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Predictors, Prevalence and Spatial Analysis of Lassa Virus among Household Rodents in a Low Socioeconomic Community in Southwestern Nigeria

[1*]**Aniekan Eric Ulor**, [1]**Godson Ana** and [2]**Adedayo Faneye**
[1]Department of Environmental Health Sciences, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.
[2]Department of Virology, UCH, Ibadan, Nigeria.
*Corresponding Author Email: aniekaneric@gmail.com

Abstract
Lassa fever is an acute viral endemic disease of public health importance in West Africa. The main reservoir for Lassa virus is the multimammate mouse, *Mastomys natalensis*. Due to increased outbreaks of Lassa fever, investigations for the presence of this virus in household rodents are pertinent. This study assessed the predictors and prevalence of the Lassa virus, providing spatial analysis of the virus among household rodents. A total of 35 households were systematically sampled. Traps were placed in each of the sampled households for four weeks and rodents captured were identified using morphological characteristics. The viral screening was done on trapped rodents using reverse transcriptase-polymerase chain reaction (RT-PCR) with primers targeting the s-gene of the virus. Maps arising from the spatial analysis were generated using ArcMap 10.1 to show rodent-infested areas and areas prone to an outbreak of the disease. The frequency of rodents captured was presented in percentages. A total of forty-four (44) rodents were captured. Three species of rodents were trapped from the selected households with *Rattus rattus* (43.2%) being the dominant species in the households followed by *Rattus fuscipes* (38.6%) and *Rattus norvegicus* (18.2%). From the PCR results, two samples of the *Rattus rattus* tested positive followed by *Rattus fuscipes* and *Rattus norvegicus* which had one rodent each, respectively testing positive for the virus. Geo-spatial maps were generated to show rat infestation density and areas prone to an outbreak.Common household rodents such as *Rattus rattus*, *Rattus fuscipes,* and *Rattus norvegicus* tested positive and these species of rodent were previously not known to be a host. Therefore, awareness should be encouraged.

Keywords:
Lassa fever,
Transmission,
Predictors,
Prevalence,
Spatial mapping.

Des Prédicteurs, la prévalence et l'analyse spatiale du virus de Lassa chez les rongeurs domestiques dans une communauté socio-économique défavorisée du sud-ouest du Nigeria

Résumé
La fièvre de Lassa est une épidémie virale aiguë d'importance de santé publique en Afrique de l'Ouest. Le principal réservoir du virus Lassa est la souris multi mammaire, Mastomys natalensis. En raison de l'augmentation des épidémies de fièvre de Lassa, les enquêtes sur la présence de ce virus chez les rongeurs domestiques sont pertinentes. Cette étude a évalué les facteurs prédictifs et la prévalence du virus de Lassa en fournissant une analyse spatiale du virus chez les rongeurs domestiques. Au total, 35 ménages ont été systématiquement échantillonnés. Des pièges ont été placés dans chacun des ménages échantillonnés pendant quatre semaines et les rongeurs capturés ont été identifiés à l'aide de caractéristiques morphologiques. Le dépistage viral a été effectué sur des rongeurs piégés à l'aide d'une réaction en chaîne par transcriptase inverse-polymérase (le RT-PCR) avec des pièges ciblant le gène's', du virus. Des cartes résultant de l'analyse spatiale ont été générées à l'aide d'ArcMap 10.1 pour montrer les zones infestées de rongeurs et les zones susceptibles à une épidémie de la maladie. La fréquence des rongeurs capturés a été présentée en pourcentages. Au total, quarante-quatre (44) rongeurs ont été capturés, trois espèces de rongeurs ont été piégées dans les ménages sélectionnés, Rattus rattus (43,2%) étant l'espèce dominante dans les ménages, suivi de Rattus fuscipes (38,6%) et

Mots-clés:
Fièvre de Lassa,
Transmission,
Prédicteurs,
Prévalence,
Cartographie spatiale

Rattus norvegicus (18,2%). D'après les résultats de PCR, deux échantillons de Rattus rattus ont été testés positifs, suivis de Rattus fuscipes et de Rattus norvegicus, qui avaient respectivement un rongeur testé positif pour le virus. Des cartes géo spatiales ont été générées pour montrer la densité d'infestation de rats et les zones sujettes à une épidémie. Les rongeurs domestiques courants tels que Rattus rattus, Rattus fuscipes et Rattus norvegicus ont été testés positifs et ces espèces de rongeurs n'étaient auparavant pas connues pour être un hôte. Par conséquent, la sensibilisation doit être encouragée.

