# Recent isolations of Lassa virus from Nigerian rodents

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*Rodents were trapped in the Benue-Plateau and North-Eastern States of Nigeria where Lassa fever had been reported in previous years. Eight Lassa virus strains were isolated from tissues and blood of rodents identified in the field as being of 3 different species:* Mastomys natalensis, Rattus rattus, *and* Mus minutoides. *All the infected rodents were collected in village habitats. These isolations indicate the presence of Lassa virus in wild rodents in Nigeria during periods when no human infections were evident.*

*Prior studies in Sierra Leone have indicated that a single rodent species,* M. natalensis, *may be the important reservoir host of Lassa virus. Since the present study indicates that other rodent species may be involved as well, the ecology of Lassa virus may be more complicated than was heretofore supposed. In view of the importance of determining the geographic and species range of rodent hosts of Lassa virus, and because of the problems inherent in rodent identification under austere field conditions, it is urgent that further studies be conducted in the same areas of Nigeria to confirm these findings.*

## INTRODUCTION

Lassa virus was isolated from rodents of the species *Mastomys natalensis* during an outbreak of Lassa fever in Sierra Leone (1). Concurrent epidemiological investigations and subsequent experimental studies (2) have strongly indicated that this species is an important reservoir host. Whether rodents of this or other species might also be the reservoirs of Lassa virus in Nigeria was investigated by testing for the presence of virus in tissues, blood, and urine of rodents trapped at various places in Benue-Plateau (BP) State and in North-Eastern (NE) State of Nigeria. The animals were collected during November and December 1972, and March and April 1973, at which times no human Lassa fever cases were known to be occurring in Nigeria.

This report describes the isolation of 8 strains of Lassa virus from tissues and blood of rodents trapped in Nigerian villages. Possible implications of the findings are considered.

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## MATERIALS AND METHODS

### *Field collection and shipment*

Rodents were captured either by snaptrap or by hand and were bled and dissected in the field. The liver, lung, kidney, and spleen of each animal were pooled and sealed in vials. Serum and urine and/or bladder from some of the animals were also saved for testing. The specimens were frozen in liquid nitrogen and shipped by air to the Center for Disease Control (CDC) in Atlanta, Georgia. At the CDC, the specimens were transferred to the Maximum Security Laboratory and kept in a mechanical freezer at −70°C until they were processed for virus isolation.

### *Cell cultures*

A continuous African green monkey kidney cell line (Vero) was used for Lassa virus isolation. The cell cultures were supplied by the Tissue Culture and Reagents Section, CDC, as monolayers grown in tissue culture tubes. In the laboratory, 3-day-old cultures were put on maintenance medium, which consisted of Eagle's minimum essential medium (MEM) to which inactivated bovine fetal serum was added in the proportion 2 ml/100 ml. The pH was adjusted with sodium bicarbonate to a level of 7.2–7.4. The cultures were used for isolation attempts 1–2 days later.

*Virus isolation*

Each tissue pool was made into a 10 g/100 ml suspension in maintenance medium and centrifuged, and 0.1 ml of the supernate was inoculated into each of 3 Vero cell culture tubes. Blood specimens were inoculated as received unless quantities were too small; in such instances they were diluted 1 : 2 or 1 : 4. The maintenance medium was changed 1 day after inoculation, and the cultures were observed every second day for 9–10 days for the appearance of a cytopathic effect (CPE). If CPE developed, the culture fluids with cells were used as antigen in the complement fixation (CF) test for virus identification (see below). A second Vero cell culture passage was made when nonspecific degeneration of the cell sheet occurred; the remaining cells were scraped from the glass surface, and 0.1 ml of a diluted suspension was inoculated into each of 3 fresh Vero cell culture tubes. The cultures were observed again for a period of 9–10 days and were then discarded if negative or used for identification if positive.

