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Seroprevalence and risk factors for Lassa virus infection in South-West and North-Central Nigeria: a community-based cross-sectional study

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Abstract

Background Understanding the level of exposure to Lassa virus (LASV) in at-risk communities allows for the administration of effective preventive interventions to mitigate epidemics of Lassa fever. We assessed the seroprevalence of LASV antibodies in rural and semiurban communities of two cosmopolitan cities in Nigeria with poorly understood Lassa epidemiology.

Methods A cross-sectional study was conducted in ten communities located in the Abuja Municipal Area Council (AMAC), Abuja, and Ikorodu Local Government Area (LGA), Lagos, from February 2nd to July 5th, 2022. Serum samples collected from participants were analyzed for IgG and IgM antibodies using a ReLASV® Pan-Lassa NP IgG/IgM enzyme-linked immunosorbent assay (ELISA) kit. A questionnaire administered to participants collected self-reported sociodemographic and LASV exposure information. Seroprevalence of LASV IgG/IgM was estimated overall, and by study site. Univariate and multivariate log-binomial models estimated unadjusted and adjusted prevalence ratios (aPRs) and 95% confidence intervals (CI) for site-specific risk factors for LASV seropositivity. Grouped Least Absolute Shrinkage and Selection Operator (LASSO) was used for variable selection for multivariate analysis.

Results A total of 628 participants with serum samples were included in the study. Most participants were female (434, 69%), married (459, 73%), and had a median age of 38 years (interquartile range 28–50). The overall seroprevalence was 27% (171/628), with a prevalence of 33% (126/376) in Abuja and 18% (45/252) in Lagos. Based on site-specific grouped LASSO selection, enrollment in the dry season (vs. wet; aPR, 95% CI: 1.73, 1.33–2.24), reported

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inconsistent washing of fruits and vegetables (aPR, 95% CI: 1.45, 1.10–1.92), and a positive malaria rapid test (aPR, 95% CI: 1.48, 1.09-2.00) were independently associated with LASV seropositivity in Abuja, whereas, only a self-reported history of rhinorrhea (PR, 95% CI: 2.21, 1.31–3.72) was independently associated with Lassa seropositivity in Lagos.

Conclusions The LASV seroprevalence was comparable to that in other areas in Nigeria. Our findings corroborate those from other studies on the importance of limiting human exposure to rodents and focusing on behavioral factors such as poor hygiene practices to reduce exposure to LASV.

Keywords Epidemiology, Lassa virus, Seroprevalence, Community-based study, Emerging infectious disease, Nigeria

Background

Lassa virus (LASV) causes Lassa fever (LF), an acute viral illness belonging to the group of viral hemorrhagic fevers (VHFs), including Dengue, Ebola, and Marburg fevers [1]. Lassa virus is a single-stranded ribonucleic acid virus (RNA) belonging to the Arenaviridae family and is considered a zoonotic virus [2]. It was first identified in the town of Lassa in North-East Nigeria and surmised, by molecular dating, to have originated in Nigeria more than a thousand years ago and spread to neighboring West African countries including Guinea, Liberia, and Sierra Leone where it is now endemic [3–6]. Although dengue fever is the most common VHF, LF ranks second in global burden [7]. An estimated three million LASV infections and up to 67,000 deaths occur annually in endemic regions [8]. Despite the high burden, it was long considered a neglected tropical disease until a record 633 confirmed cases reported in 2018, marked it as the largest outbreak to have occurred in Nigeria. This led to the declaration of a public health emergency both nationally and by the World Health Organization (WHO) [9, 10]. Due to its epidemic potential and limited medical countermeasures, in 2021, LASV was listed among the top ten priority pathogens on the WHO's research and development blueprint for a roadmap to outbreak response [11].

While LF cases are reported virtually all year, outbreaks peak annually in Nigeria during the dry season from December through April [4, 12]. Zoonotic transmission of LASV, primarily from *Mastomys* rodents, is the predominant mode of human infection. Transmission can occur via direct contact with infected animals, contact with contaminated household items or food, or inhalation of aerosolized viral particles from rodent droppings. However, person-to-person transmission also has been documented in situations with inadequate infection control practices [13, 14]. The incubation period for LF ranges from two to twenty one days [15]. Most infections are asymptomatic with approximately 20% of infected persons experiencing nonspecific symptoms such as fever, headache, sore throat, myalgia, and gastrointestinal symptoms also common to other VHFs and infectious diseases such as malaria and typhoid fever [16, 17]. Pregnant women are particularly vulnerable, with a high risk of maternal death and fetal loss in late pregnancy [18]. Although there are several candidate vaccines, there are currently no approved vaccines or immunotherapies to prevent or treat this illness [19]. Several laboratory tests like reverse transcriptase polymerase chain reaction (RT-PCR), antibody enzyme-linked immunosorbent assay (ELISA), antigen detection assays, and viral isolation in cell culture, can be used to definitively diagnose LF infection [15]. Unlike antibody tests, these methods allow for early detection of acute LF during the first week of symptoms by detecting the virus itself rather than the body's immune response [20, 21]. In West Africa, where LASV exposure is common, LASV-specific immunoglobulin M (IgM) antibodies without detectable viremia cannot be used for definitive diagnosis of acute LF [22]. IgM antibodies have been shown to persist for 532 to more than 800 days after initial LASV infection [22, 23].

Although the epidemiology of LF and exposure characteristics have been reported for several areas in Nigeria, there is little to no knowledge among healthy adult human populations in Abuja, the nation's capital, and Lagos, a major economic hub, despite the high volume of people moving in and out of these major cities [24]. National surveillance data suggests Lassa fever appears to be less prevalent in Lagos and Abuja compared to other parts of Nigeria [25]. The objectives of this study were to determine the seroprevalence of LASV infection and associated risk factors and co-infections in the Abuja Municipal Area Council (AMAC) and Ikorodu Local Government Area (LGA). The findings from this study provide useful information for future LASV vaccine development and implementation efforts.

Methods

Study area and population

We conducted a community-based cross-sectional study in rural and semiurban communities in AMAC, Abuja, the Federal Capital Territory (FCT), in North-Central Nigeria, and the Ikorodu LGA in Lagos State, South-West Nigeria (Fig. 1). Study sites in AMAC and Ikorodu LGA are hereon referred to as Abuja and Lagos, respectively. Nigeria is divided into six geopolitical

