Scientific Research Publishing

Open Journal of Medical Microbiology, 2024, 14, 120-130
https://www.scirp.org/journal/ojmm
ISSN Online: 2165-3380
ISSN Print: 2165-3372

Screening of Rodents from Rural Communities of Lagos and Ogun State Nigeria of Lassa Virus RNA

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How to cite this paper: Sarafa, F., Onyeje, E.T., Shaibu, J.O., Salu, O.B., Audu, R.A. and Omilabu, S.A. (2024) Screening of Rodents from Rural Communities of Lagos and Ogun State Nigeria of Lassa Virus RNA. *Open Journal of Medical Microbiology*, **14**, 120-130.
https://doi.org/10.4236/ojmm.2024.142010

Received: December 18, 2023
Accepted: June 24, 2024
Published: June 27, 2024

Abstract

Background: In recent times, there has been an increase in the number of Lassa fever cases resulting from the several episodes of Lassa fever epidemics ravaging Nigeria and other West African countries. The presence of Lassa virus in rodents other than the major reservoir (*Mastomys natalensis*) has been a public health concern as to the actual burden of the disease. It is therefore of a public health necessity to explore the LASV RNA habouring potential of several species of rodents in endemic as well as non-endemic areas for proper prevention of emergence of outbreaks in non-endemic areas. **Objectives:** The aim of this study was to detect the presence of LASV RNA in different species of rodents in Ikorodu, Lagos state and Abeokuta, Ogun state. **Methods:** A total of ninety one (91) rodents were captured from Ikorodu, Lagos State (61 rodents) and Abeokuta, Ogun State (30 rodents), euthanized, bled, and plasma obtained for the detection of LASV RNA by Reverse Transcriptase Polymerase Chain Reaction. **Results:** A total of 91 rodents consisting of 77 *Rattus rattus* and 14 *Crocidura spp*. The S segment of LASV RNA was not in any of the 91 rodents' plasma samples. **Conclusion:** The rodents captured within the rural communities of Ikorodu, Lagos State and Abeokuta, Ogun State were found not to habour the LASV RNA. This study is limited by the relatively small sample size. Similar studies should be encouraged both in endemic and non-endemic areas in order to understand the actual burden of Lassa fever as well as put into check future epidemics.

Keywords

Lassa Virus, Lassa Fever, *Mastomys natalensis*, Transmission, Lagos State, Ogun State

1. Introduction

Lassa virus (LASV) is an enveloped bi-segmented negative-sensed single-stranded ribonucleic acid (RNA) virus that belongs to the Arenaviridae family [1] [2]. The *Mammarenavirus* is the causative agent of Lassa fever [3], a zoonotic-borne infection and one of the viruses-causing severe viral haemorrhagic fever (VHF) in Africa. The LASV was first reported from Lassa, a village located in Borno state, Nigeria in 1969 [3]. Since then, there have been repeated outbreaks in Nigeria [4] and other West African countries, particularly Sierra Leone, Liberia, Togo, and the Republic of Benin [5] [6] [7].

The incubation period of Lassa fever is between 3 and 21 days and is presented with clinical manifestations which are usually variable and often non-specific including fever, general weakness and headache experienced in about 80% of infected individuals [8]. However, in untreated cases, disease may progress into more serious symptoms including hemorrhage (bleeding in gums, eyes, or nose), respiratory distress, repeated vomiting, facial swelling, pain in the chest, back, and abdomen, shock, and the neurological problems have also been described including hearing loss and tremors [8] [9].

The disease is prevalent in West Africa, mainly in Nigeria, Sierra Leone and Liberia. There have also been a few cases in Mali, northern Côte d'Ivoire and Guinea [10] [11]. It is estimated that it affects between 100,000 and 300,000 people yearly, approximately 100,000 - 300,000 people contract LASV, and 5000 people die from the infection [12]. Large scale outbreaks of Lassa Fever have been reported in Nigeria since 2015 with the disease occurring all year round. This is characterized by increased suspected and confirmed cases with high mortality rates especially in Ondo, Edo and Ebonyi states [13]. From 2017 to 2020, Lagos and Ogun states in Nigeria reported 13 (11 in 2017, 1 in 2018, and 1 in 2020) and 9 (7 in 2017 and 2 in 2020) confirmed Lassa fever cases with just 0.79% of the total confirmed cases in Nigeria [14].

