10. W. B. N. Berry and A. J. Boucot, *J. Geophys. Res.* **72**, 2254 (1967).
11. The Öved Sandstone has a thickness of about 25 m. There are, at most, about five horizons, totaling in thickness about 20 cm, from which bivalve impressions and leperditiocope ostracodes have been obtained, together with a depauperate fauna of dwarfed conodonts (L. Jeppsson, personal communication). Most of the Öved Sandstone is characterized by lithologies and sedimentary structures consistent with a brackish and possibly estuarine environment reminiscent of that ascribed to the Downton Castle Sandstone of the Welsh Borderland. The Öved Sandstone cannot be considered a marine unit.
12. J. B. Richardson, in *Aspects of Palynology*, R. H. Tschudy and R. A. Scott, Eds. (Interscience, New York, 1969), pp. 193–222; W. G. Chaloner, *Biol. Rev.* **45**, 353 (1970); B. Owens and J. B. Richardson, *Congr. Int. Stratigr. Geol. Carbonifere Krefeld C. R.* **1**, 325 (1972).
13. J. B. Richardson and T. R. Lister, *Palaeontology* **12**, 201 (1969).
14. H. P. Banks, in *Evolution and Environment*, E. T. Drake, Ed. (Yale Univ. Press, New Haven, Conn., 1968), pp. 73–107; *Palaeontology* **15**, 363 (1972).
15. A. J. Boucot, *Evolution and Extinction Rate Controls* (Elsevier, Amsterdam, in press).
16. The tubular, nonseptate structures, differentially thickened in either annular or spiral manner, clearly simulate tracheids of vascular plants, although they have not been demonstrated to be tracheids, and recently A. Eisenack [*Palaeontogr. Abt. A Palaeozool. Stratigr.* **139**, 64 (1972)] has speculated that structures of the same general appearance are chitinous in composition. Similar tubular structures were first noted by W. H. Lang [*Phil. Trans. R. Soc. Lond. Ser. B* **227**, 245 (1937)] in the Nematophytales, a group which he and a number of later workers have considered to be terrestrial or semiaquatic plants. O. A. Høeg [*Medd. Nor. Svalb. Unders. 83* (1942)] described such tubular structures in *Nematothallus* as "tracheidal tubes," saying that they are "very much like tracheids," a resemblance also noted by A. G. Lyon [*Trans. R. Soc. Edinb.* **65**, 79 (1962)] in reference to the "differentially thickened tubular cells" in the same group of plants. The important point about such structures is that they have apparently developed functionally in response to the transportation of water within the plant system or part of it, and that they do not appear to have developed in an aquatic, wholly submerged plant. This is the case regardless of what they are called or in which groups of plants they are found, including those of the Gotland Silurian, for which we do not claim a nematophytalean origin.
17. Supported by grants GB-29597X and GB-40502 from the National Science Foundation to the University of Oregon and by a grant from the Faculty of Science, University of Lund, to S.L.

4 March 1974

Lassa Virus Isolation from Mastomys natalensis Rodents during an Epidemic in Sierra Leone

Abstract. *Lassa fever is a severe febrile illness of man, first recognized in West Africa in 1969. During an epidemic in Sierra Leone, Lassa virus was isolated for the first time from wild rodents of* Mastomys natalensis. *A high prevalence of infected* Mastomys *was found in houses occupied by patients with Lassa fever. The data presented provide the first demonstration of an extra-human cycle of Lassa virus transmission and suggest that rodent control may be an effective method of limiting the disease.*

Lassa virus was first isolated in 1969 from three American missionary nurses who contracted a severe, hitherto undescribed febrile illness in Nigeria (*1*). Between 1969 and 1972 Lassa fever emerged as a major public health problem; four epidemics were recognized in three West African countries: Nigeria (*1, 2*), Liberia (*3*), and Sierra Leone (*4*). Although the total number of cases documented during these outbreaks was small (101 cases), the case fatality rate among hospitalized patients has been high (43 percent). In addition to the epidemic locales indicating a widespread or multifocal distribution of Lassa virus activity in West Africa, a serologic survey (*5*) has demonstrated past infection in areas where the disease has so far escaped detection. Lassa virus has a predilection for person-to-person spread within the hospital environment, with a high risk of infection in medical personnel. To date, 14 nurses and a physician have acquired Lassa fever while attending patients with the disease. The hazard to laboratory investigators is also great (*6*), and virologic studies are now confined to institutions with facilities for strict biocontainment.

