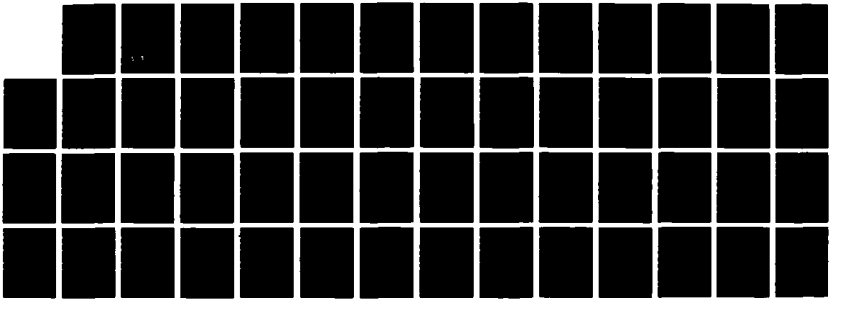
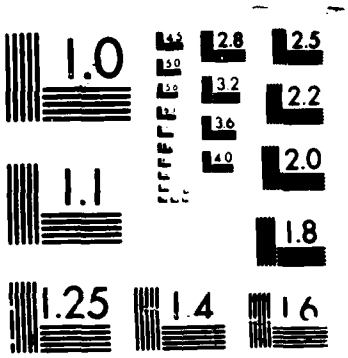
AD-A100 344 LASSA FEVER IMMUNE PLASMA(U) COLUMBIA UNIV NEW YORK 1/1
J D FRAME MAY 80 DAMD17-79-C-9024

UNCLASSIFIED F/G 6/5 NL





(12)

AD_____

LASSA FEVER IMMUNE PLASMA

ANNUAL/FINAL REPORT

John D. Frame, M.D.

May, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9024

Trustees of Columbia University
In the City of New York
New York, N.Y. 10032

DOD Distribution Statement

Approved for public release; distribution unlimited

The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A180344 / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Lassa Fever Immune Plasma	5. TYPE OF REPORT & PERIOD COVERED Annual Report 8/1/84-6/30/85, and Final Report
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John D. Frame, M.D.	8. CONTRACT OR GRANT NUMBER(s) DAMD17-79-C-9024
9. PERFORMING ORGANIZATION NAME AND ADDRESS Columbia University, 630 West 168th Street New York, N.Y. 10032	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A871.BC.093
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701-5012	12. REPORT DATE May, 1986
	13. NUMBER OF PAGES 52
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Lassa Fever
Lassa Fever, Epidemiology of
Lassa Fever Immune Plasma
Lassa Fever, Immunology

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

During the period 246 Lassa Fever Immune Plasma (LFIP) units were obtained by plasmapheresis, 106 were forwarded to USAMRIID. During the whole life of the Contract 589 LFIP units were collected and 317 forwarded to USAMRIID, of which 272 units were acceptable at a Log Neutralization Index equal to or greater than 0.3. Virological and serological testing for Lassa Fever (LF) at Curran Lutheran, Phebe and G.W. Harley Memorial hospitals identified 76 cases of LF and 14 presumptive LF. Lassa virus (LV) was isolated from 55. Since

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

#19 - Lassa Fever, Occurrence of
Liberia, Lassa Fever in
Plasmapheresis

#20 - the inception of the Contract LV has been isolated from 139 of 213 LF patients and another 71 presumptive LF cases have been identified in 3 Liberian hospitals.

Epidemiologic investigations were continued with the survey of the prevalence of LV antibody (LVA) positives among inhabitants of the Ganta Rehabilitation Center, Ganta. Two studies of the incidence of LF among staff members at CLH demonstrated an annual rate of 0.03 in each. The prevalence of LVA positives in Liberian hospital staffs ranges from 1.24 to 5.14 times that within populations of the surrounding villages, suggesting person-to-person spread of LF in the hospitals. A pilot study of the residents of households of patients hospitalized with LF revealed a prevalence of LVA among them not significantly different from among those in control households.