Although the natural reservoir for Lassa virus is the African multimammate soft-furred rat (*Mastomys natalensis*) discovered in 1974 [15], predominant throughout West Africa, there have been several reports suggesting that other species of rodents including *Mastomys natalensis*, *Hylomyscus pamfi*, *Mastomys erythroleucus*, *Rattus rattus* can also habour the LASV [16] [17] [18]. LASV is transmitted by either zoonotic transmission by the ingestion of food or materials contaminated with faeces or urine of infected rodents [19] and Human-to-human transmission by direct contact with infected tissues and body fluid including blood, semen, and breast milk [20] [21] [22]. LASV can also be transmitted via aerosols [23] [24] [25].

Major concerns for LASV cases increase in recent times in Nigeria have raised concerns regarding the actual burden of Lassa fever in the country coupled with reports suggesting other rodents other than the *Mastomys natalensis* can also be reservoirs for this LASV. Nonetheless, these reports have not provided convincing evidence through species-level identification of the rodent implicated in LASV transmission. Due to an increased incidence rate of Lassa fever in Nigeria, the role of the animal reservoir and the mode of transmission which could be vertical (zoonotic) or horizontal (human to human) cannot be overemphasized. It is therefore crucial to ascertain the ability of several species of rodents to habour LASV in both Lassa endemic and non-endemic areas for the purpose of proper and effective measures in preventing emergence of outbreaks in non-endemic areas and reoccurrence in endemic areas as well as creating more insight and future research in the virus-host relationship in Nigeria. The study sought to screen rodents in some non-endemic areas of Nigeria of the presence of LASV and the specie of rodents implicated in LASV transmission in the area.

2. Methods

2.1. Ethical Approval

The study ethical approval for this research was obtained from the Institutional Review Board (IRB) of the Nigerian Institute of Medical Research (NIMR) with IRB protocol number IRB-19-006.

2.2. Settings

This study was a cross-sectional study conducted at Igbo-olomu, Igbogbo, Ebute, Owode, Ibeshe and Ori okuta communities in Ikorodu Local Government Area, Lagos state (6.62˚N, 3.51˚E) and Olomore and Totoro communities in Abeokuta-North Local government Area, Ogun state (7.15˚N, 3.36˚E), Nigeria between April 2020 and March 2021. Although Lagos state and Ogun state are both non-endemic areas for Lassa fever, yet positive cases have been reported in these parts of the country over the years. Also, due to the inter-state border between Ogun state and Ondo state (a Lassa endemic region), and between Lagos state and Ogun state. Consequently, possible migration of rodents from endemic to non-endemic areas is possible.

2.3. Sample size

The formula of the sample size determination [26] was used to estimate the number of adults needed for this study;

$$n = \frac{z^2 pq}{e^2}$$

where, n = the minimum sample size required for this study, z = 95% confidence level (standard value of 1.96), e = margin of error at 5% (standard value of 0.05), p = prevalence of LASV in rodents estimated at 1.6% (*i.e.* 0.016) [27], $q = 1 - p =$

$1 - 0.1 = 0.984$.

The minimum sample size required for the study is estimated to be 24.

2.4. Rodent Trapping and Identification

Live traps were used to capture rodents from houses, surrounding bushes, dumpsites and marketplaces. This was carried out by placing baits into the live traps and placed in strategic locations. The traps were inspected from time to time and captured rodents were extracted from the trap for identification and sampling. All rodents captured were included in this study. Rodent captured were identified by a taxonomist by physical observation of specific body features and coloration. Features like dorsal view, ventral view, tail shape and length, body length, head shape and morphology were keys to the identification of rodents.

2.5. Specimen Collection, Handling, Transportation and Processing

Trapped rodents were then euthanized with chloroform, bled via cardiac puncture and the blood was collected in EDTA-coated blood collection tubes and centrifuged at 2200 rpm for 15 minutes. The plasma was then transferred into a clean screw-cap vial. All vials were labeled appropriately. The specimens were cold-chain transported in triple level packaging to the Centre for Human and Zoonotic Virology Laboratory (CHAZVY), Central Research Laboratory, College of Medicine of the University of Lagos (CMUL) for storage at −80˚C. Specimens were then cold chained transported to the Centre for Human Virology and Genomics, Nigeria Institute of medical Research (NIMR) for processing in a Biosafety level 3 plus Laboratory. Universal sample and handling precautions were carried out as recommended by the United States Centre for Diseases Control and Prevention [19]. All specimen transport containers were disinfected with 10% hypochlorite solution in an airtight glove box before opening.