Introduction

Lassa fever an endemic disease caused by the Lassa fever virus and is of public health concern in West Africa. The pathogen, a single-stranded RNA virus that is zoonotic or animal-borne, is a member of the virus family Arenaviridae. (1). The main reservoir for Lassa virus is the multimammate mouse, *Mastomys natalensis*. These rodents are known to live in bushes, but during the dry season, they run to houses for protection and live with humans where they deposit excreta on floors, tables, beds, and food (4). Transmission from rodents to humans occurs through direct exposure to rodent fluids such as urine, saliva, and blood or indirect exposure via surfaces and foodstuffs contaminated by these fluids (5).

Multimammate rats are ubiquitous in grasslands and cleared forests across Sub-Saharan Africa. They commonly invade the domestic environment, where transmission to humans is thought to most frequently occur (3). In Africa, Lassa fever and Mastomys distribution are associated with ecological factors such as height variability, seasonal timing of rainfalls, and other possible explanatory variables. Since most outbreaks of Lassa fever have been observed to occur in regions with annual rainfall above 1500mm, it has been suggested that the Lassa virus may survive better in humid conditions during the rainy season (1). Housing characteristics and domestic organization affect rodent density in and around households and villages and are likely to be a risk factor for Lassa fever in humans where a reservoir exists (3).

Although the multimammate mouse, *Mastomys natalensis* is the major reservoir for the Lassa fever virus, other rodent reservoirs (*Mastomys erythroleucus* and *Hylomyscus pamfi*) have also been recently identified (4). Common household rodents such as House Mouse (*Mus musculus*), the Deer mouse (*P. maniculatus*), Norway Rats (*R. norvegicus*), etc., are not known to be reservoirs of the virus but can be spotted in most homes in Nigeria and could be a potential reservoir for the virus. Therefore, to control this deleterious disease, there is a need for more information on risk factors for Lassa fever particularly those related to the predictors and prevalence that could predispose a community to the transmission of Lassa fever. This study assessed the predictors and prevalence of the Lassa virus, providing spatial analysis of the virus among household rodents.

Materials and Methods

Description of Study Area

This study was carried out in Awule Village (Figure 1) located in Akure South Local Government Area of Ondo State (7̊10'N 5̊05'E) in southwestern Nigeria, bordering Ekiti State to the north, Kogi state to the northeast, Edo State to the east, Delta State to the southeast, Ogun State to the southwest and Osun State to the northwest. The community is a nucleated one characterized by overcrowding of both houses and humans with pockets of fertile grassland, growing a variety of crops such as maize, pepper, vegetables, cocoa, and others. The topography of Awule Village can be described as undulating and hilly (22).

Study Design and Population

The study employed a descriptive cross-sectional design with a field component. Traps were placed in each of the households sampled. Morphological identification was done on rodents captured from each of the households. The target population was made up of adult men and women within the age group of 18-65 years living in Awule Village. Eligible participants should have been living in the community for a minimum period of about one year and their households were selected within the study area for the study.

Sample Size

The sample size was obtained using the cross-sectional study design formula (10). From the formula, a total of 35 households were systematically sampled for the study.

Geographical Coordinates of Trap Sites

A Garmin handheld GPS device was used to take the coordinates. The accuracy of measuring distance was set at three metres and was carried along during the field survey. It aided in marking the coordinates of sampled locations in the study area.

Rodent Density Determination

In each of the selected houses for the study 5 – 10 traps were set along a specific transect in and around the house with baits. Traps were checked every morning and night for four (4) weeks. Rodents trapped were taken to the laboratory for species identification using standard precautions (11, 12). Spatial analyses were also carried

out to assess vulnerability levels using a Geographic Information System (GIS).