Passive immunotherapy of LF patients with LFIP continued. The case fatality rate of 0.077 was not significantly different from that of untreated patients. However, the bias toward the treatment of the clinically sicker patients confounded the results.

AD_____

LASSA FEVER IMMUNE PLASMA

ANNUAL/FINAL REPORT

John D. Frame, M.D.

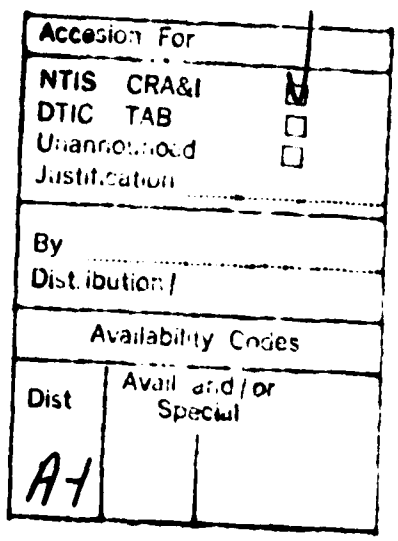
May, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9024

Trustees of Columbia University
In the City of New York
New York, N.Y. 10032



Summary

During the year plasmapheresis at Curran Lutheran Hospital (CLH) and Phebe Hospital (PH) resulted in the collection of 246 units of Lassa Fever Immune Plasma (LFIP) of which 108 were forwarded to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID); 104 units were found to have a Log Neutralization Index of 0.03 or higher, acceptable for use by USAMRIID. During the lifetime of the Contract 589 LFIP units have been obtained and 317 forwarded to USAMRIID, with 272 having LNI's of 0.3 or higher.

Testing of febrile patients for evidence of Lassa virus (LV) infection continued at CLH, PH and the G.W. Harley Memorial Hospital (GWH). Of 642 patients tested 55 were diagnosed as having Lassa Fever (LF) by virus isolation, and 21 by seroconversion. An addition 14 were diagnosed as presumptive LF on the basis of high titers to I. antibodies on single tests. From the inception of the Contract LV has been isolated from 139 cases of LF, and diagnosis made by seroconversion in another 74.

A serological investigation at a rehabilitation center near GWH revealed evidence of previous LV infection in 22 of 288 leprosy patients, 4 of 52 family members of these patients and 5 of 41 persons who were free of leprosy. The prevalences among those groups did not differ significantly. The serological responses were similar among patients with the lepromatous and mixed forms of leprosy.

This investigation was an extension of several that have been conducted in Liberian villages. The prevalence of LV antibodies in the villages showed significantly higher rates in those located beside main roads than in isolated villages "in the bush".

The first measurement in Liberia of incidences of LF in hospital staffs demonstrated an annual rate of 0.03 in two studies at CLH. The prevalence of LV antibodies in hospital staffs is 1.24 to 5.14 times that in the villages near them, suggesting person-to-person spread from LF patients to hospital personnel.

An investigation of households of patients treated for LF at CLH and of neighboring control households showed no significant differences in the number of fevers or the prevalence of LV antibody positive sera between contacts of the index cases and the controls. It was not possible to determine the relative importance of person-to-person dissemination of LF in patients' homes.

LFIP units were administered to 39 LF patients in whom the diagnosis was confirmed. The therapeutic value of the plasma was calculated by the product of the plasma volume and the LNI of the units administered. There was one death among 23 patients receiving 500 and more "Therapeutic Units", and 2 among 16 receiving less than 500. The over all case fatality rate of 0.077 was not significantly different than the rate of 0.104 among all patients with LF in CLH during the the past 6 years. However, it is believed that in general patients selected for immunotherapy were the more severely ill.

FOREWARD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Table of Contents

I. Statement of the Problem

Lassa fever (LF) was first found as a disease of high morbidity and mortality in nosocomial outbreaks in West Africa (1,2,3,4). Subsequently it was learned that Lassa virus (LV) infections are widely spread throughout the region (5). Treatment of cases, is essentially supportive; specific treatment with LF Immune Plasma (LFIP) has been used with equivocal results (6,7). Other rational therapeutic measures await better knowledge of the pathogenesis and pathology of human LV infections.