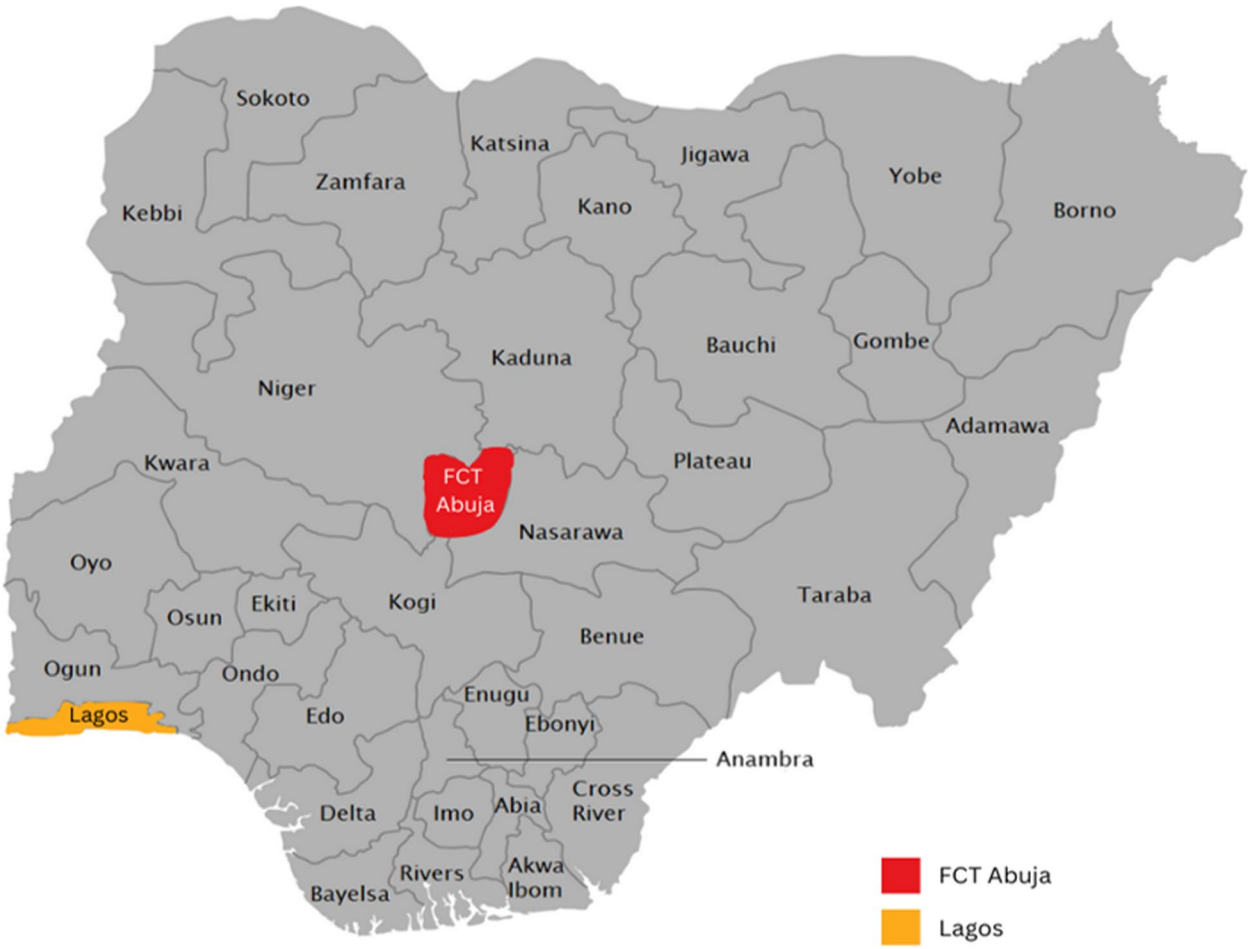

Fig. 1 Geographic map of Nigeria, with emphasis on Abuja Federal Capital Territory (FCT) and Lagos state, where recruitment communities and primary health care centers for the study were situated

regions (North-East, North-Central, North-West, South-East, South-South and South-West) with 36 states and a Federal Capital Territory, which are further divided into 774 LGAs and Area Councils, respectively, for ease of administration.

Sample size and recruitment

Enrollment for the study took place between February 2nd, 2022, and July 5th, 2022, at primary healthcare centers (PHCs) in Abuja and Lagos; an additional participant was enrolled on November 13th, 2022, to replace one who did not meet the screening criteria. The inclusion criteria were age ≥ 18 years, ability to provide written consent, willingness to provide location and contact information, and willingness to participate in study procedures.

The target sample size was achieved through a multi-stage process. In the first stage, we purposively selected the two LGAs due to their high population density, presence of a mix of urban and semi-urban communities, existing infrastructure, and established collaborations. In the second stage, following onsite assessments of PHCs for criteria such as rural/semi-urban location, functionality, community reach, collaboration, and safety, we employed random sampling to select ten PHCs from Abuja ($n=6$) and Lagos ($n=4$). Participants were recruited from communities surrounding selected PHCs and asked to meet at PHCs for study procedures. Study enrollment at each site was preceded by community engagement activities, including inaugural stakeholder meetings, advocacy visits to community heads and gatekeepers, the formation of community advisory boards, and community sensitization visits. A total of 1,271 adults in the communities were briefed about the study during the recruitment phase. In addition, individuals who routinely sought care at any of the selected PHCs were also engaged by the study staff and invited to participate. Enrollment in the study at each PHC proceeded on a sequential basis using a first-in, first-served approach. To detect city-to-city variations in Lassa seroprevalence

exceeding 5% in Nigeria from a previously estimated national prevalence of 21.3%, a sample size of 500 was needed to achieve 77% power [26]. To compensate for potential attrition of 20% due to missing data or sample loss, a target sample size of 630 was sought for an enrollment allocation of 63 (630/10) per PHC. The study involved two visits. The first visit determined eligibility and enrolled participants. The second follow-up visit provided participants with their research laboratory results and an opportunity to discuss the results with the research team. Participants were provided with compensation for their time and travel.

Ethics approval

The study was approved as minimal risk human research by the Walter Reed Army Institute of Research (study # 2760) Institutional Review Board in the United States of America (USA) and the National Health Research Ethics Committee in Nigeria. Permission was obtained from FCT/AMAC and Lagos State/Ikorodu Primary Healthcare Boards and community stakeholders to visit the communities and PHCs and perform the study procedures. Participants provided written informed consent before any study procedures were conducted. The informed consent form was reviewed with participants in detail by trained and delegated study staff before written consent was obtained.

Specimen collection and laboratory procedures

Participants were screened for potential co-infections including human immunodeficiency virus (HIV), malaria, hepatitis B, hepatitis C, and relevant conditions like pregnancy. Blood (venous and capillary) and urine specimens were collected from each participant. Venous blood and urine specimens were labelled with unique identifiers and transported under appropriate temperature conditions to the Clinical Research Centre (CRC) laboratory in Abuja or the 68 Nigerian Army Reference Hospital Yaba (68 NARHY) in Lagos for processing, testing, and storage. Routine urinalysis and malaria tests for all participants and urine pregnancy testing for female participants were performed at the CRC and 68 NARHY laboratories. Rapid HIV tests were performed on site at the PHCs. Results were provided to the participants on the same day and included pre- and post-HIV test counseling. Serum was separated and stored at -80 degrees Celsius until screening for LASV IgM and IgG antibodies, hepatitis B virus (HBV) surface antigen (sAg) and hepatitis C virus (HCV) total antibody was performed at The Defence Reference Laboratory (DRL), Abuja.

Rapid HIV testing was performed in accordance with Nigeria's national HIV rapid testing algorithm which comprised (1) Determine HIV-1/2 (Abbott, California (CA), USA) for screening followed by (2) Unigold HIV-1/2 (Trinity Biotech Plc., Ireland) for confirmation, and, (3) Statpak HIV-1/2 (Chembio Diagnostic Systems, Inc., New York, USA) if test results for (1) and (2) were discordant [27]. Malaria infection was detected with a USA Food and Drug Administration-cleared rapid diagnostic test (RDT; BinaxNOW™ Malaria, Abbott). The test also differentiated malaria infection with *Plasmodium falciparum* from less virulent panmalarial infections due to *Plasmodium vivax*, *Plasmodium ovale*, *or Plasmodium malariae*. Urine specimens were tested with a Sure-Vue® STAT Serum/Urine hCG Test Kit (Fisher Scientific, Waltham, Massachusetts, USA) for the detection of pregnancy status. Additionally, urine specimens were tested with Multistix® 10 SG reagent strips (Siemens Healthineers, Malvern, Pennsylvania, USA) for routine urinalysis.