Handling and Transportation of Samples

Plastic air-tight and leak-proof containers were used to transport the trapped rodents. The external surface of the container was disinfected with 3% sodium hypochlorite. All samples were labelled with unique sample identification. Specimens were accompanied by a documentation sheet (packaged separately from the sample) including the sample's unique identification, date/time/place of sampling, and type of specimen.

Identification of Rodents/Dissection and Collection of Organs

Each sample (rodents) collected from the study area was identified using morphological characteristics and then dissected (11, 12). Organ harvesting was carried out on the rodents. Vital organs such as the heart and kidneys were harvested from the rodents and macerated. The tissues obtained were placed in Phosphate Buffer Saline (PBS) and stored at -80°C until analysis.

Molecular Detection of Lassa Fever Virus

Molecular Detection of Lassa Fever Virus: Total RNA was extracted from the stored tissues from the harvested organs using a commercially available total RNA purification kit by Jena Biosciences (Jena, Germany) according to the manufacturer's instruction. The extracted RNA was transcribed to cDNA with random hexamers using cDNA synthesis kit by Jena Biosciences (Jena Germany). The cDNA was then tested for Lassa fever virus by PCR using primers targeting the S-gene (36E2: AAC GGG GAT CCT AGG CAT TT, and LVS-339-Rev: GTT CTT TGT GCA GGA MAG GGG CAT KGT CAT to give an amplicon size of 320bp) of the Lassa fever virus genome in a 25µl reaction following the protocol described thus; Five microliters of the 5X red load taq mix produced by Jena Biosciences (Jena Germany) and one microliter each of 0.06 'PM of forward and reverse primers, five microliters of cDNA template and 13µl of PCR grade water. The PCR was performed in the Applied Biosystems 9700 thermal cycler programmed as; thus, 950C for 5 minutes followed by 40 cycles of 950C for 30 seconds, 520C for 30 seconds and 720C for 30 seconds and one cycle of 720C for 7 minutes. The amplicon was taken through agarose gel electrophoresis to assess amplification. The positive sample has the expected 336 base pairs bands when viewed under the transilluminator.

Ethical Considerations

Ethical clearance (UI/EC/18/0697) with a registration number (NHREC/05/01/2008a) was obtained from the University of Ibadan/UCH Research Ethics Committee before the commencement of the fieldwork. Consent was obtained from community stakeholders and heads of households. Permission to commence work in the households was received from community leaders after proper information dissemination about the project. Assent was also obtained from each of the study households. There was no form of coercion, and confidentiality was maintained with each household given a specific identification number.

Results

Rodent density in households

The distribution of rodent species found in households in the study area is shown in Table 1. A total of 44 rodents were captured; three species of rodents were trapped from the selected households with *Rattus rattus* (43.2%) being the dominant species in the homes of the study area followed by *Rattus fuscipes* (38.6%) and *Rattus norvegicus* (18.2%). The highest number of rodent samples captured in a house was between 5-6 samples and the least number of rodents captured was 1-2 samples throughout the study period. Fig. 3 shows the frequency of rodents captured according to geographical zones. The highest number of rats was captured in the northeastern part of the study area while the eastern part of the study area had the lowest number of rats captured.

Lassa fever virus screening

Of the 44 rodents from households tested for Lassa fever virus by PCR, four (4) had the virus gene representing 0.09% of the total rat density. *Rattus rattus* had the highest number (2) of rodents testing positive for Lassa fever virus, while *Rattus fuscipes* and *Rattus norvegicus* had one rodent sample each testing positive for the virus (Table 1). Rodents that tested positive for the Lassa fever virus were caught in houses 23, 24, 25, and 31 representing 11.4% of the total number of households sampled.

Geospatial maps showing rat-infested areas and Lassa fever hotspots

Global Positioning System (GPS) coordinates of trapped rodents were transferred to Arc GIS 10.1 software for spatial analysis. The maps (Figures 6 & 7) show the ranges of rodents caught per trap site. The size of the dots reflects the number of rodents caught per trap site and how the rodents are distributed in the study area (Figure 7). The map showing hotspots, levels of severity, vulnerable areas for a possible outbreak of Lassa fever in the study area is shown in Figure 7.