*Identification of isolates*

Lassa virus isolates were identified by the CF test method described by Casey (3), modified for use of microtitre plates. The " infected " culture fluid of each possible isolate was diluted in twofold steps starting with a dilution of 1 : 2. A standard dilution (1 : 80) of guinea-pig immune serum with a CF titre of 1 : 320–1 : 640 against the L. P. (Pinneo) strain of Lassa virus was added to all wells. As positive control antigen, a Vero cell culture harvest of the L. P. strain of Lassa virus was used and titrated in the same way as the harvest of the possible isolate. A new isolate was considered to be typed as Lassa virus when it had about the same titre (usually 1 : 16 or 1 : 32) as the L. P. antigen. When weak fixation of complement occurred, the possible isolate was passed once more in Vero cell cultures and evaluated again. A normal Vero cell culture antigen and a normal guinea-pig serum were included in each test.

## RESULTS

Rodents were trapped in or near houses, but also on farmland, such as rice fields and corn plots, and in rocky areas.

Table 1 presents the results of attempts to isolate Lassa virus from 151 rodents trapped in or near human dwellings. In all, 150 tissue pools, 29 blood specimens and 44 urine specimens were inoculated into Vero cell cultures. Lassa virus was isolated from tissue pools and blood specimens of 5 rodents field-

Table 1. Lassa virus isolations from rodents trapped in or near human dwellings in the Benue-Plateau State and the North-Eastern State of Nigeria

| Time | Location | Rodent species[a] | | | | |
|---|---|---|---|---|---|---|
| | | *Mastomys natalensis* | *Rattus rattus* | *Aethomys stannarius* | *Dasymys incomtus* | *Mus minutoides* |
| Nov.-Dec. 1972 | *BP State :* | | | | | |
| | Du | 0/7 | | 0/7 | | |
| | Langtang | | | 0/6 | | |
| | Ner-Pankshin | 5/20 | 0/1 | | | |
| | Rukuba | 0/2 | | 0/3 | | |
| Mar.-Apr. 1973 | *BP State :* | | | | | |
| | Foron | | 0/9 | 0/4 | | |
| | Fusa | | 0/1 | 0/1 | | 0/16 |
| | Miango | 0/1 | 0/2 | 0/2 | | |
| | Naraguta | | 0/2 | | 0/1 | |
| | Vom | 0/2 | 2/10 | 0/4 | 0/3 | 0/1 |
| | *NE State :* | | | | | |
| | Bauchi | | 0/6 | | | 1/2 |
| | Gogombi | | | | | 0/2 |
| | Gombe | | | | 0/12 | |
| | Lassa | | | 0/5 | 0/8 | |
| | Michika | | 0/4 | | 0/2 | 0/2 |
| | Uba | | 0/2 | | 0/1 | |
| Total | | 5/32 | 2/37 | 0/32 | 0/27 | 1/23 |

[a] Numerator: number of Lassa virus isolations; denominator: number of rodents tested.

Table 2. Rodents trapped on farmland and bush in the Benue-Plateau State and the North-Eastern State of Nigeria and found to be negative for Lassa virus

| Time | Location | Rodent species | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Mastomys natalensis* | *Rattus rattus* | *Aethomys stannarius* | *Dasymys incomtus* | *Mus minutoides* | *Cricetomys gambiamus* | *Arvicanthus niloticus rufinus* | *Lemnisomys striatus* | *Myomys daltoni* |
| Nov.-Dec. 1972 | *BP State :* | | | | | | | | | |
| | Ner-Pankshin | 7 | | | | 2 | | | 1 | |
| | Langtang | 7 | | 38 | | | | | | 1 |
| Mar.-Apr. 1973 | *BP State :* | | | | | | | | | |
| | Foron | | | | 1 | | | | | |
| | Miango | | 1 | 2 | 2 | 4 | | | | |
| | Toro | | | | | | 3 | | | |
| | Vom | 2 | | 3 | | | | | | |
| | *NE State :* | | | | | | | | | |
| | Bara | | 7 | | | | | | | |
| | Bauchi | | | | 4 | 4 | | | | |
| | Biu | 3 | | | | | | | | |
| | Gogombi | | | | | | | 1 | | |
| | Lassa | | | | 4 | | 3 | | | |
| | Uba | | | | | | 7 | 5 | | |
| | Womdiu | | | | 14 | | | | | |
| Total | | 19 | 8 | 43 | 25 | 10 | 13 | 6 | 1 | 1 |