Prevention of ~~LF~~ requires elucidation of its epidemiology, and will likely require the preparation and use of a vaccine. However, investigations into LV infection carry a definite risk ~~(8)~~, and measures should be available to protect investigators as well as patients.

Thus, research into the epidemiology and pathogenesis of LF, in the nature of the virus, and in the development of a preventive vaccine appear to be of high priority if this hemorrhagic fever is to be brought under control.

II. Background

An outbreak of LF in the Curran Lutheran Hospital (CLH), in Zorzor in 1972 demonstrated its presence in Liberia (3). A pilot study conducted from 1976 to 1979 revealed high prevalences of LV antibodies (LVA) in members of hospital staffs in Liberia, and the feasibility of working with Liberian hospitals in further investigations of LF in that country (9). It also persuaded the Republic of Liberia to agree to ongoing research in LF. On the basis of the preliminary findings, a joint program to procure LFIP and to study the epidemiology of LF was entered upon by Columbia University (CU), the Liberian Institute for Biomedical Research (LIBR) and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) under Contract DAMD17-79-C-9024 awarded by U.S. Army Medical Research and Development Command (USAMRDC).

Early studies of the LFIP units demonstrated that protective neutralizing antibody (NA) titers were not developed until 3 to 9 months after the onset of illness, that titers might rise and fall in some donors, and that plasma with adequate protective titers against one serotype of LV might not have them against another strain (10,11). Thereafter convalescents from LF were tested periodically for NA titers, generally against two strains of LV, and plasmapheresis begun after a Log Neutralization Index (LNI) of at least 0.3 was attained against one of the strains (12).

Virological and serological investigations among hospitalized patients have shown that in northwestern Liberia LF

is the cause of illness in 10 to 15% of febrile patients, in some cases being the most common cause of fever among them (13). LV has been isolated from many patients, and classification of the virus isolated by serotype has been begun at USAMRIID (14). Village surveys have shown prevalences of LV antibodies (LVA) of about 1% to 14% in villages of Lofa County (15). Surveys of hospital personnel have demonstrated that LV infections are endemic in all regions of Liberia (16). However, most investigations have been conducted in the vicinity of CLH, Zorzor, and Phebe Hospital (PH), near Gbarnga, in Lofa and Bong Counties, respectively.

III. Approach to the Problem

Plasma donors were identified by continued serological testing of febrile patients in hospitals, and by attempts at virus isolation. Potential donors were requested to submit sera for testing for LV NA, and those with adequate LNI were asked to submit to plasmapheresis.

Epidemiological investigations were carried out among patients found to have LF, and by means of village surveys for the prevalence of LF; villages were selected for differences that might elucidate factors contributing to varying prevalences of LVA.

IV. General Narrative

In July, 1984 the Field Investigator, Mr. J. E. Yalley-Ogunro, visited CLH and PH to conduct plasmapheresis; he returned to CLH in October, November and December, 1984, and March and May, 1985 for the same purpose. In March 1985 he attempted plasmapheresis at PH but found the refrigerated centrifuge not functioning. He obtained help of a person familiar with electronic equipment, but repairs could not be completed, and the assemblies found by trouble-shooting to be the most likely non-functional were returned to the United States for repair or replacement.

The Principal Investigator, John D. Frame, M.D., traveled to Liberia in November, 1984 and May, 1985. On both occasions he visited CLH and PH to review the status of specimen collections, and to review with staff members of the hospitals the criteria for the treatment of patients with LFIP. He assisted in the conduct of plasmapheresis at CLH. In May he visited the Leprosy Rehabilitation Center at Ganta and reviewed with the staff the results of the serological survey of patients conducted the previous June.

Treatment of patients with the clinical diagnosis of LF was conducted at CLH and PH under the supervision of the medical personnel of the two institutions. Dr. Mark Monson, Chief Medical Officer at CLH, was on leave in the United States, and work in that hospital was carried out by Dr. Korathu Alexander, Director,

and Dr. Kokulo Waiwaiku; at Phebe Hospital treatment was supervised by Dr. John Fredell, Chief of the Medical Staff. Dr. Andrew Cole, Clinical Investigator, was absent for 4 months while he attended a course in epidemiology in Kenya.