In three of the four epidemics, Lassa virus was spread from person to person within a hospital environment, after introduction of the virus by a single infected patient (*1–3*). Because these were nosocomial (intrahospital) epidemics, the primary source and cycle of virus transmission in nature were not elucidated.

In September and October 1972 we investigated an outbreak of Lassa fever in the villages of Panguma and Tongo, Eastern Province, Sierra Leone (*4, 7*). In contrast to the previous nosocomial epidemics, most human cases arose in the affected communities. Although person-to-person transmission probably occurred, a nonhuman source of infection was also considered likely.

Our attention centered on wild vertebrates, since the epidemiology of viruses related to the agent of Lassa fever was known to involve rodents and bats. Lassa virus is morphologically indistinguishable from lymphocytic choriomeningitis (LCM) virus (*8, 9*) and eight other agents formerly included in the Tacaribe virus group. By complement-fixation (CF) tests, minor serologic cross reactions have been demonstrated between Lassa, LCM, and several Tacaribe group viruses (*10*). These viruses have thus been placed in a new taxonomic group designated the arenaviruses (*9*). A number of arenaviruses have been isolated from wild rodents, including: LCM virus from *Mus musculus*; Machupo virus (the agent of Bolivian hemorrhagic fever) from *Calomys callosus* in Bolivia (*11*); Junin virus (the agent of Argentinian hemorrhagic fever) from *Calomys laucha* and other species in Argentina (*12*); Tamiami virus from *Sigmodon hispidis* in Florida (*13*); and Pichinde and Amapari viruses from cricetine rodents in Colombia (*14*) and Brazil (*15*), respectively. Tacaribe virus has been isolated from *Artibeus* bats in Trinidad (*16*).

During investigations in Sierra Leone, we collected a total of 641 small vertebrates. Bats were caught in nylon mist nets set at the fringes of the affected villages. Rodents and shrews were either captured in live traps set in houses and surrounding farms and forests, or were purchased from local native trappers. We removed samples of lung, heart, spleen, kidney, blood, and, if possible, bladder urine and ectoparasites. Specimens were frozen in liquid nitrogen and returned to the Maximum Security Laboratory at the Center for Disease Control, Atlanta, Georgia, whereupon they were transferred to a mechanical freezer (−78°C). For attempts at virus isolation, the organ pools from each animal were thawed, ground in a mortar with modified (*17*) Eagle's medium, and lightly centrifuged. We tested (*3, 7*) the supernatant fluids for virus in tube cultures of African green monkey kidney stable (Vero) cells. Cultures were inoculated with 0.1 ml of 10 percent (weight to volume) organ suspensions; 45 minutes later, maintenance medium was added, and the tubes were checked daily for cytopathic effect (CPE). Positive cultures showed typical Lassa virus CPE by day 5 or 6, whereupon they were harvested by freeze-thawing and identified by CF test. The identification of Lassa virus isolates by CF test has been described (*3, 7, 18*). The neutralization test has not been found useful for virus identification, since, in our hands, antiserums of sufficient neutralizing potency have not been produced.

Table 1. Numbers of wild vertebrate species collected and tested for Lassa virus, Sierra Leone in 1972.

Species	Collected (No.)	Lassa virus (positive/tested)
Rodents		
Mus musculus	195	0/141
Mus musculoides	35	0/27
Rattus rattus	73	0/50
Mastomys natalensis	109	14/82
Lophuromys sikapusi	29	0/20
Dasymys incomtus	3	0/2
Hybomys univattatus	1	0/1
Hylomyscus simus	5	0/4
Praomys tullbergi	7	0/5
Myomys daltoni	3	0/3
Uranomys ruddi	1	0/1
Cricetomys gambianus	15	0/10
Euxerus erythropus	5	0/1
Funisciurus pyrrhopus	1	0/1
Thryonomys swinderianus	2	0/1
Unidentified	1	0/1
Total	475	14/350
Bats		
Rousettus aegyptiacus	52	0/37
Epomops buettikoferi	50	0/33
Micropteropus pusillus	6	0/3
Hipposideros caffer	1	0/1
Hipposideros ruber	1	
Pipistrellus nanus	2	0/1
Total	112	0/75
Insectivores		
Crocidura spp.	32	0/24
Primates		
Cercopithecus spp.	10	0/3
Reptiles		
Turtle	1	0/1
Lizard	1	

Lassa virus was isolated from the organs of 14 of 350 rodents tested but not from 75 bats, 24 shrews, 3 monkeys, or 1 turtle. No urine or ectoparasite samples were available from any of the virus-positive animals. Table 1 shows the distribution of vertebrate species collected and the frequency of virus isolations. All of the Lassa virus isolates were from a single murine species, *Mastomys natalensis* A. Smith, 1847. Serologic identification of two strains isolated from this rodent is shown in Table 2.