Table1: Distribution of rodent species and number of Lassa virus cases in the study area

Rodent Species	Frequency	No of Positive
Rattus rattus	19 (43.2%)	2 (50%)
Rattus fuscipes	17 (38.6%)	1 (25%)
Rattus norvegicus	8 (18.2%)	1 (25%)
Total	**44 (100%)**	**4 (100%)**

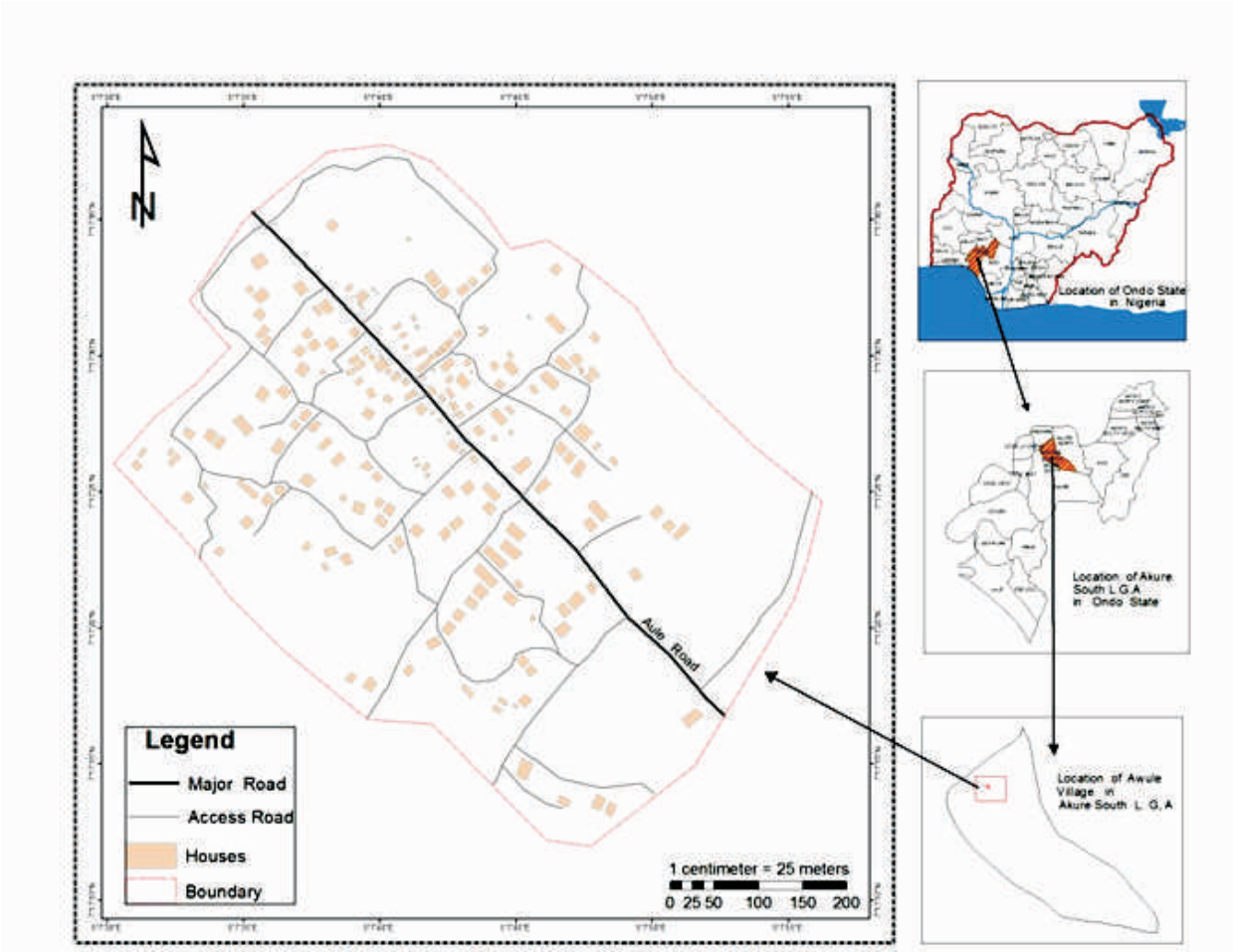

Figure 1: Map of the study area (Awule community)

