M, Dzelalija B, Punda-Polic V, Zoric I. High prevalence of antibodies to lymphocytic choriomeningitis virus in a murine typhus endemic region in Croatia. J Med Virol 2006;78(12):1643–7.

el Karamany RM, Imam IZ. Antibodies to lymphocytic choriomeningitis virus in wild rodent sera in Egypt. J Hyg Epidemiol Microbiol Immunol 1991;35(1):97–103.

Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med 2006;354(21):2235–49.

Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient evolution of mammarenaviruses: adaptation via changes in the L protein and no evidence for host-virus codivergence. Genome Biol Evol 2018;10(3):863–74.

Gonzalez JP, Emonet S, de Lamballerie X, Charrel R. Arenaviruses. Curr Top Microbiol Immunol 2007;315:253–88.

Ishii A, Thomas Y, Moonga L, Nakamura I, Ohnuma A, Hang'ombe BM, et al. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. J Gen Virol 2012;93(Pt 10):2247–51.

Kallio-Kokko H, Laakkonen J, Rizzoli A, Tagliapietra V, Cattadori I, Perkins SE, et al. Hantavirus and arenavirus antibody prevalence in rodents and humans in Trentino, Northern Italy. Epidemiol Infect 2006;134(4):830–6.

Knust B, Macneil A, Wong SJ, Backenson PB, Gibbons A, Rollin PE, et al. Exposure to lymphocytic choriomeningitis virus, New York, USA. Emerg Infect Dis 2011;17 (7):1324–5.

Knust B, Ströher U, Edison L, Albariño CG, Lovejoy J, Armeanu E, et al. Lymphocytic choriomeningitis virus in employees and mice at multipremises feeder-rodent operation, United States, 2012. Emerg Infect Dis 2014;20(2):240–7.

Laakkonen J, Kallio ER, Kallio-Kokko H, Vapalahti O, Vaheri A, Henttonen H. Is there an association of Pneumocystis infection with the presence of arena-, hanta-, and poxvirus antibodies in wild mice and shrews in Finland?. Parasitology 2006;132(Pt 4):461–6.

Ledesma J, Fedele CG, Carro F, Lledó L, Sánchez-Seco MP, Tenorio A, et al. Independent lineage of lymphocytic choriomeningitis virus in wood mice (Apodemus sylvaticus), Spain. Emerg Infect Dis 2009;15(10):1677–80.

Leibler JH, Zakhour CM, Gadhoke P, Gaeta JM. Zoonotic and vector-borne infections among urban homeless and marginalized people in the United States and Europe, 1990–2014. Vector Borne Zoonotic Dis 2016;16(7):435–44.

Li K, Lin XD, Wang W, Shi M, Guo WP, Zhang XH, et al. Isolation and characterization of a novel arenavirus harbored by Rodents and Shrews in Zhejiang province, China. Virology 2015;476:37–42.

Lledó L, Gegúndez MI, Saz JV, Bahamontes N, Beltrán M. Lymphocytic choriomeningitis virus infection in a province of Spain: analysis of sera from the general population and wild rodents. J Med Virol 2003;70(2):273–5.

Nadine ND, Berthet N, Rougeron V, Mangombi JB, Durand P, Maganga GD, et al. Evidence of lymphocytic choriomeningitis virus (LCMV) in domestic mice in Gabon: risk of emergence of LCMV encephalitis in Central Africa. J Virol 2015;89(2):1456–60.

Nidom CA, Nakayama E, Nidom RV, Alamudi MY, Daulay S, Dharmayanti IN, et al. Serological evidence of Ebola virus infection in Indonesian orangutans. PLoS One 2012;7(7)e40740.

Parson W, Pegoraro K, Niederstätter H, Föger M, Steinlechner M. Species identification by means of the cytochrome b gene. Int J Legal Med 2000;114(1–2):23–8.

Reiserová L, Kaluzová M, Kaluz S, Willis AC, Závada J, Závodská E, et al. Identification of MaTu-MX agent as a new strain of lymphocytic choriomeningitis virus (LCMV) and serological indication of horizontal spread of LCMV in human population. Virology 1999;257(1):73–83.

Riera L, Castillo E, Del Carmen Saavedra M, Priotto J, Sottosanti J, Polop J, et al. Serological study of the lymphochoriomeningitis virus (LCMV) in an inner city of Argentina. J Med Virol 2005;76(2):285–9.

Stephensen CB, Blount SR, Lanford RE, Holmes KV, Montali RJ, Fleenor ME, et al. Prevalence of serum antibodies against lymphocytic choriomeningitis virus in selected populations from two U.S. cities. J Med Virol 1992;38(1):27–31.

Takagi T, Ohsawa M, Morita C, Sato H, Ohsawa K. Genomic analysis and pathogenic characteristics of lymphocytic choriomeningitis virus strains isolated in Japan. Comp Med 2012;62(3):185–92.

Tani H, Shiokawa M, Kaname Y, Kambara H, Mori Y, Abe T, et al. Involvement of ceramide in the propagation of Japanese encephalitis virus. J Virol 2010;84(6):2798–807.

The World Bank. https://data.worldbank.org/indicator/SP.DYN.CBRT.IN?end=2018&locations=GA&start=2018&view=map [Accessed 17 January 2021].

Urata S, Yun N, Pasquato A, Paessler S, Kunz S, de la Torre JC. Antiviral activity of a small-molecule inhibitor of arenavirus glycoprotein processing by the cellular site 1 protease. J Virol 2011;85(2):795–803.

Zhang L, Li S, Huang SJ, Wang ZD, Wei F, Feng XM, et al. Isolation and genomic characterization of lymphocytic choriomeningitis virus in ticks from northeastern China. Transbound Emerg Dis 2018;65(6):1733–9.