V. Results

A. Plasmapheresis

1. Annual

Early in 1984 a refrigerated centrifuge suitable for plasmapheresis was installed at PH. Following delays in the electrical connection of the centrifuge plasmapheresis was conducted in July and 24 units obtained from 12 donors. Testing of the plasma NA was delayed because of the renovation of the secure laboratory at the USAMRIID. An attempt at further plasmapheresis at PH in March 1985 was frustrated by the malfunction of the centrifuge. Attempts at repair were not successful, and the panels which trouble-shooting indicated were at fault were returned to the United States for replacment. Thus, no further plasmapheresis was done at this hospital.

Plasmapheresis at CLH continued as in former years and in all, 222 units were obtained from 40 donors, all of whom has contracted LF at least six months prior to plasmapheresis. Thus, at PH and CLH 246 units were collected and 108 forwarded to USAMRIID. 104 were found to have LNI's of 0.3 or higher, and were acceptable for use by USAMRIID. The remainder were retained in Liberia to be used for the treatment of LF patients (See below).

Information regarding the donors, the dates of plasma donation, the IFA titers and LNI's and the disposition of the units is summarized in Appendix A.

2. Final Summary

Initially plasampheresis for LFIP was conducted by gravity separation of the plasma from the blood; subsequently, refrigerated centrifuges were installed at CLH and PH. After some early anxiety regarding plasmapheresis donors have been found willing to submit to repeated plasmapheresis, and plasma units have been obtained with increasing ease.

Early there was uncertainty as to the criteria for the acceptance of donors. It was found by Dr. Peter Jahrling that the titer of IFA did not reflect the protective power of LFIP by *in vitro* neutralization tests (10), and that the NA titer, as determined by the LNI, was essential as a measure of the potency of the plasma. He also found that adequate levels of NA did develop within three to nine months after the onset of the donor's illness; this presented the field staff a guideline for the beginning of plasmapheresis on convalescents, and has led to the obtaining of a high proportion of adequate LFIP units for use in

treatment, and acceptable to USAMRIID.

During the period of research under Contract DAMD17-79-C-9024 589 units of LFIP have been obtained by plasmapheresis at the Swedish Free Pentacostal Mission Clinic (SMC) at Foya Kamara, at CLH in Zorzor, both in Lofa County and at PH in Suakoko, Bong County. Of these, 317 have been forwarded to USAMRIID and 272 were found to meet the standard of containing NA at an LNI of 0.3 or higher, suitable for the purpose of USAMRIID.

A summary of the number of units collected during this investigation, the number forwarded to USAMRIID and those meeting the criteria for acceptance by USAMRIID is given in Table 2, Appendix A.

B. Hospital Patient Surveys

During the year serological testing of febrile patients for evidence of LF continued; routine virus isolation was not attempted because of the pressure of other investigations involving the containment laboratory at USAMRIID. The diagnosis of "Lassa fever" was made on the basis of sero-conversion or a 4-fold rise in LVA as determined by the IFA technique. "Presumptive Lassa fever" was diagnosed by the presence of IFA titers of 1:64 or more, either on a single specimen or in both members of serum pairs. Serological testing was performed at the LIBR; previous studies have demonstrated no significant differences between the results of IFA testing or the LIBR and USAMRIID.

1. Annual

a. Curran Lutheran Hospital

Between May, 1984, and June, 1985, 426 patients with fever at CLH were tested; 65 cases of LF and 8 of presumptive LF were found, for a total of 73 patients, an incidence of 0.171. In an additional 40 patients LVA were present at lower than diagnostic titers. See Appendix B, Table B-1.

b. Phebe Hospital

Among 188 patients tested at Phebe Hospital from April, 1984, through June, 1985, eight LF and five presumptive LF patients were diagnosed for a total of 13, an incidence of 0.070; 16 others had LVA at non-diagnostic titers. Serum pairs were obtained in only about one-fourth of the patients (Appendix B, Table B-1).

c. Other Hospitals

Of 28 patients at the G.W. Harley Memorial Hospital in Ganta, Nimba County three were diagnosed as LF and one as presumptive LF for an incidence of 0.143. No LF was diagnosed among 12 patients at the Tellewoyan Memorial Hospital in Voinjama, Lofa County.