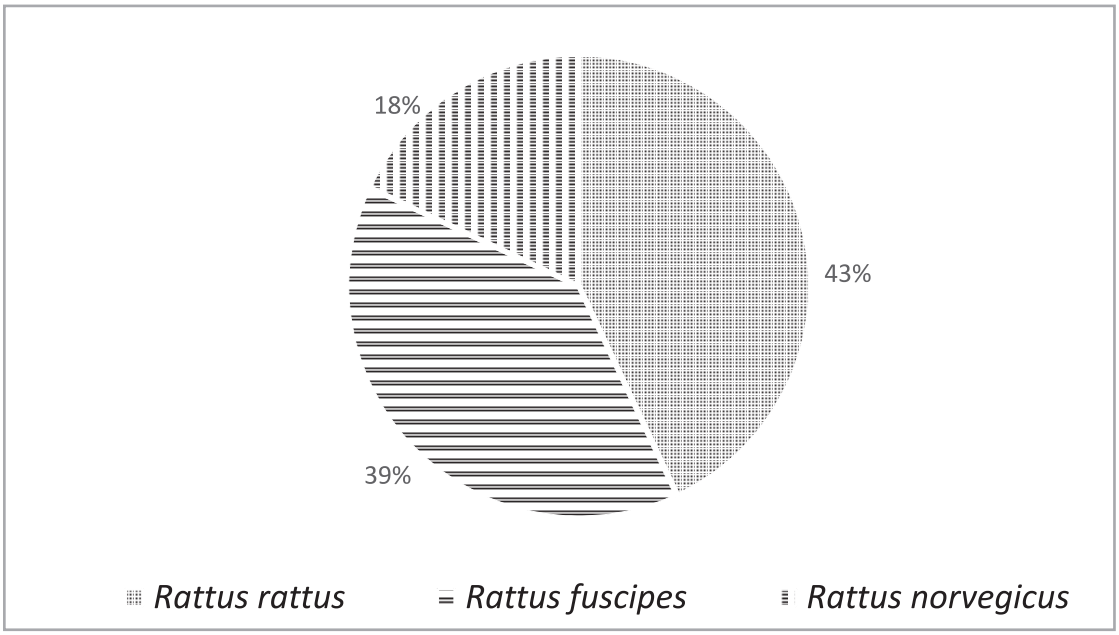

Figure 2: Chart distribution of rodent species in the study area

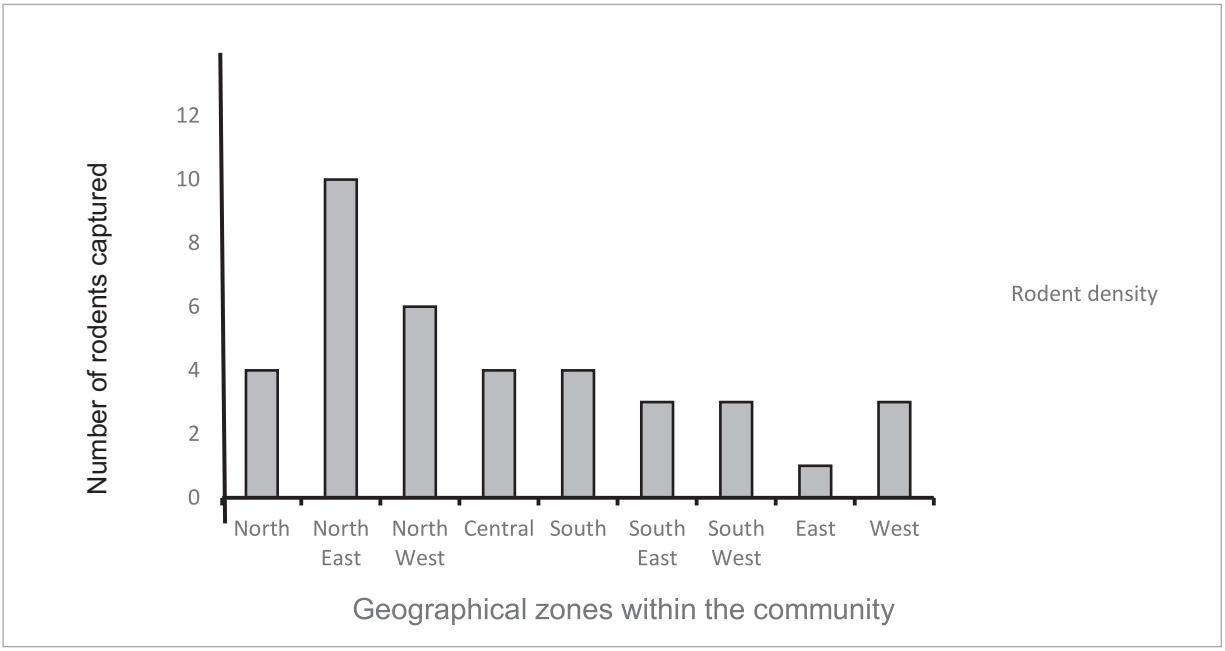

Figure 3: Number of rodents captured according to geographical zones in the study area