An attempt was also made to test the serums of animals collected in Sierra Leone for CF antibodies to Lassa virus; nearly all serums contained high titers of anticomplementary substances. Neutralization testing has not yet been performed.

The results clearly implicated *Mastomys natalensis* as the reservoir of Lassa virus in Sierra Leone, but did not elucidate the mode of virus transmission to man. Infection of man with the other rodent-borne pathogenic arenaviruses (LCM, Machupo, Junin) is known to depend upon close associations between rodent and man and contamination of the environment (for example, food, dust, and air) with infected rodent urine or other excreta. We were thus interested in the ecology and habits of *Mastomys natalensis* and in the distribution of infected animals in relation to human cases of Lassa fever.

Only four of the rodent species were present within houses in the epidemic area: *Mus musculus, Rattus rattus, Mastomys natalensis*, and (very rarely) *Hylomyscus simus*. Of these, *Mus musculus* and *Rattus rattus* were exclusively commensal in their habits, whereas *Mastomys natalensis* was trapped mainly in houses, but also in surrounding gardens and fields. When the two villages, Panguma (population 3,159) and Tongo (11,456), most severely affected by the epidemic were compared, differences were noted both in the prevalence of commensal rodent species and in the frequency of virus isolation. In Panguma *Mus* and *Rattus* predominated, comprising respectively 75 and 17 percent of the commensal rodent population; only 6 of the 84 rodents captured in Panguma houses were *Mastomys*. In contrast, 39 of 53 commensal rodents (73.5 percent) in Tongo were *Mastomys; Mus* (24.5 percent) and *Rattus* (2 percent) were less common. The frequency of virus isolation from Tongo *Mastomys* (13/35 positive) was also higher than that from Panguma. Only 1 of 46 *Mastomys* captured in houses and obtained from sources other than household trapping in Panguma was positive. These observations appeared to correlate with the results of a human serologic survey (*4*), which showed a higher prevalence of Lassa CF antibodies in Tongo residents (39/346, 11.3 percent) than in Panguma (4/115, 3.5 percent).

In two households in Tongo village occupied by recent Lassa fever cases, an extraordinary rate of virus recovery from *Mastomys* was obtained. Ten of 12 rodents of this species yielded virus. Of six *Mus musculus* (the only other species trapped in these households), none were positive. Although it is possible that *Mastomys* were secondarily infected from a human source, it seems more likely that the primary epidemiological event was a rodent enzootic or epizootic. The data suggested that the virus was active in household foci within the epidemic zone rather than being widely diffused in the rodent population.

Since Lassa virus has a rather low pathogenicity for most laboratory rodents (*5, 10*), it is unlikely that the observations on relative rodent species prevalence and frequency of infection can be explained by a high mortality among infected *Mus* or *Rattus*. It appears that these species either resist infection or avoid contact with infected *Mastomys*.

Table 2. Identification of two Lassa virus strains (A44 and A206) isolated from *Mastomys natalensis* rodents in Sierra Leone in 1972.

Antigen†	Antiserum*		
	Lassa (Pinneo)	LCM (Armstrong)	Broad Tacaribe
A44	512/8‡	0	0
A206	1024/16	Trace	0
Lassa (Pinneo)	512/16	Trace	0
LCM (Armstrong)	Trace	256/32	0
Machupo (Carvallo)	Trace	0	64/32

* Lassa (Pinneo) hyperimmune antiserum was prepared in guinea pigs. LCM and Broad Tacaribe antiserums were prepared as hyperimmune mouse ascitic fluids. † Antigens for Lassa, Pinneo strain, and rodent isolates were prepared from infected Vero cells. LCM and Machupo antigens were prepared from infected mouse brains. ‡ CF titer of antiserum (numerator) and antigen (denominator). All serums were negative in control tests with normal Vero and mouse brain antigens. 0 indicates that both serum titer and antigen titer were < 4.

The pathogenesis of Lassa virus infection in its natural host, *Mastomys natalensis*, remains unknown. Since LCM (*19*), Machupo (*20*), and perhaps other arenaviruses are known to induce a chronic virus carrier state in their natural rodent hosts, possibly through virus-induced immune suppression (*21*), it is reasonable to expect a similar mechanism for Lassa virus. Baby white mice infected with Lassa virus have been shown to excrete the virus in their urine for at least 82 days (*10*). Machupo and LCM viruses can be passed both horizontally from rodent to rodent (and rodent to man) and vertically in rodents by congenital infection, whereupon lifelong persistent infections develop.

