Prevalence of Lassa virus among rodents trapped in three South-South States of Nigeria

D.E. Agbonlahor¹-², A. Erah³, I.M. Agba³, F.E. Oviasogie⁴, A.F. Ehiaghe¹-³, M. Wankasi², O.A. Eremwanarue¹, I.J. Ehiaghe¹, E.C. Ogbu¹, R.I. Iyen¹, S. Abbey², M.Y. Tatfeng² & J. Uhunmwangho⁵

¹Lahor Research Laboratories and Medical Centre, Benin City, Edo State; ²Department of Medical Laboratory Science, College of Health Sciences, Niger Delta University, Amassoma, Bayelsa State; ³Department of Medical Laboratory Science, Ighemedion University, Okada; ⁴Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City; ⁵Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

ABSTRACT

Background & objectives: Lassa fever has been endemic in Nigeria since 1969. The rodent Mastomys natalensis has been widely claimed to be the reservoir host of the Lassa virus. This study was designed to investigate the distribution of species of rodents in three states (Edo, Delta and Bayelsa) of Nigeria and to determine the prevalence of Lassa virus amongst trapped rodents in the selected states.

Methods: Rodents were trapped during November 2015 to October 2016 from the three states in South-South region of Nigeria. Total RNA was extracted from the blood collected from the trapped rodents. Reverse transcription polymerase chain reaction (RT-PCR) was used to confirm the presence of Lassa virus in the rodents.

Results: The results revealed that six species of rodents were predominantly present in these geographical locations. Mus musculus (39.4%) had the highest prevalence, closely followed by Rattus rattus (36.1%), R. fuscipus (20.3%), M. natalensis (2%), Myosoricinae soricidae (1.2%) and R. norvegicus (1%). The overall positivity (carrier rate) of Lassa virus was 1.6% amongst the 1500 rodents caught in the three states. In Edo and Delta States, the RT-PCR results showed presence of Lassa virus in R. rattus, M. musculus and M. natalensis. On the other hand, only M. natalensis was detected with the virus, amongst the species of rodents caught in Bayelsa State. M. natalensis recorded the highest Lassa virus among rodents trapped in Edo (87%), Delta (50%) and Bayelsa (11%) States respectively.

Interpretation & conclusion: The rather low Lassa virus positive among rodents in Bayelsa State of Nigeria may explain the absence of reports of outbreak of Lassa fever over the past 48 yr in the state. The results also confirmed that apart from Mastomys natalensis, other rodents such as Rattus rattus and Mus musculus may also serve as reservoirs for Lassa virus. From the findings of this cross-sectional study, it was concluded that a more comprehensive study on rodents as reservoir host, need to be undertaken across the entire states of Nigeria, for better understanding of the epidemiology and endemicity of Lassa fever.

Key words Bayelsa; Delta; Edo; Lassa virus; Mastomys natalensis; Nigeria; rodent

INTRODUCTION

Lassa fever is a viral haemorrhagic fever that was first recorded in 1969 in the town of Lassa in the northeast of Nigeria¹. It is endemic in the West African countries including Nigeria, Sierra Leone, Guinea and Liberia²-³. It is caused by a single stranded RNA virus, which is a member of Arenavidae family and its primary natural host is the rodent, Mastomys natalensis, which live in close proximity to humans⁴. The infected Mastomys rodents drop urine/excreta containing the virus on floors and uncovered food items⁴-⁵. The virus can be transmitted to humans through direct contact with infected materials⁶.

The uncertainty about the precise natural host of Lassa virus is considered a major obstacle in the control of the disease. Although, Mastomys natalensis is majorly associated with the spread of Lassa virus⁴-⁶, some reports suggest that other species of rodents could also be possible reservoir hosts for Lassa virus⁷-⁸. Okoror et al⁹ reported an incidence of 46.79% Lassa virus seroconversion in Mastomys natalensis among rodents trapped in a Lassa fever endemic village of Ekpoma in Edo State, Nigeria. Outbreaks of Lassa fever have been reported annually from different states of Nigeria including Edo and Delta States; however, it is not reported from Bayelsa¹⁰. This study was designed to investigate the distribution of rodent species in the above three States of Nigeria and also attempts were made to confirm the presence of Lassa virus amongst trapped rodents from these states.
MATERIAL & METHODS