All serum samples were screened for LASV IgG and IgM antibodies using a commercially available ELISA assay (Research Use Only (RUO), ReLASV® Pan-Lassa Combo NP/ Prefusion GP IgG/IgM ELISA Kit, Zalgen Labs, Frederick, Maryland, USA) [28]. To detect a wider range of Lassa virus infections, the assay targets both prefusion glycoprotein (GP) and nucleoprotein (NP) antigens specific to Lassa virus lineages II (Nigeria) and IV (Guinea, Liberia, and Sierra Leone) [29]. Four lineages (I-III and VI/Kako strain) have been identified in Nigeria [30]. Both IgM and IgG are considered markers of prior exposure to Lassa virus since LASV-specific IgM antibodies are not an independent surrogate marker for acute or recent infection and can persist in healthy populations for months to years after infection [22]. Thus LASV seropositivity was defined as positivity on either IgM or IgG testing. Assays were performed according to the manufacturer's guidance and methods used previously for assay evaluation for laboratory diagnostics for a vaccine development program [28, 29]. Following established methodology from a prior Nigerian study, cutoffs were determined based on the sample data set's optical density (OD) values [29]. Consistent with the reference, the negative cutoff was set at the 95th percentile ($OD < 0.250$), and the positive cutoff was set at twice the negative cutoff ($OD \geq 0.500$). Samples with OD values between these cutoffs were considered indeterminate. All other serologic assays were conducted with the following: GS HBsAg EIA 3.0 (BioRad Laboratories, Hercules, CA, USA) for screening for HBsAg, GS HBsAg Confirmatory Assay 3.0 (BioRad Laboratories) for confirmation of GS HBsAg EIA 3.0-reactive specimens, Ortho® HCV Version 3.0 ELISA (Chiron Corporation, Emeryville, CA) for screening for antibodies to HCV (anti-HCV),

and INNO-LIA™ HCV Score (RUO, Fujirebio, USA) for confirmation of anti-HCV reactive specimens.

Data collection, management, and statistical analysis

At enrollment, a physical examination was conducted, and questionnaires were administered to obtain information such as current sociodemographics, potential LASV exposures, and medical history including past and current symptoms [31, 32]. Sociodemographic data included age, sex, tribe/ethnicity, marital status, occupation, level of education, and residence/housing information. Potential LASV exposures in the past 2 years included animal and other environmental exposures, food hygiene practices, hand hygiene practices, sick contacts, health worker or other occupational exposure, participation in funerals and travel history. For analysis, food and hand hygiene practices were collapsed to a two-level categorical variable ('Always' or 'Other') from the six-level ordinal variable (coded as 'never', 'rarely', 'sometimes', 'usually', 'almost always', 'always') in the questionnaire. For animal vector exposures in the past 2 years, specific information was elicited about the presence of rodents at home, contact with rodents or rodent excreta, viewing rodent excreta on food and water/drink, rodent consumption, and history of rodent bites. Physical examination included vital signs (height, weight, body temperature, heart rate, blood pressure, and respiratory rate), whereas medical history intake included self-reported prior and current medical history and comorbidities, and self-reported prior and current LF-related symptoms. All the data collected from the hard-copy questionnaires were coded with a unique participant identification number and manually entered into a password-protected REDCap web-based database (Bethesda, Maryland) [33, 34].

Sociodemographic characteristics, LASV exposure and symptom history were described using frequencies and percentages. The seroprevalence of Lassa IgG and IgM, HIV, and HCV antibodies and HBsAg and malaria was calculated by dividing the number of participants with positive test results by the total number of participants tested. Univariate statistical testing was used to identify independent characteristics associated with Lassa seropositivity. For univariate analyses, we assessed associations between characteristics of interest and Lassa seropositivity by using prevalence ratios (PRs) with 95% confidence intervals (CIs) from log-binomial regression. We used prevalence ratios over odds ratios since odds ratios can inflate estimates of the effects of variables when the prevalence is > 10% [35].

Because our study contained a total of 109 initial predictors of interest, variable selection was performed using grouped Least Absolute Shrinkage and Selection Operator (LASSO) regression analyses, performed separately by study site using the glmnet package in R. During the regularization procedure, grouped LASSO shrinks the beta coefficients of variables without predictive power toward zero. Characteristics with nonzero beta coefficients were then selected as predictors. Grouped LASSO expands upon LASSO by selecting or not selecting variables preselected to be in a group. Dummy coded variables were grouped together for the selection process including number of pregnancies, number of live births, pregnancy outcome (live birth, spontaneous abortion/miscarriage, still birth, terminated pregnancy), and age at enrollment and place of food preparation (indoor, outdoor, indoor, and outdoor). Because predictor selection is highly variable depending on fold randomization, we iterated the grouped LASSO across 100 randomly generated tenfold partitions. The value for lambda was selected using the minimum cross validation error (MCVE) method across each iteration. Using MCVE is more conservative and selects a smaller number of predictors than the method using one standard error above the MCVE. While neither of the lambda selection techniques demonstrate greater accuracy, the conservative MCVE approach reduces false discovery rates for predictors [36, 37]. Predictors selected by grouped LASSO fifty or more times were included in a log-binomial generalized linear model (GLM) and adjusted for other selected variables by site for estimation of adjusted prevalence ratios (aPRs) [38, 39]. All *p*-values less than 0.05 were considered to indicate statistical significance. All data were managed and analyzed using SAS® (SAS Institute, Cary, North Carolina, USA, version 9.4) or R Studio software (version 4.0.3, Boston, Massachusetts, USA).

Results

Among 630 participants enrolled in the study, 628 provided blood specimens for the assessment of Lassa IgG and IgM antibodies and were included in the analysis. The participants had a median age of 38 years (interquartile range (IQR) 28–50) and were predominantly female (434, 69%) or married (459, 73%). Almost half (294, 47%) had not completed secondary school. The most common occupations reported were commerce or business (176, 28%) followed by skilled trade (145, 23%). The participants came from low socioeconomic backgrounds. Their median weekly income was ₦8,000 (IQR ₦5,000-₦15,000) (equivalent to roughly USD 6.20 (IQR 3.80–11.50) on April 26th, 2024). Typically, households had a median of 5 other occupants (IQR 4–7) living in a median of 2 rooms (IQR 1–3).

Overall, 27% (171/628) of participants were Lassa seropositive, with significantly more seropositive participants from Abuja (126/376, 33%) than from Lagos (45/252, 18%) ($p < 0.05$, Table 1). Abuja showed a seasonal

Table 1 Laboratory findings for Abuja and Lagos, Nigeria, 2022

Laboratory test	Overall		Abuja		Lagos		*p*-value
	n	(%)	*n*	(%)	*n*	(%)	
Lassa IgG antibody							< 0.0001
Positive	89	(14)	75	(20)	14	(6)	
Indeterminate	132	(21)	92	(24)	40	(15)	
Negative	407	(65)	209	(55)	198	(79)	
Lassa IgM antibody							0.0058
Positive	115	(18)	81	(21)	34	(13)	
Indeterminate	160	(25)	102	(27)	58	(21)	
Negative	353	(56)	193	(51)	160	(63)	
Lassa IgG and IgM antibodies							< 0.0001
Both negative	270	(43)	130	(35)	140	(56)	
Both indeterminate	42	(7)	30	(8)	12	(5)	
Both positive	33	(5)	30	(8)	3	(1)	
IgG negative, IgM indeterminate	95	(15)	56	(15)	39	(15)	
IgG negative, IgM positive	42	(7)	23	(6)	19	(7)	
IgG indeterminate, IgM negative	50	(8)	34	(9)	16	(6)	
IgG indeterminate, IgM positive	40	(6)	28	(7)	12	(5)	
IgG positive, IgM negative	33	(5)	29	(8)	4	(2)	
IgG positive, IgM indeterminate	23	(4)	16	(4)	7	(3)	
Lassa IgG or IgM antibodies							< 0.0001
Positive	171	(27)	126	(33)	45	(18)	
Negative/indeterminate	457	(73)	250	(66)	207	(82)	
Rapid HIV test result							0.3608
Positive	17	(2)	12	(3)	5	(2)	
Negative	611	(97)	364	(97)	247	(98)	
Rapid malaria test result							< 0.0001
Positive, *P. falciparum*	47	(7)	46	(12)	1	(0)	
Negative	580	(92)	329	(87)	251	(100)	
Not performed	1	(0)	1	(0)	0	(0)	
Hepatitis B surface antigen							0.0004
Positive	56	(9)	30	(8)	26	(10)	
Negative	550	(87)	324	(86)	226	(90)	
Missing	22	(4)	22	(6)	0	(0)	
Hepatitis C antibody							< 0.0001
Positive	45	(7)	40	(11)	5	(2)	
Negative	549	(87)	302	(80)	247	(98)	
Indeterminate	2	(0)	2	(1)	0	(0)	
Missing	32	(5)	32	(9)	0	(0)	
Urine pregnancy							< 0.0001
Positive	35	(6)	27	(7)	8	(3)	
Negative	396	(63)	210	(56)	186	(74)	
Missing	4	(1)	4	(1)	0	(0)	
Not applicable	193	(31)	135	(36)	58	(23)	