2. Final

Since the inception of this investigation LF and presumptive LF have been diagnosed in 284 patients in Liberia (Appendix B, Table B-2). By this means the incidence of LF among febrile hospital patients and the case-fatality rate (Appendix B, Table B-3) have been determined. The investigations have lead to the isolation of numerous strains of LV, and to the finding that there are several types of LV circulating in the region, as reported by Dr. Peter Jahrling at USAMRIID. The study of febrile aptients has also identified potential donors of LFIP.

In the Annual Summary Report of August, 1984 (17), it was noted that in a hospital such as CLH with a staff alert to the diagnosis of LF and with ordinary refrigerator-freezers, virus isolation was possible in about one-half the patients in whom a diagnosis of LF was eventually made. Between October, 1983 and April, 1984 an ultra-freezer was available at CLH, particular efforts were made to ensure that specimens did not thaw at any time between collection and attempted virus isolation, and virus isolation of all specimens were attempted. Under such "ideal" conditions virus isolation established the diagnosis of LF in 85% of LF patients and 76% of LF and presumptive LF cases (Appendix B, Table B-3). Serological diagnosis was made in 50% of LF cases, and 73% of LF and presumptive cases.

Virus isolation likely failed in some cases when specimens were obtained late in the illness when viremia was no longer present. In some instances there were also likely lapses in technique, with specimens remaining warm for excessive periods of time. Serodiagnosis fails whent he staff does not obtain second sera, a common occurrence because of death or discharge of the patient, or negligence or the pressure of ordinary hospital activities, or if the time between the collection of specimens is too short.

Virus isolation is not possible for most hospitals not a part of a research program. Even where it is available virus isolation is associated with the disadvantage that diagnosis is not available to the clinician at the time he is treating a LF patients. It is hoped that a technique such as the enzyme-linked immunosorbent assay may prove on field testing to meet the need for a test available for the diagnosis of LF in the hospitals where it occurs.

C. Village Surveys

1. Annual

In June 1984 a serological survey for the prevalence of LVA was conducted in the Ganta Rehabilitation Center (GRC), a village associated with the G.W. Harley Memorial Hospital (GWH) there. Most of the residents in this community are patients with leprosy,

though a few others with other chronic illnesses are housed there as well. There are also a number of staff members and their families residing in the village.

The survey was conducted as described in the Annual Summary Report, June, 1983 (18). Briefly, the village leadership was approached through GWH and agreed to a serological survey of the residents in the village. Those to be tested were enrolled on a roster with the record of their age and sex, and blood was obtained by finger prick and collected on discs of filter paper. After the discs dried they were placed in a freezer, and subsequently tested after reconstitution in phosphate buffered saline for LVA by the IFA technique at the LIBR. Previous experiments have demonstrated that the screening titer by this technique is 1:8.

After serological testing was completed the diagnoses of those who were surveyed was supplied by Sister Mary Chambers, M.D., the GRC physician, who is a qualified leprologist. She divided the patients into lepromatous, borderline and unclassified cases; the few tuberculoid cases in Liberia do not live in the GRC.

There were 381 people tested of whom 288 were leprosy patients, 52 members of their families and 41 staff members or otherwise free of leprosy (Appendix C, Table C-I). Of the patients with leprosy 0.076% (22/288) were positive for LVA; the prevalence of positive LVA was 6.079% (4/52) in members of their families. Five of 41 or 0.122 of those without leprosy and not in the families of leprosy patients were positive; the difference between them and the others was not statistically significant.

Among the various classifications of leprosy no significant differences of LVA were found. There was no difference between the prevalences of LVA positives among males and females, nor among the various age groups (Table C-2), even when the various diagnostic categories were taken into account (Table C-3).