Mastomys natalensis is ideally suited to disseminate Lassa virus in rural Africa. It is a common species widely distributed in a variety of biotopes south of the Sahara, is a prolific breeder, and is adapted to life both within houses and in the fields. Its prevalence in a given area is determined by density-dependent variations in mortality, seasonal factors, and competition with other rodent species. In the rainy months, *Mastomys* may abandon the fields and seek shelter and food within houses, perhaps explaining in part the peak incidence of human cases in Sierra Leone during the rainy season of 1972 (*4*). The low level of sanitation, the customary storage of grains

and other foodstuffs within houses, and the ample harborage for rodents in houses constructed of mud and thatch all contribute to contacts between rodents and humans.

The results of our investigations provide a basis for controlling Lassa fever in West Africa. Because no vaccine is available, rodent control and human quarantine are the only potentially effective means of limiting a community-based Lassa fever outbreak. Rodent populations may be reduced by limiting the availability of food and shelter, by various biological control methods, or, more directly, by trapping and poisoning. *Mastomys natalensis* populations have been successfully reduced by the use of an anticoagulant rodenticide (warfarin) in villages in the Sudan (*22*). Trapping and rodent-proofing of food stores have been used to stop epidemic spread of Machupo virus by *Calomys callosus* in Bolivia (*23*).

The apparent interspecific competition between *Mastomys natalensis* and the more aggressive species *Rattus rattus* demonstrated in Sierra Leone suggests a possible means of biological control but, more importantly, warns against the indiscriminate reduction of rodent populations through trapping and poisoning. Reduction of *Rattus rattus* populations in villages where this species predominates may allow introduction of *Mastomys*, which would then enter a period of logarithmic population growth.

The epizootiologic role of *Mastomys natalensis* in areas other than Sierra Leone remains to be proved. Meanwhile, investigations of high priority for zoologists and public health authorities include studies of the distribution, population dynamics, and behavior of this species in West Africa.

THOMAS P. MONATH
VERNE F. NEWHOUSE
Vector-Borne Diseases Division, Bureau of Laboratories, Center for Disease Control, Public Health Service, Fort Collins, Colorado 80521
GRAHAM E. KEMP
Tropical Diseases Laboratory, Bureau of Laboratories, Center for Disease Control, Public Health Service, San Juan, Puerto Rico 00936
HENRY W. SETZER
Division of Mammals, National Museum of Natural History, Smithsonian Institute, Washington, D.C. 20560
ANTHONY CACCIAPUOTI
Virology Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia 30333