classified as *M. natalensis*. They were collected in December 1972 at Ner-Pankshin in BP State. Virus was not isolated from the urine of these rodents; however, only 1 urine specimen from an infected *Mastomys* rat was submitted for testing. Lassa virus was also isolated from tissue pools of 2 rodents classified as *Rattus rattus*, trapped at Vom, BP State, in March 1973. Blood and urine were available from only 1 of them; the blood specimen yielded Lassa virus but the urine did not. Another Lassa virus strain was isolated from the tissues of a rodent classified as *Mus minutoides*, trapped at Bauchi, NE State, in April 1973. Blood and urine from this animal were not tested.

Lassa virus could be reisolated from all positive tissue suspensions. Lassa virus was not isolated from blood specimens of any animals that did not also have infected tissue pools.

A total of 126 rodents were trapped in areas outside villages. From these, 124 tissue pools, 16 blood specimens, and 46 urine specimens were tested for presence of Lassa virus (Table 2). None was found positive.

## DISCUSSION

Wild rodents were known to be infected with a variety of different viruses, especially arboviruses, in regions of Nigeria where Lassa fever has been reported (4). However, attempts to isolate Lassa virus from Nigerian rodents had previously been unsuccessful. The survey described here was designed to investigate in a preliminary way the incidence of Lassa virus infection in rodents in parts of Nigeria where Lassa fever had been reported in the past. Since *M. natalensis*, the multimammate mouse, had been strongly implicated in the maintenance and spread of Lassa virus in Sierra Leone (1), it seemed likely that this species and possibly other rodent species might serve as reservoirs in Nigeria.

Lassa virus was isolated from 8 wild rodents in our study. Five isolations came from animals identified in the field as *M. natalensis*, 2 were from *R. rattus*, and 1 from *Mus minutoides*. All the infected rodents were trapped in or near human dwellings.

With the difficulties inherent in rodent classification under field conditions, the possibility that misidentifications may have occurred must be strongly considered. At least two distinct forms of *Mastomys* have been found in Nigeria, one having a colour phase resembling *R. rattus*. The finding of Lassa virus in a highly domesticated species such as *R. rattus* would be of great potential epidemiological significance. The isolations from animals identified as *Rattus* must be confirmed by further field work and the identity of each rodent tested must be verified by examination of skull and skin or the preserved carcass.

Regardless of the specific identification of the infected rodent species, the findings reported here show definitely that Lassa virus infects rodents in Nigeria and, most significantly, that these infections can occur during periods when there are no outbreaks in humans.

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## RÉSUMÉ

ISOLEMENTS RÉCENTS DU VIRUS DE LASSA À PARTIR DE RONGEURS NIGÉRIANS

Des rongeurs ont été capturés au Nigéria, dans les Etats de Bénoué-Plateau et du Nord-Est, tant à l'intérieur et à proximité des habitations qu'en plein champ. On a réuni, dans une fiole scellée, le foie, le poumon, le rein et la rate de chaque animal. En outre, on a conservé pour analyse le sérum et l'urine et/ou la vessie de quelques animaux. Les spécimens ont été congelés dans de l'azote liquide puis expédiés par air au Center for Disease Control d'Atlanta (Géorgie).