difference in seropositivity, with higher rates in the dry season than in Lagos, which did not exhibit seasonal variation (Fig. 2). Compared to those in Lagos, the prevalence of *Plasmodium falciparum* malaria (12% vs. 0%) and HCV antibodies (11% vs. 2%) in Abuja was significantly greater ($p < 0.05$, Table 1). Conversely, HBsAg prevalence was greater in Lagos (10%) than in Abuja (8%) ($p < 0.05$), while HIV prevalence was similar in both cities (Abuja

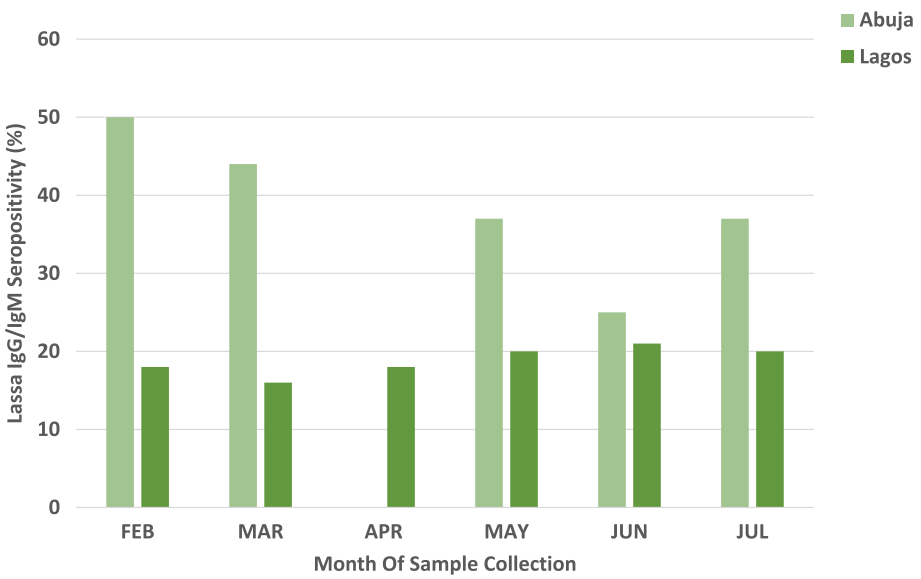

Fig. 2 Seroprevalence of Lassa IgG/IgM by month of sampling and by site, 2022

3%, Lagos 2%, $p=0.3608$). Urine pregnancy tests showed a positivity rate of 7% among women in Abuja, and 3% among women in Lagos (Table 1). Overall, LASV seroprevalence was 23% (8/35) among the pregnant women tested (Table 1).

In Abuja, ethnicity, electricity in a residence, cleanliness and storage practices in the kitchen, seasonality, prior medical history of an upper respiratory tract infection (URI), and a positive malaria RDT were significantly associated with Lassa seroprevalence in unadjusted analyses ($p<0.05$) (Tables 2, 3 and 4). Participants who reported ethnicities other than Hausa/Igbo/Yoruba had 33% lower seroprevalence of Lassa antibodies (PR, 95% CI: 0.67, 0.50–0.89) (Table 2). Participants without electricity in their residence had a 40% greater Lassa seroprevalence (PR, 95% CI: 1.40, 1.00-1.94) than did those with electricity (Table 2). Compared to participants who reported always cleaning their cooking environment or utensils after use, those who reported inconsistent or never cleaning had 40–44% greater prevalence of Lassa (PR, 95% CI: cooking environment 1.44, 1.04–2.01; cooking utensils 1.40, 1.02–1.93) (Table 3). Participants who reported inconsistently washing fruits and vegetables thoroughly before consumptions had a prevalence of Lassa that was 49% (PR, 95% CI: 1.49, 1.12–1.99) greater than participants who reported always washing fruits and vegetables before consumption (Table 3). Compared to participants who reported that they stored food without a cover, those who reported storing food with a cover had a 36% lower prevalence (PR, 95% CI: 0.64, 0.44–0.91) (Table 3). Participants enrolled in the dry season had 68% (PR, 95% CI: 1.68, 1.27–2.22) higher exposure to Lassa virus compared to those who were enrolled in the wet season (Table 3). No past or current symptoms were significantly associated with Lassa seroprevalence ($p>0.05$) (Table 4). However, participants who had a self-reported medical history of URIs had a greater Lassa seroprevalence (PR, 95% CI: 1.83, 1.08–3.10) than did those who did not (Table 4). Additionally, participants who had a positive malaria RDT at enrollment had 60% (PR, 95% CI: 1.60, 1.15–2.22) greater Lassa seroprevalence compared to those who tested negative for malaria.

After variable down-selection by grouped LASSO regression and adjustment for other down-selected characteristics in the GLM, only dry season enrollment (aPR, 95% CI: 1.73, 1.33–2.24) compared to wet season, the practice of inconsistently washing fruits and vegetables before consumption (aPR, 95% CI: 1.45, 1.10–1.92), and a positive malaria test at enrollment (aPR, 95% CI: 1.48, 1.09-2.00) were independently associated with Lassa seroprevalence (Table 5). Although marital status, ethnicity, electricity in a residence, food preservation by sun drying on the roadside or other surfaces, and food storage without a cover were selected in the grouped LASSO, these variables were not included in the final multivariate model because they did not meet the >50 selection criteria (Fig. 3A).

In Lagos, contact with a sick animal, food storage methods, and prior self-reported rhinorrhea symptoms were significantly associated with Lassa seroprevalence in unadjusted analyses ($p<0.05$) (Tables 2, 3 and 4). Participants with contact with a sick animal had at least a twofold greater (PR, 95% CI: 2.04, 1.12–3.69) seroprevalence than participants without contact with a

Table 2 Prevalence of IgM/IgG antibodies to Lassa virus by select socio-demographic characteristics of participants recruited from Abuja and Lagos, Nigeria, 2022[a]