2.6. Nucleic Acid Extraction and Reverse Transcriptase-Polymerase Chain Reaction

Plasma samples were collected from the freezer and thawed at room temperature. Viral nucleic acids were then extracted employing the Jena Bioscience viral RNA + DNA preparation kit (Jena Bioscience GmbH, LoebstedterStrasse, Germany) in a Class IIA biological safety cabinet according to the manufacturer's instructions. After the extraction of viral nucleic acid, S segment of the RNA genome, 3' non-coding region and 5' non-coding region of the nucleic acid of LASV were amplified in quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and discrete reverse transcriptase polymerase chain reaction (RT-PCR) with primers as listed in Table 1. The quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed for each of the RNA samples employing a RealStar Lassa Virus RT-PCR kit 2.0 (Atona Diagnostics, Hamburg Germany). The conventional reverse transcriptase polymerase chain reaction (RT-PCR) was also performed on the nucleic acid extracts. Complimentary DNA (cDNA) synthesis was carried out using Jena Biosciencekit (Jena Bioscience GmbH, LoebstedterStrasse, Germany). RT-PCR was then performed on the cDNAs using the MiniAmp Plus thermocycler with the programmed cycling conditions shown in **Table 2**. A shorter fragment of the amplicons from the primary PCR was further amplified in a nested RT-PCR using the MiniAmp Plus thermocycler with cycling condition as the initial amplification. Subsequently, PCR amplicons were subjected to 1.8% agarose gel electrophoresis with 1X SYBR®Safe DNA gel staining dye (Invitrogen, Carlsbad, California, United States) for 30 min at 120 V/400mA and images of amplicon bands under UV light were taken with a BioDocAnalyze 2.0 (Biometra, Goettingen, Germany). The positive control used for Lassa assays were previously detected Lassa samples from Irrua, Edo State, Nigeria with accession number GU481078 NIG 08-A47 2008 IRRUA.

3. Result

A total of 61 (66.9%) and 30 (33.1%) rodents were captured within Ikorodu, Lagos state and Abeokuta, Ogun state respectively. **Figure 1** shows the distribution of rodents into species where 14 (15.3%) of rodents captured were *Crocidura* spp while 77 (84.7%) were *Rattus rattus.* Of the Rattus rattus trapped, 47 (61%) were trapped from Ikorodu, Lagos state while 30 (39%) where trapped from Abeokuta, Ogun state as shown in **Table 3**. No *Crocidura* spp was captured in Ogun state where. As presented in **Table 4**, analysis of rodents' plasma by RT-PCR (both qRT-PCR and conventional RT-PCR) showed that none 0/91 (0%) of the rodents captured both in Ikorodu, Lagos state and Abeokuta, Ogun state was positive for LASV-RNA. The expected amplicons band size of approximately 320

Table 1. RNA sequence of primers.

Name	Sequence	Amplicon size
Fwp	5'CTT TTA TGT TGA GAA MAG TGG CTT GGG GTG 3'	~320 bp
Rvrs-p	5'CCT GTA AAT GGA CGC CCC C 3'	

Table 2. Cycling conditions of polymerase Chain reaction.

	Temperature (˚C)	Time (min: sec)	Cycles
RT inactivation/denaturation	94	02:00	1
Denaturation	94	00:30	40
Annealing	57	00:30	40
Extension	72	01:30	40
Final extension	72	02:00	1

Table 3. Distribution of the rodents by trapping location.

Species	LAGOS	OGUN	Total (%)
Rattus rattus	47 (61)	30 (36)	77 (100)
Crocidura spp	14 (100)	0 (0)	14 (100)
Total	61 (67)	30 (33)	91 (100)

Table 4. Distribution of the rodents by species.

Species	LASV RNA POSITIVE (%)	LASV RNA NEGATIVE (%)	Total (%)
Rattus rattus	0 (0.0)	77 (84.7)	77 (84.7)
Crocidura spp	0 (0.0)	14 (15.3)	14 (15.3)
Total	0 (0.0)	91 (100.0)	91 (100.0)