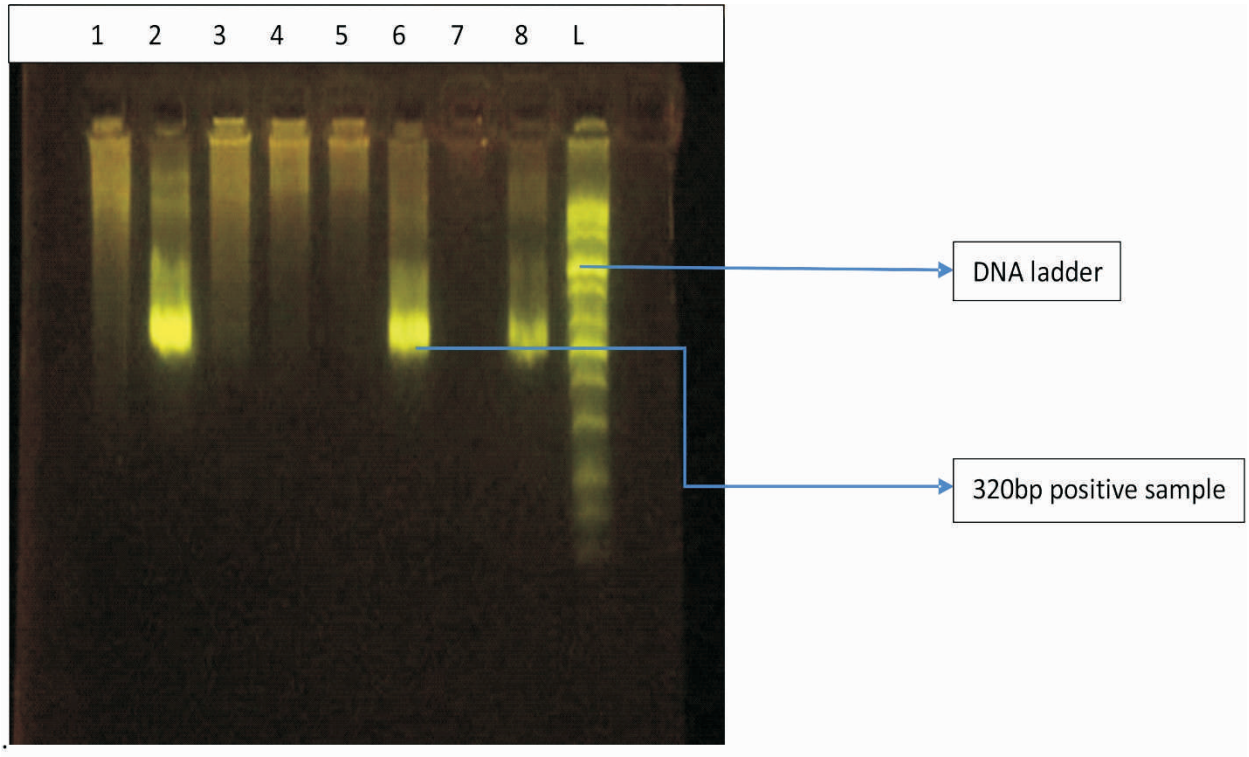

Figure 4: RT- PCR results showing expected band size for the amplified fragment of Lassa fever virus (Agarose gel electrophoresis: Lanes 2, 6 and 8 have the expected 320bp positive band while lane L is the 50 base pair Ladder).