Variable	Abuja								Lagos							
	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value
	N	%	N	%	%				N	%	N	%	%			
Sex																
Female	238	(63)	74	(59)	31.1	Referent	—	—	196	(78)	39	(87)	19.9	Referent	—	—
Male	138	(37)	52	(41)	37.7	1.21	(0.91, 1.61)	0.1878	56	(22)	6	(13)	10.7	0.54	(0.24, 1.21)	0.1325
Age																
18-24	84	(22)	27	(21)	32.1	Referent	—	—	17	(7)	1	(2)	5.9	Referent	—	—
25-29	58	(15)	17	(13)	29.3	0.91	(0.55, 1.51)	0.7210	22	(9)	7	(16)	31.8	5.41	(0.73, 39.86)	0.0976
30-39	102	(27)	32	(25)	31.4	0.98	(0.64, 1.49)	0.9105	62	(25)	12	(27)	19.4	3.29	(0.46, 23.55)	0.2356
40-49	54	(14)	23	(18)	42.6	1.33	(0.85, 2.05)	0.2085	65	(26)	10	(22)	15.4	2.62	(0.36, 19.04)	0.3425
50-59	52	(14)	23	(18)	44.2	1.38	(0.89, 2.13)	0.1508	49	(19)	10	(22)	20.4	3.47	(0.48, 25.13)	0.2182
≥ 60	26	(7)	4	(3)	15.4	0.48	(0.18, 1.24)	0.1299	37	(15)	5	(11)	13.5	2.3	(0.29, 18.18)	0.4307
Current marital status																
Married	262	(70)	96	(76)	36.6	Referent	—	—	197	(78)	37	(82)	18.8	Referent	—	—
Other	114	(30)	30	(24)	26.3	0.72	(0.51, 1.02)	0.0608	55	(22)	8	(18)	14.5	0.77	(0.38, 1.56)	0.4763
Highest level of education																
Completed Secondary School (or above)	179	(48)	61	(48)	34.1	Referent	—	—	155	(62)	26	(58)	16.8	Referent	—	—
No Schooling or Less than Secondary School	197	(52)	65	(52)	33.0	0.97	(0.73, 1.29)	0.8241	97	(38)	19	(42)	19.6	1.17	(0.68, 1.99)	0.5695
Ethnicity																
Hausa, Igbo, or Yoruba	89	(24)	40	(32)	44.9	Referent	—	—	226	(90)	37	(82)	16.4	Referent	—	—
Other Ethnicity	287	(76)	86	(68)	30.0	0.67	(0.50, 0.89)	**0.0062**	26	(10)	8	(18)	30.8	1.88	(0.98, 3.59)	0.0561
Total household income per week																
<=7500	214	(57)	69	(55)	32.2	Referent	—	—	88	(35)	18	(40)	20.5	Referent	—	—
>7500	162	(43)	57	(45)	35.2	1.09	(0.82, 1.45)	0.5486	164	(65)	27	(60)	16.5	0.80	(0.47, 1.38)	0.4284
Type of residence currently being occupied																
Other	117	(31)	38	(30)	32.5	Referent	—	—	0	(0)	0	(0)	0.00	Referent	—	—
House or Apartment	259	(69)	88	(70)	34.0	1.05	(0.77, 1.43)	0.7767	252	(100)	45	(100)	17.9	—	—	
Number of rooms in current residence																
>1 Rooms	273	(73)	86	(68)	31.5	Referent	—	—	151	(60)	28	(62)	18.5	Referent	—	—
1 Room	103	(27)	40	(32)	38.8	1.23	(0.91, 1.66)	0.1700	101	(40)	17	(38)	16.8	0.91	(0.53, 1.57)	0.7288
Number of years living in current residence																
Under 15 years	243	(65)	87	(69)	35.8	Referent	—	—	198	(79)	35	(78)	17.7	Referent	—	—
15+ years	133	(35)	39	(31)	29.3	0.82	(0.60, 1.12)	0.2113	54	(21)	10	(22)	18.5	1.05	(0.56, 1.98)	0.8858

Table 2 (continued)

Variable	Abuja								Lagos							
	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value
	N	%	N	%	%				N	%	N	%	%			
Number of other adults and children living in residence																
0-4	113	(30)	39	(31)	34.5	Referent	—	—	142	(56)	22	(49)	15.5	Referent	—	—
5+	263	(70)	87	(69)	33.1	0.96	(0.71, 1.30)	0.7863	110	(44)	23	(51)	20.9	1.35	(0.80, 2.29)	0.2665
Electricity in residence																
Yes	317	(84)	100	(79)	31.5	Referent	—	—	242	(96)	43	(96)	17.8	Referent	—	—
No	59	(16)	26	(21)	44.1	1.40	(1.00, 1.94)	**0.0471**	10	(4)	2	(4)	20.0	1.13	(0.32, 4.00)	0.855
Washing facilities in residence																
Yes	132	(35)	43	(34)	32.6	Referent	—	—	167	(66)	29	(64)	17.4	Referent	—	—
No	244	(65)	83	(66)	34.0	1.04	(0.77, 1.41)	0.7783	85	(34)	16	(36)	18.8	1.08	(0.62, 1.88)	0.7745
Water in residence																
Yes	170	(45)	63	(50)	37.1	Referent	—	—	208	(83)	37	(82)	17.8	Referent	—	—
No	206	(55)	63	(50)	30.6	0.83	(0.62, 1.10)	0.1851	44	(17)	8	(18)	18.2	1.02	(0.51, 2.04)	0.9506

[a] Prevalence of antibodies to Lassa virus by other socio-demographic characteristics can be found in supplemental table 1

Table 3 Prevalence of IgM/IgG antibodies to Lassa virus by select self-reported animal, and environmental exposure histories of participants recruited from Abuja and Lagos, Nigeria, 2022[a]

Variable	Abuja								Lagos							
	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value	Overall		Positive	Prevalence	Unadjusted Prevalence Ratio	(95% CI)		*p*-value
	N	%	N	%	%				N	%	N	%	%			
Wet or dry season																
Wet	252	(67)	69	(55)	27.4	Referent	—	—	69	(27)	14	(31)	20.3	Referent	—	—
Dry	124	(33)	57	(45)	46.0	1.68	(1.27, 2.22)	**0.0002**	183	(73)	31	(69)	16.9	0.83	(0.47, 1.47)	0.5329
Works as unskilled laborer																
Yes	27	(7)	13	(10)	48.1	Referent	—	—	25	(10)	5	(11)	20.0	Referent	—	—
No	349	(93)	113	(90)	32.4	0.67	(0.44, 1.02)	0.0639	227	(90)	40	(89)	17.6	0.88	(0.38, 2.03)	0.7657
Contact with sick animal																
No	298	(79)	99	(79)	33.2	Referent	—	—	221	(88)	35	(78)	15.8	Referent	—	—
Yes	78	(21)	27	(21)	34.6	1.04	(0.74, 1.47)	0.8153	31	(12)	10	(22)	32.3	2.04	(1.12, 3.69)	**0.0189**
Place of food preparation																
Indoor	198	(53)	62	(49)	31.3	Referent	—	—	173	(69)	34	(76)	19.7	Referent	—	—
Outdoor	97	(26)	38	(30)	39.2	1.25	(0.91, 1.73)	0.1735	39	(15)	3	(7)	7.70	0.39	(0.13, 1.21)	0.1032
Indoor and Outdoor	81	(22)	26	(21)	32.1	1.03	(0.70, 1.50)	0.8978	40	(16)	8	(18)	20.0	1.02	(0.51, 2.03)	0.9603
Cleaning of cooking environment																
Always	321	(85)	101	(80)	31.5	Referent	—	—	162	(64)	30	(67)	18.5	Referent	—	—
Other	55	(15)	25	(20)	45.5	1.44	(1.04, 2.01)	**0.0296**	90	(36)	15	(33)	16.7	0.90	(0.51, 1.58)	0.7141
Cleaning of cooking utensils after use																
Always	310	(82)	97	(77)	31.3	Referent	—	—	191	(76)	35	(78)	18.3	Referent	—	—
Other	66	(18)	29	(23)	43.9	1.40	(1.02, 1.93)	**0.0367**	61	(24)	10	(22)	16.4	0.89	(0.47, 1.70)	0.7335
Handwashing with soap and water before cooking																
Always	156	(41)	53	(42)	34.0	Referent	—	—	82	(33)	9	(20)	11.0	Referent	—	—
Other	220	(59)	73	(58)	33.2	0.98	(0.73, 1.30)	0.8724	170	(67)	36	(80)	21.2	1.93	(0.98, 3.81)	0.0586
Washing of fruits and vegetables thoroughly before consumption																
Always	284	(76)	85	(67)	29.9	Referent	—	—	204	(81)	36	(80)	17.6	Referent	—	—
Other	92	(24)	41	(33)	44.6	1.49	(1.12, 1.99)	**0.0070**	48	(19)	9	(20)	18.8	1.06	(0.55, 2.05)	0.8570
Food preservation by sun drying by the roadside or other surfaces																
Yes	121	(32)	33	(26)	27.3	Referent	—	—	45	(18)	5	(11)	11.1	Referent	—	—
No	255	(68)	93	(74)	36.5	1.34	(0.96, 1.87)	0.0872	207	(82)	40	(89)	19.3	1.74	(0.73, 4.16)	0.2136
Food storage in container with cover																
Yes	237	(63)	73	(58)	30.8	Referent	—	—	238	(94)	40	(89)	16.8	Referent	—	—