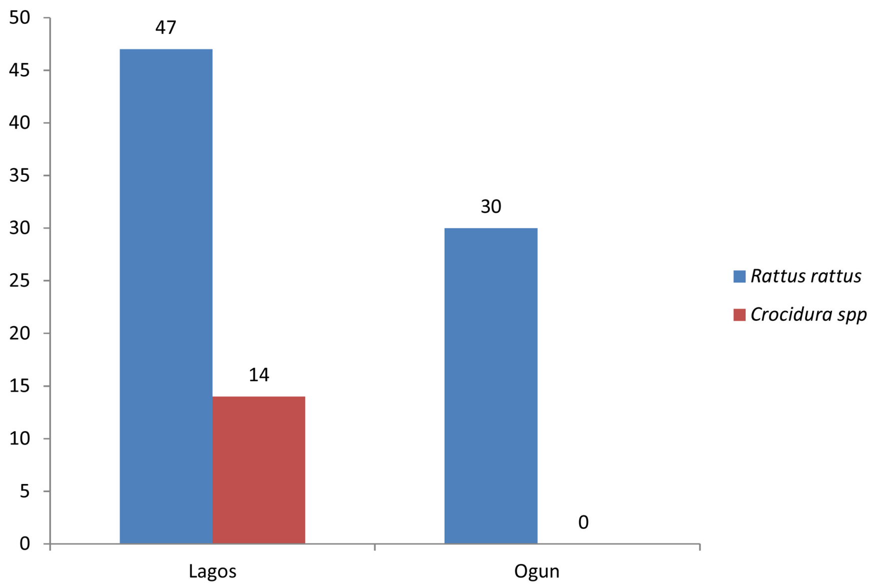

Figure 1. Distribution of the rodents between study locations.

base pairs (bp) of the S segment of the RNA genome for Lassa virus (LASV) were not detected as shown by the agarose gel electrophoresis analysis as shown in **Figure 2**.

4. Discussion

The persistent emergence and re-emergence of Lassa fever outbreak has placed a burden on vulnerable populations in West Africa and Nigeria in particularly due to the progressive increase in suspected and confirmed cases of LASV infection in recent times. It continues to be a public health burdens and remains a disease of global health importance based on the possibilities of its importation from endemic to non-endemic regions or countries where it has never been experienced before. The investigation, focusing on the study of the Lassa virus reservoir,

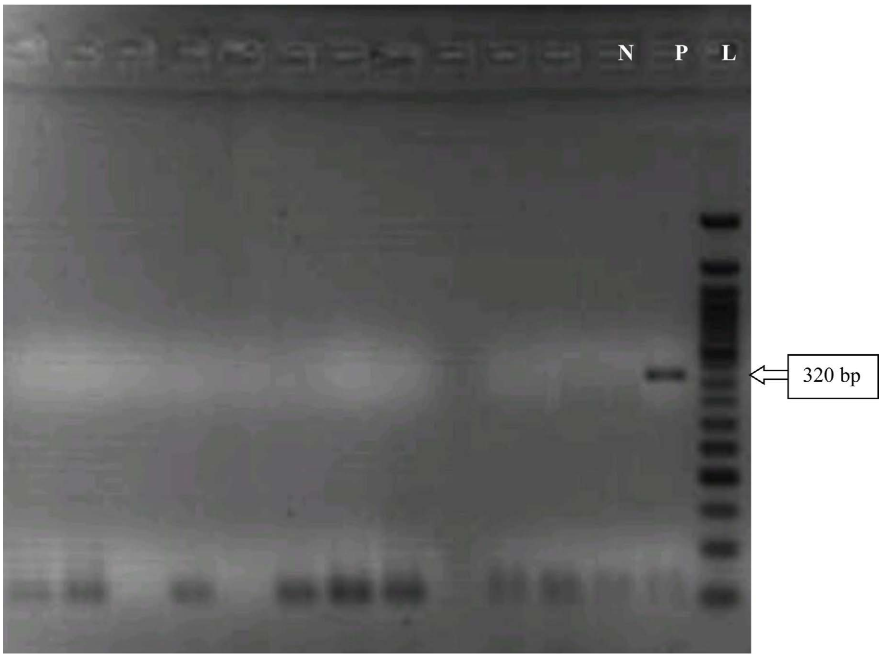

Figure 2. Gel electrophoresis picture of the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) detection of S gene fragment of LASV in rodents of the rural communities of Ikorodu and Abeokuta, Lagos state. The rodents samples shows a negative reverse transcription polymerase chain reaction for Lassa fever virus on agarose gel. Key: N-Negative control; P-Positive control; L-Ladder or Biomarker.

carries significant implications for both the epidemiology and control of Lassa fever. This is due to the notable variations in the ecology of different rodent species. While Mastomys natalensis, a primary carrier of LASV, has distinct ecological characteristics, such as being commensal, the same cannot be said for species like Hylomyscus pamfi (forest dwelling), Mastomys erythroleucus (more of a generalist) [18], Crocidura spp (insectivore and generalist), and Rattus rattus (commensal) [26].