C'est le Laboratoire de sécurité maximale qui a traité ces échantillons. Une suspension à 10% a été préparée à partir de chaque groupe de tissus et inoculée dans des tubes de culture de cellules Vero. Les spécimens de sang et d'urine ont été inoculés dès la réception. Le milieu d'entretien (Eagle MEM additionné de 2 ml de sérum fétal bovin par 100 ml de milieu) a été changé un jour après l'inoculation et les cultures ont été gardées en observation pendant 9-10 jours pour rechercher l'apparition d'un effet cytopathique (ECP). Lorsqu'un ECP s'est manifesté, on a utilisé le liquide de culture contenant les cellules comme antigène dans l'épreuve de fixation du complément pour l'identification du virus. Quand il y avait dégénération non spécifique du film cellulaire, on a opéré un second passage sur culture de cellules Vero.

Huit souches de virus de Lassa ont été isolées à partir des tissus et du sang de rongeurs appartenant à trois espèces différentes: *Mastomys natalensis*, *Rattus rattus* et *Mus minutoides* (identifiées sur le terrain). Tous les rongeurs infectés avaient été capturés dans des habitations villageoises. Ces isolements indiquent la présence du virus de Lassa au Nigéria chez les rongeurs sauvages à un moment où aucune infection humaine n'était patente.

Des études antérieures faites en Sierra Leone (1) avaient indiqué qu'une seule espèce de rongeur, *M. natalensis*, pouvait être le réservoir essentiel du virus de Lassa. Comme il ressort de la présente étude que d'autres espèces peuvent également être vectrices, il se pourrait que l'écologie du virus de Lassa soit plus complexe qu'on ne le supposait. Pour pouvoir déterminer l'aire géographique et la série d'espèces de rongeurs qui hébergent le virus de Lassa et compte tenu du problème que pose l'identification du rongeur dans les conditions difficiles du terrain, il est urgent de faire d'autres recherches dans les mêmes régions du Nigéria en vue de confirmer ces observations.

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## DISCUSSION

ISAÄCSON: I was very interested to see the large number of species of rodent that have been tested. I believe *Cricetomys gambianus* formed part of your survey and was found to be negative. There is very little difficulty in identifying this large animal; the only other animal with which it can be confused is the so-called cane rat. I am interested in this animal because it has a peculiar ectoparasite known as *Hemimrus talpoides* and its geographic distribution exactly parallels that of Lassa fever. It might perhaps be worth while to do some further

investigation on this ectoparasite. Another rodent species in your survey, *Dasymys incomptus*, is also fairly easy to recognize because of its shaggy fur. Again, its distribution in West Africa rather closely parallels that of Lassa fever. *Mus minutoides*, which was mentioned by Dr Wulff, has not quite the same distribution.

SETZER: The identification of African rodents in general is in a state of great confusion. For many years I have suspected, along with other mammalogists, that there are two sympatric species of *Mastomys* in West Africa. We have recently been able to arrive at a rather nice separation of these two forms by the use of sophisticated statistical methods. We have a large series from near Durban, the type locality of *Mastomys natalensis*. Using only males of one age group, we made a tentative separation of species with a correlation coefficient of about 0.62. These were then separated as samples and the animals examined individually using discriminate function analysis. We found a very clean separation, but the characters are esoteric, the differences being confined to the rostral portion of the skull. We feel confident at this stage that we have identified two species in South Africa. We did a quick test on Ivory Coast *Mastomys* and found the same sort of thing. However, identification of rodents in the field is much more difficult.

MONATH: What about mistaking *Rattus rattus* for *Mastomys* in the field? This is an important question, because of the isolation of Lassa virus from *Rattus* collected and identified in Nigeria.

SETZER: A number of years ago we were working on the Jos Plateau, and we collected what we assumed to be extremely melanistic *Rattus rattus* until we caught a mature female, which turned out to be *Mastomys*. It is a rather unusual situation in this area. The two species can be readily confused in the field.

SHOPE: Dr Wulff, would your isolation technique detect Marburg virus? Were any viruses other than Lassa virus isolated from the rodents?

WULFF: We inoculated Vero cell cultures only. Arboviruses causing cytopathic effect in Vero cell cultures should have been detected, because as I mentioned we kept the cultures for 9–10 days; no such isolations were made, however.