Table 3 (continued)

Variable	Abuja								Lagos							
	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value	Overall		Positive	Prevalence	Unadjusted Prevalence Ratio	(95% CI)		*p*-value
	N	%	N	%	%				N	%	N	%	%			
No	139	(37)	53	(42)	38.1	1.24	(0.93, 1.65)	0.1423	14	(6)	5	(11)	35.7	2.12	(1.00, 4.53)	0.0511
Food storage in container without cover																
Yes	36	(10)	18	(14)	50.0	Referent	—	—	8	(3)	1	(2)	12.5	Referent	—	—
No	340	(90)	108	(86)	31.8	0.64	(0.44, 0.91)	**0.014**	244	(97)	44	(98)	18.0	1.44	(0.23, 9.2)	0.6983
Food storage inside locker																
Yes	28	(7)	9	(7)	32.1	Referent	—	—	11	(4)	4	(9)	36.4	Referent	—	—
No	348	(93)	117	(93)	33.6	1.05	(0.6, 1.83)	0.8746	241	(96)	41	(91)	17.0	0.47	(0.20, 1.07)	0.0728
Food storage in cellophane bags																
Yes	63	(17)	19	(15)	30.2	Referent	—	—	24	(10)	6	(13)	25.0	Referent	—	—
No	313	(83)	107	(85)	34.2	1.13	(0.76, 1.7)	0.5452	228	(90)	39	(87)	17.1	0.68	(0.32, 1.45)	0.3211
Food storage in sacs																
Yes	206	(55)	66	(52)	32.0	Referent	—	—	34	(13)	6	(13)	17.6	Referent	—	—
No	170	(45)	60	(48)	35.3	1.10	(0.83, 1.46)	0.5051	218	(87)	39	(87)	17.9	1.01	(0.46, 2.21)	0.9726
Food storage inside cupboard																
Yes	47	(13)	19	(15)	40.4	Referent	—	—	60	(24)	10	(22)	16.7	Referent	—	—
No	329	(88)	107	(85)	32.5	0.80	(0.55, 1.18)	0.2623	192	(76)	35	(78)	18.2	1.09	(0.58, 2.07)	0.7838
Food storage by unlisted method																
Yes	23	(6)	7	(6)	30.4	Referent	—	—	6	(2)	3	(7)	50.0	Referent	—	—
No	353	(94)	119	(94)	33.7	1.11	(0.59, 2.09)	0.7523	246	(98)	42	(93)	17.1	0.34	(0.15, 0.80)	**0.0128**
Contact with a sick person?																
No	181	(48)	54	(43)	29.8	Referent	—	—	150	(60)	24	(53)	16.0	Referent	—	—
Yes	195	(52)	72	(57)	36.9	0.81	(0.61, 1.08)	0.1484	102	(40)	21	(47)	20.6	0.78	(0.46, 1.32)	0.3501
Contact with sick person, dead or alive?																
No	181	(48)	54	(43)	29.8	Referent	—	—	150	59.52	24	53.33	16.0	Referent	—	—
Yes	195	(52)	72	(57)	36.9	1.24	(0.93, 1.65)	0.1484	102	40.48	21	46.67	20.6	1.29	(0.76, 2.18)	0.3501

[a] Prevalence of antibodies to Lassa virus by other self-reported animal, and environmental exposure histories of participants can be found in supplemental table 2

Table 4 Prevalence of IgM/IgG antibodies to Lassa virus by select self-reported medical and symptom history, and study laboratory results of participants recruited from Abuja and Lagos, Nigeria, 2022[a]

Variable	Abuja								Lagos							
	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value	Overall		Positive	Prevalence		Unadjusted Prevalence Ratio	(95% CI)	*p*-value
	N	%	N	%	%				N	%	N	%	%			
Medical History																
High blood pressure																
No	327	(87)	104	(83)	31.8	Referent	—	—	207	(82)	37	(82)	17.9	Referent	—	—
Yes	49	(13)	22	(17)	44.9	1.41	(1.00, 2.00)	0.0524	45	(18)	8	(18)	17.8	0.99	(0.50, 1.99)	0.9878
Malaria requiring hospitalization or physician diagnosed																
No	265	(70)	89	(71)	33.6	Referent	—	—	140	(56)	25	(56)	17.9	Referent	—	—
Yes	111	(30)	37	(29)	33.3	0.99	(0.73, 1.36)	0.9624	112	(44)	20	(44)	17.9	1.00	(0.59, 1.70)	1.0000
Typhoid requiring hospitalization or physician diagnosed																
No	324	(86)	114	(90)	35.2	Referent	—	—	212	(84)	40	(89)	18.9	Referent	—	—
Yes	52	(14)	12	(10)	23.1	0.66	(0.39, 1.10	0.1103	40	(16)	5	(11)	12.5	0.66	(0.28, 1.58)	0.3515
Symptom history																
Currently Sick	1	(0)	0	(0)	0.0											
No	319	(85)	110	(87)	34.5	Referent	—	—	192	(76)	34	(76)	17.7	Referent	—	—
Yes	56	(15)	16	(13)	28.6	0.83	(0.53, 1.29)	0.4032	60	(24)	11	(24)	18.3	1.04	(0.56, 1.91)	0.9120
Upper respiratory tract infection																
No	366	(97)	120	(95)	32.8	Referent	—	—	207	(82)	34	(76)	16.4	Referent	—	—
Yes	10	(3)	6	(5)	60.0	1.83	(1.08, 3.10)	**0.0246**	45	(18)	11	(24)	24.4	1.49	(0.82, 2.71)	0.1930
Previous sore throat																
No	366	(97)	124	(98)	33.9	Referent	—	—	230	(91)	39	(87)	17.0	Referent	—	—
Yes	10	(3)	2	(2)	20.0	0.59	(0.17, 2.06)	0.4077	22	(9)	6	(13)	27.3	1.61	(0.77, 3.37)	0.2081
Previous rhinorrhea (runny nose)																
No	363	(97)	119	(94)	32.8	Referent	—	—	171	(68)	22	(49)	12.9	Referent	—	—
Yes	13	(3)	7	(6)	53.8	1.64	(0.97, 2.77)	0.0636	81	(32)	23	(51)	28.4	2.21	(1.31, 3.72)	**0.0029**
Any previous symptoms																
No	124	(33)	39	(31)	31.5	Referent	—	—	8	(3)	1	(2)	12.5	Referent	—	—
Yes	252	(67)	87	(69)	34.5	1.10	(0.80, 1.50)	0.5564	244	(97)	44	(98)	18.0	1.44	(0.23, 9.2)	0.6983
Laboratory Results																
Rapid HIV test																
Negative	364	(97)	122	(97)	33.5	Referent	—	—	247	(98)	45	(100)	18.2	Referent	—	—
Positive	12	(3)	4	(3)	33.3	0.99	(0.44, 2.24)	0.9895	5	(2)	0	(0)	0.0	0	(0, .)	0.9998

Table 4 (continued)