Lepromatous leprosy is characterized by a diminished cell-mediated immunity to Mycobacterium leprae, and in general shows diminished cellular responsiveness to infecting agents. However, leprosy patients with varying degrees of cellular immunity are apparently able to mount similar humoral immunologic responses to LV.

2. Final Summary

Serological surveys of village populations have been conducted to determine the prevalence of LV activity in Liberia, northwest Liberia, and to help elucidate the epidemiology of LF in that region.

Blood for serological testing was obtained by finger prick. The technique described in the Annual Summary Report, June 1983

(18) permits an IFA screening titer of 1:8. Prevalence of LVA among the populations in Lofa County ranged from 0.021 in a village "in the bush" in Kolahun District, to a high of 0.141 in a village adjacent to the SMC, in Foya, also in Kolahun District.

Eight villages were pre-selected for comparisons of the prevalences of LVA in villages along the highway and those away from the main road and "in the bush". The results are given in Table C-4 (Appendix C), and demonstrate that the prevalences of LVA are higher in the villages near the highway than they are in villages removed relatively isolated from the main road.

Comparisons were also made between the prevalences in various age groups for the eight villages and two near Foya Kamara. They demonstrated significantly different rates among the various age groups. The lowest rate is among adolescents with four LVA positive of 184 tested, for a prevalence of 0.022; 14 of 610 children from 5 to 12 years of age were seropositive for a prevalence of 0.023. In children under 5 the prevalence was 0.046, and among adults, 0.055% (See Table C-5), Appendix C).

It is known that IFA tend to drop with time. About one-half fall to levels too low to measure in about 5 years. Thus, the presence of measurable IFA indicates a relatively recent infection. The variations in prevalences by age group may be explained by hypothesizing that small children, living close to the ground, will acquire LV infections relatively easily. As they mature the IFA will gradually fall, though new cohorts of infections will lead to develop of new cases of LV infections among them through adolescence. Among adults it appears that new LV infections occur among susceptibles, though it is not clear at this time where they acquire the infections. Our investigations have not shown significant differences in the prevalence of LVA between males and females.

D. Hospital Staff Surveys

1. Annual

a. Tellewoyan Memorial Hospital, Voinjama

In June 1984 the staff of the Tellewoyan Memorial Hospital (TMH) in Voinjama, Lofa County, was investigated to determine the prevalence of LVA by the IFA technique. As in previous staff surveys hospital personnal were invited to submit to venepuncture on a voluntary basis. Sera were separated, transported to CLM 60 miles distant and there stored until they could be taken to the LIBR for testing.

Specimens were obtained from 89 staff members. Diagnostic titers of LVA were obtained from 11, for an incidence of .126; three others were questionably positive. Previous experience suggests that they, too had had LF; if these results are considered positive the prevalence of previous LV infections in

the staff was .157. (Appendix D, Table D-1).

b. Incidence of LF in CLH staff

Previous surveys of staff members have shown prevalences of 12% to 23% at CLH; however, there have been no investigations of the incidence of LF among them. Beginning in 1983 all new students in the midwifery and practical nursing programs at CLH have been tested for LVA upon entry into their courses of study and annually thereafter. The results of the testing to date are given in Appendix D, Table D-2; they demonstrate a single sero-conversion in a group of 24 who had completed 16 months in their education, an annual incidence of 0.03.

Since 1979 there have been 14 cases of LF recognized among hospital staff members; there was one death in a pregnant aide. On the average there are 100 employees at the hospital. Previous surveys conducted at the hospital in 1977 and 1979 have demonstrated that 12% to 23% have LVA indicating previous infection; no surveys have been done since 1979. One may estimate that 15% of the staff are immune to LF; thus, over the period of 5 1/2 years there was a total of 467.5 person-years of exposure to LF by staff members susceptible to it. The incidence of LF among them was 0.03, and the case fatality rate 0.07.