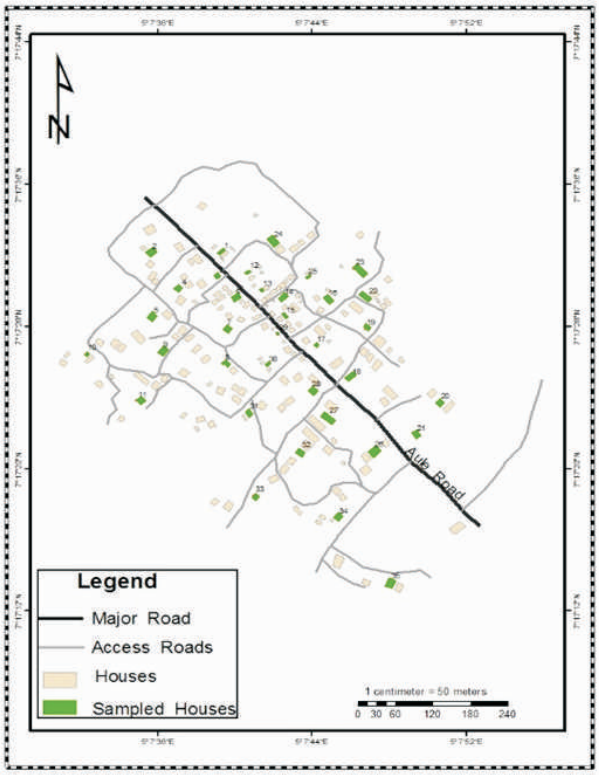

Figure 5: Map showing the sampled houses in the study area

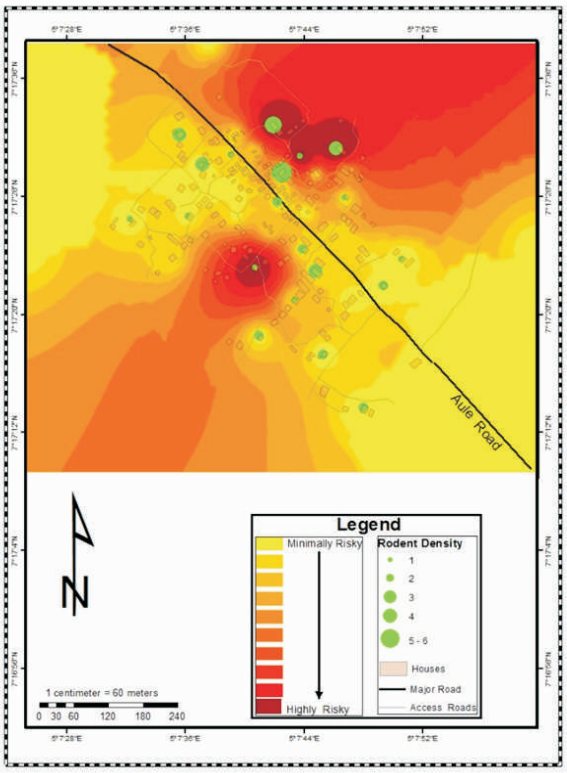

Figure 7: Geospatial map showing hotspots and vulnerable areas for a possible outbreak of Lassa fever in the study area