References and Notes

1. J. D. Frame, J. M. Baldwin, Jr., D. J. Gocke, J. M. Troup, *Am. J. Trop. Med. Hyg.* **19**, 670 (1970).
2. D. E. Carey, G. E. Kemp, H. A. White, L. Pinneo, R. F. Addy, A. L. M. D. Fom, G. Stroh, J. Casals, B. E. Henderson, *Trans. R. Soc. Trop. Med. Hyg.* **66**, 402 (1972).
3. T. P. Monath, P. E. Mertens, R. Patton, C. F. Moser, J. J. Baum, L. Pinneo, G. W. Gary, Jr., R. E. Kissling, *Am. J. Trop. Med. Hyg.* **22**, 773 (1973).
4. D. W. Fraser, C. C. Campbell, T. P. Monath, P. A. Goff, M. B. Gregg, *ibid.*, in press.
5. B. E. Henderson, G. W. Gary, Jr., R. E. Kissling, J. D. Frame, D. E. Carey, *Trans. R. Soc. Trop. Med. Hyg.* **66**, 409 (1972).
6. E. Liefer, D. J. Gocke, J. Bourne, *Am. J. Trop. Med. Hyg.* **19**, 677 (1970).
7. T. P. Monath, M. Maher, J. Casals, R. E. Kissling, A. Cacciapuoti, *ibid.*, in press.
8. R. W. Speir, O. Wood, H. Liebhaber, S. M. Buckley, *ibid.* **19**, 692 (1970).
9. W. P. Rowe, F. A. Murphy, G. H. Bergold, J. Casals, J. Hotchin, K. M. Johnson, F. Lehmann-Grube, C. A. Mims, E. Traub, P. A. Webb, *J. Virol.* **5**, 651 (1970).
10. S. M. Buckley and J. Casals, *Am. J. Trop. Med. Hyg.* **19**, 680 (1970).
11. K. M. Johnson, M. L. Kuns, R. B. Mackenzie, P. A. Webb, C. E. Yunker, *ibid.* **15**, 103 (1966).
12. A. S. Parodi, J. R. Rugiero, D. J. Greenway, N. Mettler, A. Martinez, M. Boxaca, H. M. de la Barrera, *Prensa Med. Argent.* **46**, 554 (1959).
13. C. H. Calisher, T. Tzianabos, R. D. Lord, P. H. Coleman, *Am. J. Trop. Med. Hyg.* **19**, 520 (1970).
14. H. Trapido and C. Sammartin, *ibid.* **20**, 631 (1971).
15. F. P. Pinheiro, R. E. Shope, A. H. Paes de Andrade, G. Bensabath, G. V. Cacios, J. Casals, *Proc. Soc. Exp. Biol. Med.* **122**, 531 (1966).
16. W. G. Downs, C. R. Anderson, L. Spence, T. H. G. Aitken, A. H. Greenhall, *Am. J. Trop. Med. Hyg.* **12**, 640 (1963).
17. R. E. Kissling and D. Reese, *J. Immunol.* **91**, 362 (1963).
18. A. W. Woodruff, T. P. Monath, A. F. Mahmoud, A. K. Pain, C. A. Morris, *Br. Med. J.* **3**, 616 (1973).
19. F. Lehmann-Grube, *J. Clin. Pathol.* **25** (Suppl. 6), 8 (1971).
20. G. Justines and K. M. Johnson, *Nature (Lond.)* **222**, 1090 (1969).
21. C. A. Mims and S. Wainwright, *J. Immunol.* **101**, 717 (1968); M. Hanaoka, S. Suzuki, J. Hotchin, *Science* **163**, 1216 (1969).
22. J. Schmutterer, *Pests of Crops in Northeast and Central Africa* (Fischer, Stuttgart, Germany, 1969).
23. R. B. Mackenzie, M. L. Kuns, P. A. Webb, *Pan Am. Health Org. Sci. Publ. 147* (1967), p. 260.
24. We thank M. D. Sanderlin for technical assistance in testing virological specimens. Drs. Jordi Casals, David W. Fraser, and C. C. Campbell assisted in many phases of the field studies.

27 March 1974

Thyrotropin-Releasing Hormone: Regional Distribution in Rat Brain

Abstract. *A sensitive and specific radioimmunoassay has been used to measure the distribution of thyrotropin-releasing hormone (TRH) in rat brain. All areas of brain tested, except cerebellum, contained readily measurable amounts of TRH. The hypothalamus contained only 31.2 percent of the total brain content of TRH. These results support recent suggestions of central actions for TRH in addition to its hypophysiotropic functions.*

Hypothalamic thyrotropin-releasing hormone (TRH), whose structure is pyroglutamyl-histidyl-prolineamide (*1*), has been shown to stimulate thyrotropin (*1*), prolactin (*2*), and, at times, growth hormone secretion (*3*) from the pituitary gland. It may have significant effects on behavior and on mood state in addition to its more well established effects on pituitary hormone release (*4–6*). For example, TRH has been shown to potentiate the phenomenon of behavioral excitation induced by L-dopa in mice (*4*). This potentiation was also observed in hypophysectomized animals. Several groups have reported that TRH has mood elevating effects in depressed women (*5*). In one study, normal women were also found to respond to TRH administration with a transient elevation in mood (*6*). In light of these newer observations of the behavioral effects of TRH, a series of experiments was performed to investigate the possibility that TRH is a normal constituent of extrahypothalamic brain tissue. The results indicate that this is indeed the case. Similar results have been presented by other investigators (*7*).

Normal rats (Sprague-Dawley) weighing 200 to 250 g were decapitated and the brains were rapidly removed. By a modification of the technique described by Glowinski and Iversen (*8*), the brains were dissected into six pieces. The hypothalamus was removed first by freeing the sides of its lateral borders. With the optic chiasm as the anterior border, a cut 2 mm deep was made under the hypothalamus, allowing it to be lifted up from the third ventricle. Next an arbitrary cut was made from the anterior border of the hypothalamus straight down through the cortex. This cut yielded a piece, identified here as forebrain, which contained both frontal and some temporal cortex, as well as some anterior diencephalic tissue. The brainstem and cerebellum were separated by blunt dissection. Finally, the remainder of the cortex, called posterior cortex, was peeled back from the remaining posterior diencephalic tissue. In most experiments, pieces of liver, kidney, or