The incidence calculated from all surveys, including the both studies reported above must be considered minimal. It is known that in a proportion of people who have had LF IFA titers drop with time, though NA may still be found when IFA titers are no longer discernible. The number of truly susceptible persons is likely to be lower than the number in whom no LVA were found on testing. Thus, the denominator in determining the incidence would likely be smaller and the calculated incidence higher than would be the case if the records of previous LV infections were available.

2. Final Summary

The testing of hospital personnel for LVA has proved useful in the determination of areas of high incidence of LF. At the beginning of LF research in Liberia the high prevalences of LVA in the staffs of CLH and SMC was instrumental in the selection of Lofa County as the site of ongoing investigations. Relatively high LVA prevalences in the staffs of PH and GWH have also been associated with subsequent discoveries of much LF among patients in those hospitals. LF has been relatively uncommon in institutions with lower numbers of LVA positives among hospital personnel. Thus, only one case of LF has been clinically in the last six years recognized at ELWA hospital near Monrovia, where the prevalence of LVA among staff members was .054 in 1976.

The variations in prevalence of LVA among hospital staff members, confirmed in some localities by concurrent investigations of patients, has made possible an estimate of the distribution of

LV activity in Liberia. LV infections are found in all parts of the country, but become increasingly common as one moves from the Southeast to the Northwest, and from the coast inland (Appendix D, Table D.).

The investigations of hospital staffs for the presence of LVA has also cast some light on the epidemiology of LF and the significance of person-to-person spread as compared with infections from common sources. Comparisons may be made of the prevalences of LVA in hospital staffs with those in neighboring communities, determined from village surveys (Appendix D, Table D-4). In these studies only village adults are included, to permit valid comparisons with the hospital staff members. Among the hospital staff only those with IFA titers of 1:8 or higher are included, inasmuch as the village surveys were performed at this screening titer.

The prevalences of LVA at CLH, TMH, SMC and GWH were 3.38, 1.24, 5.14 and 1.65 times those of the respective adjoining communities. The increased risk of the acquisition of LV infections among personnel in these institutions is likely due to infections from two sources from patient contact as well as from the communities where they live. The results give some indication of the significance of person-to-person infection in the spread of LF in a community.

E. Lassa Fever in Households of Hospital Cases

In the spring of 1985 a pilot study was undertaken to elucidate the pattern of LV infections and transmission in the families of hospital patients clinically diagnosed as having LF. Serological investigations using venous blood were conducted in the households suspected of nine LF patients; 10 neighboring control households were investigated as well.

The protocol used in the household epidemiological survey is given in Appendix E. In brief, households were visited and permission for the survey obtained. The numbers of rooms in the home and the family members sleeping in each room were determined, and the incidents of febrile illness among them was recorded. Blood was drawn from members of the index and control households, serum separated and subsequently tested for IFA at the LIBR.

Inasmuch as the diagnosis of LF was clinical, it was only later that it was confirmed or rejected. Of the nine patients studied 7 were confirmed as having LF, 6 by virus isolation and 1 by seroconversion. The results of the tests are given in Table E.1, Appendix D. Of the 47 living in the 7 households of cases there was one seropositive person among the 19 sharing the index cases' bedrooms, and 5 IFA positives among the 18 living in the other rooms of the house. There There were 4 and 2 cases with a history of recent fever in the two groups, respectively. The results were not significantly different from those in control households.

The results do not permit any conclusion regarding the transmission of LF in the households, whether from a common source or person to person.

F. Passive Immunotherapy of Lassa Fever

Passive immunotherapy of clinically diagnosed LF patients using LFIP has been conducted at CLH in 88 cases since the first patient was treated with LFIP in April, 1981. Laboratory confirmation of all instances followed treatment by several weeks or months; thus, the diagnosis was not confirmed by in some instances. Thiry-three cases were eventually confirmed as LF by virus isolation, or serologically by seroconversion or 4-fold elevation of IFA titer. Earlier it has been noted some cases of LF will be missed by both virus isolation and serological testing, so that some others who were administered LFIP may have actually had LF, even though they are not included in the results of this experiment.