Variable	Abuja								Lagos							
	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value	Overall		Positive		Prevalence	Unadjusted Prevalence Ratio	(95% CI)	*p*-value
	N	%	N	%	%				N	%	N	%	%			
Rapid malaria test																
Negative	330	(88)	103	(82)	31.2	Referent	—	—	251	(100)	44	(98)	17.5	Referent	—	—
Positive, *P. falciparum*	46	(12)	23	(18)	50.0	1.60	(1.15, 2.22)	0.0141	1	(0)	1	(2)	100.0	4.58	(0, .)	1.000
Hepatitis B surface antigen																
Negative	324	(86)	111	(88)	34.3	Referent	—	—	226	(90)	40	(89)	17.7	Referent	—	—
Missing	22	(6)	6	(5)	27.3	0.80	(0.40, 1.60)	0.5224						—	—	—
Positive	30	(8)	9	(7)	30.0	0.88	(0.50, 1.54)	0.6342	26	(10)	5	(11)	19.2	1.09	(0.47, 2.51)	0.8458
Hepatitis C antibody																
Negative	302	(80)	105	(83)	34.8	Referent	—	—	247	(98)	44	(98)	17.8	Referent	—	—
Indeterminate	2	(1)	2	(2)	100.0	2.00	(2.00, 2.00)	.						—	—	—
Missing	32	(9)	7	(6)	21.9	0.82	(0.56, 1.19)	0.2955						—	—	
Positive	40	(11)	12	(10)	30.0	0.93	(0.68, 1.27)	0.6549	5	(2)	1	(2)	20.0	1.12	(0.19, 6.61)	0.8982
Urine pregnancy																
Negative	210	(56)	69	(55)	32.9	Referent	—	—	186	(74)	36	(80)	19.4	Referent	—	—
Not applicable	135	(36)	49	(39)	36.3	1.10	(0.82, 1.48)	0.5091	58	(23)	7	(16)	12.1	0.62	(0.29, 1.33)	0.2196
Missing	4	(1)	2	(2)	50.0	1.52	(0.56, 4.13)	0.4100						—	—	—
Positive	27	(7)	6	(5)	22.2	0.68	(0.33, 1.41)	0.2948	8	(3)	2	(4)	25.0	1.29	(0.38, 4.44)	0.6848

[a] Prevalence of antibodies to Lassa virus by other self-reported medical and symptom histories can be found in supplemental table 3

Table 5 Lassa virus antibody (IgM/IgG) prevalence ratio by site (adjusted for covariates), Abuja and Lagos, Nigeria, 2022

Variable	Comparison	Adjusted Prevalence Ratio	(95% CI)	*p*-value
Abuja				
Season	Dry vs. wet	1.73	(1.33, 2.24)	**< 0.001**
Washing fruits and vegetables before consumption	Other vs. always	1.45	(1.10, 1.92)	**0.0085**
Rapid malaria test	Positive vs. negative	1.48	(1.09, 2.00)	**0.0112**
Lagos[a]				
Previous rhinorrhea (runny nose)	Yes vs. no	2.21	(1.31, 3.72)	**0.0029**

[a] Prevalence ratio at Lagos was not adjusted for other covariates

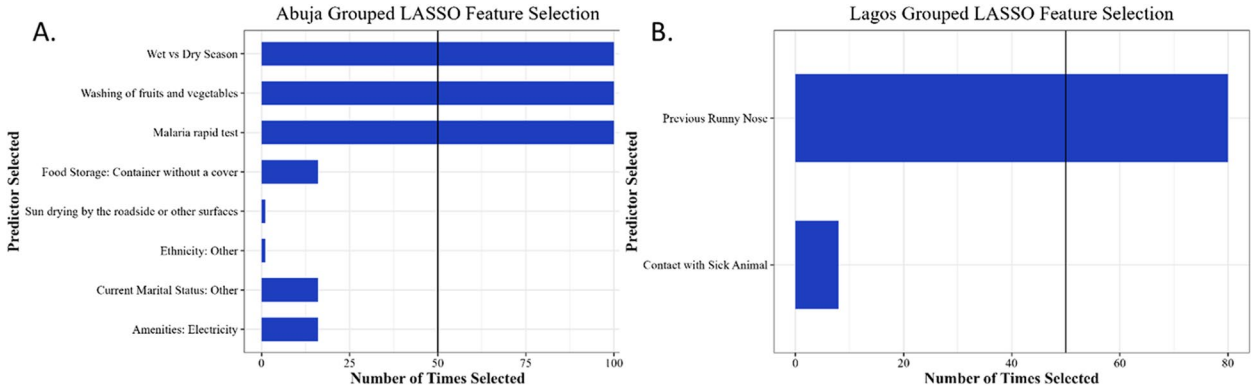

Fig. 3 **A** Variable selection for Abuja by LASSO regression analysis. **B** Variable selection for Lagos by LASSO regression analysis

sick animal (Table 3). Participants who reported having rhinorrhea (runny nose) in the past had more than two times greater (PR, 95% CI: 2.21, 1.31–3.72) Lassa seroprevalence than participants who did not report having a runny nose in the past (Table 4). In grouped LASSO regression (Table 5), only reported previous rhinorrhea was independently associated with Lassa seroprevalence, although contact with a sick animal was selected by grouped LASSO but did not meet the final selection criteria (> 50 times) (Fig. 3B).

Discussion

This community-based cross-sectional seroprevalence study was conducted to determine the extent of previous exposure to LASV and the risk factors associated with LASV infection. The overall seroprevalence was 27% and almost twofold greater in Abuja than in Lagos, with a prevalence of 33% and 18%, respectively. Seasonality, food washing before consumption, diagnosis of malaria at enrollment, and history of rhinorrhea were linked to LASV exposure.

The burden of LASV exposure estimated in this study is comparable to that in other reports from Nigeria. In 1988 the overall seroprevalence of LASV infection, measured by indirect immunofluorescence antibody testing, was estimated to be 21.3% (range of 13.4–37.5%) in the general population, hospital personnel and their contacts from areas such as Benue, Ondo, Plateau, and Gongola (present day Adamawa and Taraba states) in central, southwestern, and northeastern Nigeria [26]. A review of LF outbreaks occurring in Nigeria from 1952 to 2020 indicated that North-Central states (which include the study site of Abuja) experienced outbreaks for more years (an average of 11 years) compared to 6.8 years in South-Western states (including Lagos) [40]. Lassa virus has historically been found in the drier savannas of northern Nigeria [26]. However, LASV is prevalent in many countries in Africa with variations in population, exposure, and geographic region. A meta-analysis of 82 LASV prevalence studies in 25 sub-Saharan African countries revealed an overall prevalence of 8.7% (95% CI: 6.8–10.8%) with only West African countries having deaths due to LASV [41]. In meta-analysis, the prevalence of LASV was based on studies using various diagnostic tests, such as immunofluorescence, complement fixation, viral culture, RT-PCR, or ELISA, and included acute and convalescent samples.

The seasonality of LF is well known with outbreaks mirroring the ecology of the zoonotic reservoir, the *Mastomys* rat [42–44]. *Mastomys* populations flourish during

the wet season, providing vegetation cover and facilitating increased reproduction [45]. Human land-use practices such as clearing land for planting and harvesting crops increase human-rodent contact. The resulting food scarcity during the dry season heightens human-rodent contact, by driving rodents to seek nourishment inside human homes thereby increasing exposure to Lassa virus. This may explain the observed increase in prevalence of Lassa among participants enrolled in the dry season (versus the wet season) in Abuja compared to Lagos, where the wet and dry seasons are less distinct.