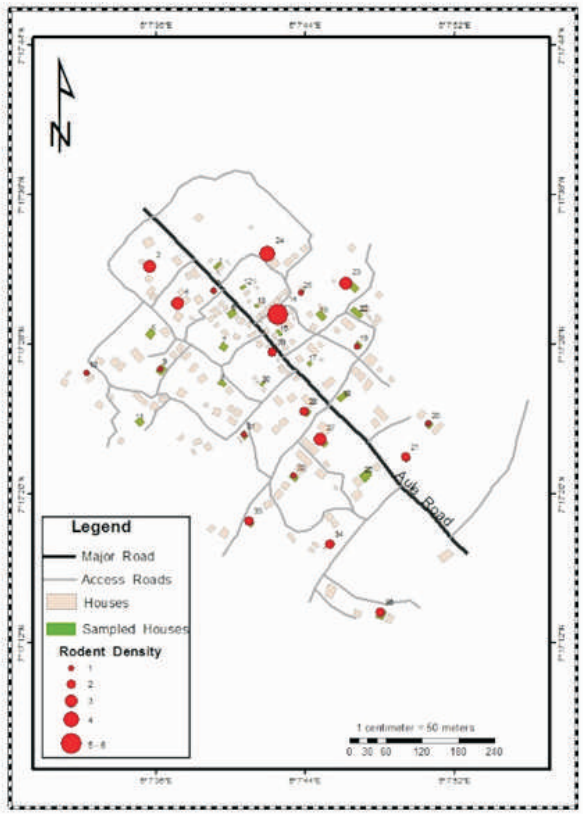

Figure 6: Map showing rodent density in households in the study area

Discussion

This study evaluated the rodent frequency in households associated with the spread of the Lassa fever virus in households and communities in Southwestern Nigeria using Awule Village. *Mastomys natalensis* is the primary host of the virus but was not found among the species captured in households in this study. Other species caught tested positive for the virus as reported previously (23). Apart from *Mastomys natalensis* other rodents such as R*attus rattus,* and *Mus musculus* may also serve as reservoirs for the Lassa virus (23).

From the geo-spatial analyses, it is obvious that most houses in the northeastern part of the community had a higher proportion of rodents in their homes. Ecology studies suggest that rodent abundance in houses doubles during the dry season indoors, possibly as a result of limited food supply outdoors and increased food supply indoors (14). Geographical Information System (GIS) has been extensively utilized in determining the locations of epidemics of infectious diseases, and in the estimation of the presence of diseases or vectors at non-sampled locations (18). The geo-spatial analysis carried out in the study area showed a predictive overview of areas minimally at risk to areas that are highly at risk of infections (Fig 7). ArcGIS has the capability of using interpolation tools to

measure points and estimate related features at unmeasured sites (20).

GIS has been used to find the relationship between a particular disease, environmental factors, and the spread of disease over a particular time in a particular location (19). The spatial analysis made in this study showed a predictive map of vulnerable areas in the community where preventive measures should be highly implemented. Such maps help public health policies and research in targeting disease control and studies in potentially infected areas (18). The map (Fig 7) predicted that there is a possibility of rodent to human transmission in this area of the community with reference to the close proximity of the households where the rodents captured tested positive. GIS often functions as a decision support system because it aids in data analysis and may give new insights to stakeholders and help in their decision making. Also, it is a powerful tool that enhances the measurement, monitoring, mapping, and modelling of geographic data (19). From the findings made from this study, prompt measures should be carried out immediately to prevent any immediate outbreak of the disease in the community.

Conclusion

Lassa fever is a severe acute infection caused by the Lassa virus. Common household rodents such as *Rattus rattus*, *Rattus fuscipes,* and *Rattus norvegicus* tested positive. These species of rodent were previously not known to be a host showing that *Mastomys natalensis* is not the only reservoir for the virus. Geographical maps were generated to show rat infestation density and areas prone to an outbreak of the disease. The geo-spatial analysis predicted possible transmission of the disease. Persons living in the study area are prone to an outbreak, hence awareness and effective surveillance should be encouraged.

Conflict of interests

The authors declared no conflict of interest.

Acknowledgments

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