From this study, none of the rodent was found to carry the LASV RNA. In contrary to this finding, a study by Agbonlahor *et al*. (2017) [27] which investigated the presence of Lassa virus amongst trapped rodents from Edo, Delta and Bayelsa states in Nigeria reported a total prevalence of Lassa virus to be 1.6% with individual prevalence to be 3.4% (Edo), 1.2% (Delta) and 0.2% (Bayelsa) [27]. This absence of LASV in this area may be due to the relatively low sample size of this study. Also, the fact that no *Mastomys natalensis* was trapped in this study may contribute to the outcome, although *Rattus* rattus has also been implicated in LASV transmission [16]. A study by Wulffet al, confirmed the isolation of LASV in *Rattus rattus* which has since been of a great epidemiological significance proving its implication in LASV spread [16]. A study by Olayemi *et al*. detected the presence of LASV RNA in *Hylomyscus pamfi* captured from Kako, southwestern part of Nigeria [18]. Their study supports the hypothesis that *H. pamfi* and *M. erythroleucus* are full-fledged reservoirs and not just incidental hosts thereby increasing insight into the genetic and ecological complexity of the virus.

In the study by Agbonlahor *et al.* (2017) [27], 1 (0.2%) of the rodent captured in the Bayelsa, a Lassa fever non-endemic area carried the Lassa virus RNA while none of the rodents captured in Lagos and Ogun (Lassa non-endemic areas) carried the Lassa virus RNA in this current study. This may be due to difference in sample size as well as ecological, environmental and virological difference. Although, Lagos and Ogun state, Nigeria had only reported a relatively low confirmed cases in from 2017 to 2020 [14], Based on the outcome of this study, it can be deduced that cases reported in this geographical location during this period were imported cases from other endemic parts of the country rather than vertical transmission from rodents to human as revealed by the sequence data generated from some isolates recovered from patients suspected of LASV infection in 2018/2019 by Omilabu, S.A (unpublished data).

The primary consideration in controlling Lassa fever is the mode of transmission. This study indicates that cases in this region are likely imported, emphasizing the ongoing threat of horizontal transmission (human-to-human transmission). To mitigate this risk, effective control measures should focus on enhancing environmental and household hygiene [28]. Maintaining a vigilant awareness for suspected cases and promptly referring individuals displaying signs and symptoms to designated Lassa treatment centers is essential. Additionally, implementing proper infection control practices in healthcare facilities within the localities is crucial for effectively managing and preventing the spread of this infection [29]. Nevertheless, effective rodent control in areas close to living environment, avoiding contact with rodents and the non-consumption of it should still be encouraged to limit infections.

Findings from this study might not be sufficient to generalize the fact that rodents in Ikorodu, Lagos and Abeokuta, Ogun states do not habour the LASV-RNA. Regardless, this study has given a pointer as to the possible transmission route suggesting possible migration of infected individuals from Lassa endemic to non-endemic areas. As a response to the upsurge in the Lassa fever cases in the country, it is imperative to implement means preventing future outbreaks in non-endemic regions. Conducting more studies of this nature would reveal the potential emergence of Lassa fever in novel niches beyond the current occurrences in Nigeria and West Africa as a whole. This information is crucial for developing comprehensive strategies to monitor and control the spread of Lassa fever.

This study explored the transmission dynamics of this virus in the rodent population in a bid to unravel the proportion of the changing epidemiology of Lassa virus in Nigeria. It also offers new opportunities in understanding rodent to rodent interactions in the harbouring of Lassa virus and how they co-exist within our communities as markers for strengthening the Lassa fever outbreak early detection and surveillance, warning alerts and rapid response implementation in endemic and vulnerable settings.

5. Conclusion

This study suggested that the rodents present in Lagos and Ogun state do not habour the LASV RNA. The consequence is that future outbreak emanating from zoonotic transmission in the study area is unlikely, although potential transfer of the diseases from endemic geographic region is still possible which can be responsible for events of future outbreaks. Despite the findings from this study, the availability of these rodents and their roles in the carriage and transmission of Lassa virus requires further evaluations.

Limitation of Study

This study is limited by the relatively small sample size to the large geographical area of the sample location. Similar studies should be encouraged both in endemic and non-endemic study in order to understand the actual burden of Lassa fever as well as put into check future epidemics.

Challenges

We were only able to sample ninety one subjects which is a small sample size compared to the magnitude of the objective and relatively long sampling period. This was due to the series of lockdown episodes in Nigeria during the Covid 19 pandemic.

Funding

This work was financially supported by the seed grant (1NIMREX0002-19-01) from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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