Food safety may be more of a concern for geographical areas where human land-use practices support rodent populations in homes. In Abuja, food hygiene practices (washing of fruits and vegetables) were associated with LASV seropositivity, which could be due to heightened contact with zoonotic vectors from seasonal variations in animal vector populations. However, in both cities, surprisingly, there was no connection between exposure to rodents (presence, contact, droppings, consumption, bites) and LASV infection. Unintentional and unsought contact with animal excreta has been associated with LASV seropositivity in cross-sectional population-based studies in Nigeria and Guinea. Houses with poor hygiene scores studied in a peri-urban settlement in Edo State in southern Nigeria had 50 times greater odds of reporting cases of LF than did houses with good hygiene scores [46]. In Guinea, uncovered food storage along with other factors was associated with increased LASV seropositivity [47]. Interestingly, in a cross-sectional LASV seroprevalence study in forested regions of Guinea, Kernéis et al. did not find that contact with rats or mice was a major risk factor [48]. Instead, two risk factors were identified: receiving an injection in the past year and living with someone who had bleeding symptoms. The investigators hypothesized that person-to-person transmission, perhaps in healthcare settings or close household contact, might be more important than previously thought. Although food hygiene practices and certain living conditions may be associated with the risk of LF infection, the role of rodents in transmission remains unclear. This finding might be due to limitations of our observational study design, and the established route of transmission through *Mastomys* rodents should not be discounted. The observed association between food hygiene and LASV seropositivity may be due to an indirect effect of food attracting rodents, rather than direct contact with them [49, 50].

Self-reported rhinorrhea was independently associated with Lassa infection in Lagos, but not in Abuja. Similar associations along with other indistinct symptoms such as fever, pharyngitis, and a clinical presentation with general systemic, respiratory or gastrointestinal symptoms have been reported in other LF studies conducted in West Africa [51, 52]. In a retrospective study analyzing surveillance data from Lassa patients identified in 2018–2019 from all 36 states and FCT in Nigeria, clinical presentations with general systemic, chest/respiratory, ear/nose/throat, or gastrointestinal symptoms were associated with laboratory-confirmed Lassa diagnoses as were occupations in business, trading, farming or agriculture, and male sex [53]. Since LASV infection does not have characteristic symptoms, rhinorrhea and other nonspecific symptoms can be symptoms of LF infection as well as any other respiratory illnesses that occur in the region.

Malaria (*Plasmodium falciparum*) diagnosis at enrollment was independently associated with Lassa seropositivity in Abuja but not in Lagos. This may represent an incidental association since risk factors for malaria in Abuja likely overlap with those for Lassa infection, despite seasonal variation in malaria burden with higher prevalence in the wet season [54]. Notably, a prior study conducted in Southern Nigeria, reported a high prevalence (37%) of co-infection with malaria in LF patients, but no statistically significant impact of malaria on LF outcome was observed [55]. Risk factors for malaria include poverty, less education, and poor housing conditions [56, 57]. People with lower socioeconomic status likely have limited access to preventive measures and live in housing that is not properly sealed or screened allowing mosquitoes to enter more easily, thus increasing the risk of malaria infection. Further investigation is needed to determine whether the observed association between malaria diagnosis and Lassa seropositivity in Abuja is due to confounding factors, such as socioeconomic status, which can influence both malaria and LF risk.

Our study has a few limitations. The cross-sectional design and reliance on self-reported risk factors limit our ability to definitively establish the temporal relationships between exposures and Lassa infection. Additionally, the lack of Lassa antigen/RNA testing prevents differentiation between acute/recent and past infections. Consequently, the observed associations might include a combination of both types of infections. Furthermore, indeterminate results, potentially due to early infection, low-level antibody presence, non-specific cross-reacting antibodies, or technical variability, were combined with negative results, which may underestimate the true prevalence of Lassa virus exposure. Similarly, while the ReLASV® Pan-Lassa Combo NP/ Prefusion GP IgG/IgM ELISA Kit is designed to detect a wide range of Lassa virus infections, it is important to note that four lineages (I-III and VI/Kako strain) have been identified in Nigeria [30], which could potentially impact the assay's sensitivity and specificity. Future studies may benefit from incorporating additional assays targeting these specific

lineages. While this study observed a trend of higher LF seroprevalence among participants recruited during the dry season, the limited recruitment window (February 2nd, 2022 – July 5th, 2022) likely restricts definitive conclusions regarding seasonality and necessitates further investigation across a full annual cycle to capture potential peak and trough periods. The voluntary nature of the study and purposive selection of LGAs raises concerns about its generalizability to broader LGA communities. Participation may be skewed toward individuals with a history of LF, those motivated by the offered compensation, or those who found participation convenient due to a coinciding healthcare facility visit. Future research could explore a broader range of LGAs and randomized selection of participants to enhance generalizability. Finally, restricting the study to adults only provides an incomplete picture of LASV exposure, transmission dynamics, and risk factors, potentially leading to skewed findings. Children may play a role in transmission within households and communities and may have unique risk factors for LASV infection due to their behavior, immune system development, or reliance on caregivers who might be exposed.

Conclusions

Although LASV has long been endemic to countries in West Africa, it is of global consequence due to the ease of international travel and the potential for the use of LASV as a biological weapon. This study fills a knowledge gap for two major metropolitan areas in Nigeria where LASV exposure was previously unknown. By highlighting priority populations, geographic areas, and preexisting immunity levels, our findings can inform LASV vaccine research and development, and vaccine design and testing strategies. Study findings reinforce prior literature on limiting human-rodent contact to prevent LASV transmission, although our findings focus on behavioral factors such as poor hygiene. Since proper food hygiene protects against various infectious pathogens, not just LASV, educational programs should emphasize this practice for broader public health benefits.

Abbreviations

AMAC	Abuja Municipal Area Council
anti-HCV	Antibodies to hepatitis C virus
aPRs	Adjusted prevalence ratios
CA	California
CI	Confidence interval
CRC	Clinical Research Centre
DRL	Defence Reference Laboratory
ELISA	Enzyme-linked immunosorbent assay
FCT	Federal Capital Territory
GLM	Generalized linear model
GP	Glycoprotein
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Inc	Incorporated
IQR	Interquartile range
LASSO	Least Absolute Shrinkage Selection Operator
LASV	Lassa virus
LF	Lassa fever
LGA	Ikorodu Local Government Area
MCVE	Minimum cross validation error
₦	Naira
NARHY	Nigerian Army Reference Hospital
NP	Nucleoprotein
OD	Optical density
PHC	Primary healthcare center
Plc	Place
PR	Prevalence ratio
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
RUO	Research use only
sAg	Hepatitis B surface antigen
USA	United States of America
USD	United States dollar
VHF	Viral hemorrhagic fever
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1.

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Disclaimer

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Authors' contributions

KM, MOI, ABT, LAE, PP, and ZFP initiated the study. ABT, AZ, EB, DB, KM, LAE, MM, MOI, OA, ORA, PP, SSM, SH, TM, YF, and ZFP designed the research. MJ created new software used in the study. ABT, AO, CA, CE, DE, EI, FA, JF, MA, MM, MOI, NA, NDC, NO, OA, ORA, PD, RA, SSM, TA, VA, YA gathered the data. KL, KM, LAE, NDC, NO, PP, and RA analyzed laboratory or other data. SH, OF, SF, and GS statistically analyzed all data presented in the manuscript. ABT, GS, KL, KM, LAE, NDC, VA, and SH interpreted the data. SH wrote the manuscript and MOI, ABT, AZ, EB, JF, LAE, MA, OA, ON, ORA, PD, SSM, TM, and ZFP contributed to revisions. All authors read and approved the final manuscript.

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Availability of data and materials
The anonymized data used in this study are publicly available from the Harvard Dataverse online data repository: https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/9TN21Y.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Walter Reed Army Institute of Research Institutional Review Board in the USA and the National Health Research Ethics Committee in Nigeria. Administrative approval was obtained from the Federal Capital Territory/Abuja Municipal Area Council and Lagos State/Ikorodu Local Government Area Primary Healthcare Boards and community stakeholders. Written informed consent was obtained from each participant before any study procedures were conducted. The informed consent form was reviewed with volunteers in detail by trained and delegated study staff before written consent was obtained.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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