Rodent trapping

Rodents were trapped during November 2015 to October 2016 from homes, neighbouring environments and dump sites, using cages, rat gums and local traps. A total of 1500 rodents were trapped from three states in South-South Region of Nigeria, viz. Edo, Delta and Bayelsa (500 rodents from each state). Two (Edo and Delta) of the selected are endemic for Lassa fever. Edo State divided into three senatorial districts (Edo North, Edo Central and Edo South). From Edo North (Auchi), 100 rodents were trapped, while 200 each were trapped from Edo Central (Ekpoma and Irrua) and Edo South (Benin and Okada) respectively. Delta State divided into three Senatorial district, viz. Delta North Senatorial District (DNSD), Delta Central Senatorial District (DCSD) and Delta South Senatorial District (DSSD). A total of 200 rodents were trapped from DNSD (Agbor) and DCSD (Efurrun), respectively while 100 rodents were trapped from DSSD (Oleh). Bayelsa State is also divided into three Senatorial districts (Bayelsa West, Bayelsa Central and Bayelsa East). A total of 200 rodents were captured from Bayelsa West (Sagbama and Ekeremor) and Bayelsa Central Senatorial Districts (Yanagoa and Amasaoma) respectively while, 100 rodents were trapped from Bayelsa East Senatorial Districts (Ogbia and Otoke).

Sample collection, preparation and rodents’ phenotypic characterization

Blood was collected from the trapped rodents through cardiac puncture and was preserved at 4°C for total RNA extraction as described by Christine et al. After blood collection, the rodents were preserved in 10% formal in for characterization. Morphological characteristics such as colour, length of rodent, length of tail and body weight were used to identify trapped rodents as described by Fichet-Hoch et al.

Polymerase chain reaction

RNA extraction: Total RNA was extracted using the whole-blood RNA Mini Prep (Zymo Research, Irvine, CA) as previously described by Ehiaghe et al. A 70 µl of the extracted RNA was transferred into a RNA stable tube supplied by Biomatrica, Inc., San Diego (Catalog No. 93221-001) for storage at room temperature after proper drying.

Reverse transcriptase polymerase chain reaction (RT-PCR): The extracted RNA was reverse-transcribed and amplified using one Taq one-step RT-PCR kit (catalog No. NEB E5315S), supplied by New England Bio Labs Incorporation (Massachusetts), according to the manufacturer’s specification. Forward and reverse primers (ATATAATGATGATGACTGTGTGTTTTTGTGCA; ACAGGGAGTCTAGGATTATT) were used to target Lassa virus glycoprotein complex template using Peltier thermal cycler PCR machine at the Lahor Research Laboratories, Benin City, Nigeria. The PCR was performed in a 50 µl reaction mixture containing 25 µl one Taq one-step reaction master mix (2×), 2 µl one Taq one-step enzyme mix (2×), 2 µl of each gene-specific forward primer (10 µM), 2 µl of each gene-specific reverse primer (10 µM), 9 µl of nuclease-free water and 10 µl of the RNA template was then added. The PCR was programmed as reverse transcription at 48°C for 30 min; initial denaturation at 94°C for 1 min; denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec; and extension at 68°C for 1 min, repetition of the denaturation step for 39 cycles; final extension at 68°C for 5 min and final holding at 4°C. A total of 5 µl of the amplified PCR products were analyzed on 1.5% agarose gel containing ethidium bromide in 1×Tris EDTA buffer. Electrophoresis was performed at 90 V for 30 min with the EDVOTEK tetra source electrophoresis machine, Bethesda, USA. The targeted genes were visualized using Wealtec (USA) Dolphin-Doc UV trans-illuminator and photographed. Molecular weights were calculated using molecular weight standard marker (100–1000 bp).

Ethical approval

The study was approved by the various local Governments health authorities in Edo, Delta and Bayelsa States. Ethical approval was also obtained from the Ethics Committee of Lahor Research Laboratories and Medical Centre, Benin City, Edo State, Nigeria with reference number LRL/005/034.

RESULTS

The overall distribution of rodent species found in the three states (Edo, Delta and Bayelsa) is shown in Table 1. In total six species of rodents were trapped, with M. musculus (39.4%) having the highest prevalence for Lassa virus, closely followed by R. rattus (36.1%), R. fuscipus (20.3%), M. natalensis (2%), Myosoricinae soricidae (1.2%) and R. norvegicus (1%). The Table 2 summarizes a comparative data of distribution of rodent species in the study states according to their Lassa virus positivity. A total of rodent species were trapped in Edo State, whereas six species of rodents were trapped in Delta and Bayelsa.
States. Comparatively, R. rattus had the highest prevalence in the three states. The RT-PCR results were positive for R. rattus, M. musculus and M. natalensis in Edo and Delta State (Table 2 and Fig. 1). Positive RT-PCR result was obtained only from M. natalensis rodent caught in Bayelsa State. Overall, M. natalensis was observed to be the most potent carrier of Lassa virus in Edo (87%), Delta (50%) and Bayelsa (11%) states respectively. This study also revealed an overall positivity of 1.6% for Lassa virus amongst the 1500 rodents trapped in the three states. The Edo State showed highest positive cases of Lassa virus with a prevalence of 3.4%, closely followed by Delta (1.2%) and Bayelsa State (0.2%).

**DISCUSSION**

The results of the study showed that six species of rodents are commonly found in Edo, Delta and Bayelsa States of Nigeria, with M. musculus (39.4%) having the highest prevalence of Lassa virus, closely followed by R. rattus (36.1%), R. fuscipus (20.3%), M. natalensis (2%), M. soricidae (1.2%) and R. norvegicus (1%). During the study, R. fuscipus was not found in the Edo State. This finding is in tandem with an earlier study conducted by Demby et al14 and Keenlyside et al15 wherein, their reported prevalence varied from of 0 to 9% in different places. According to them the prevalence of rodent species in Africa depends on the geographic location. In this study, the overall prevalence of positivity of Lassa virus was 1.6% amongst the 1500 trapped and studied rodents. Positive RT-PCR results for Lassa virus from M. natalensis were detected from the three states. In addition, Lassa virus was detected in R. rattus and M. musculus in Edo and Delta States, which suggests that other rodent species could be involved in the transmission of Lassa virus in this region. This is in conformity with earlier findings, which showed that other species of rodents might also serve as possible reservoir host for the maintenance and transmission Lassa virus7-8. These findings might explain the endemicity, wide-spread nature and the perennial outbreaks of this 48-yr-old deadly disease in Nigeria. One such very devastating and widest spread outbreak of Lassa fever oc-
occurred in 2012 in Nigeria. The states affected were Edo, Delta, Ondo, Rivers, Ebonyi, Kano, Yobe, Benue, Kaduna, Kogi, Bauchi, Adamawa, Abia, Anambra, Imo States and the Federal Capital Territory, Abuja. Earlier studies have shown that rodent population density influence the distribution and transmission of Lassa virus in varying geographic locations. Proper identification and documentation of prevailing reservoir rodents should be an important pre-requisite in any meaningful effort aimed at controlling the menace of Lassa fever in an endemic nation such as Nigeria.

The *M. natalensis* species showed highest positivity (carrier rate) for Lassa virus in the present study. The positivity of Lassa virus was relatively low in rodents trapped in Bayelsa State. This might explain the reason behind less outbreaks of Lassa fever in Bayelsa State as compared to other states in Nigeria including Edo and Delta states. The arenaviruses infect their respective rodent hosts either by vertical virus transmission (dams-to-progeny) or horizontal transmission, while fighting for the dominance or survival. Eradication of arenaviruses is not a feasible goal because these pathogens (Lassa virus) are usually harboured by wild rodent population. However, if exposure of infected rodents can be reduced by avoiding rodent-human contact, the infection spread would drastically reduce. Prevention is always better than cure; hence, it should be obligatory to consider the sustainable preventive strategies that would ensure the reduction of rodent-human contact through sustained Lassa fever enlightenment programme, prevention of rodents from entering homes, ensuring that all food items must remain properly covered in homes and those working with clinical specimens wear protective clothing (such as masks, gloves, gowns and goggles), and safeguarding that infected patients are isolated from unprotected persons until the disease completes its course. These measures are very important because outbreaks of Lassa fever in Nigeria between 1969 and 2016 has claimed over 100,000 lives and its still counting.

**CONCLUSION**

Based on the study results it was concluded that apart from *M. natalensis*, other rodent species such as *R. rattus* and *M. musculus* may also serve as reservoirs for Lassa virus. Hence the nations, such as Nigeria, which are endemic to Lassa fever, should mandatorily put in place sustainable preventive strategies that would ensure the reduction of rodent-human contact.

**Conflict of interest**

The authors declare that they have no conflict of interests.

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*Correspondence to:* Prof. D.E. Agbonlahor, Department of Medical Laboratory Science, College of Health Sciences, Niger Delta University, Amassoma, Bayelsa State, Nigeria.

E-mail: deagbonlahor@yahoo.com

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