Besides the diagnostic ambiguity at the time of treatment each patient there was also a gree of uncertainity regarding the efficacy of the plasma used. Initially, the hospital staff did not always identify the LFIP donor in the patient's hospital chart. Furthermore, some units were given that had been collected for other purposes by the hospital laboratory, and were not from among the LFIP units obtained by plasmapheresis of known convalescents. In some instances information

from USAMRIID received after plasma infusion indicated that the plasma LNI's were low, with NA titers of doubtful value to the receipient. Thus, the effect of treatment was likely to vary a great deal from patient to patient.

Experience in Nigeria has suggested that if LFIP is to be administered to it should be given no later than the 10th day of illness, as accurately as the time can be determined (7). The Nigerian experience confirms that with Junin convalescent plasma in Argentinian hemorrhagic fever - the administration of plasma late in the course of illness may actually result in a higher mortality than if no immunotherapy at all is given (19).

At CLH two units were used for each patient in most instances; in general, one unit was administered to a small child, less in a few cases. In attempting to compare the effect of treatment among patients we have calcualted arbitrary "Therapeutic Units" (TU), the result of multiplying the LNI of each plasma unit by 250, the average volume in milliliters. If two units were administered the TU of the units were added to indicate the total treatment each person received.

The results of treatment with LFIP's given in Appendix F, Table F-1. Three of 39 confirmed cases of LF receiving LFIP died. Two were among the 8 who received plasma of uncertain origin, in most cases with very low LNI. One did who had received 700 TU.

The case fatality rate, 0.077, is not different from that among all patients treated at CLH during the same period (See Appendix B, Table B-3). It should be noted, however, that in during much of this time the supplies of LFIP were limited, and immunotherapy was administered only to patients who were clinically considered to be severely ill.

It is not known what does of TU can be considered clinically effective. Among 16 patients who received plasma of uncertain potency or less than 500 TU 2 died; one died among those who had received 500 TU or more.

VI. Conclusion

The first Technical Objective of Contract DAMD17-79-C-9024 was the collection of LFIP units, and this has been accomplished. During the course of this effort potential plasma donors were identified by means of virus isolation and serological testing. Plasmapheresis and ultra-freezing equipment were installed in two centers, at CLH and PH. Appropriate donors were persuaded to contribute plasma, and a roster to ensure regular plasma collection was established. Through experiments conducted at USAMRIID criteria for the selection of donors were developed as the project continued.

Surveys of hospital staffs have demonstrated that LF infections are endemic throughout Liberia. Where investigated, it has been found that infection among hospital staff occurs at rates several times that

in the nearby communities, suggesting person-to-person transmission in the hospital environment.

Surveys of patients indicate that in the Liberian Northwest Lassa fever is common, and in some places the leading cause of illness among febrile patients admitted to hospitals.

During the course of the investigation hospital and village health personnel have been recruited into programs of diagnosis and treatment; there is now a strong cadre of physicians, physicians' aids and nurses familiar with the disease, and prepared for further efforts in the elucidation and managements of LV infections in their invironment. The capability of serological testing for LV antibodies, and of conducting epidemiological investigations and of supervising and managing field diagnostic and therapeutic programs has been developed in the person of the Field Investigator and Resident Head of the project at the LIBR, Mr. J. E. Yalley-Ogunro.

The progress under the Contract has thus made significant contributions toward understanding the scope of LF in Liberia. Furthermore, personnel and facilities are in place in Liberia to permit further work there, and, it is hoped, to assist in the ultimate control of LF in Africa.

VII. References

1. Frame, J.D., Baldwin, J.M., Jr., Gocke, D.J., & Troup, J.M. Lassa fever, a new virus disease of man from West Africa. Clinical description and pathological findings. Am. J. Trop. Med. Hyg. 19: 670-675 (1970).

2. White, H.A. Lassa fever. A study of 23 hospital cases. Trans. Roy. Soc. Trop. Med. Hyg. 66: 390-401 (1972).

3. Monath, T.P., Mertens, P.E., Patton, R., Moser, C.R., Baum, J.J., Pinneo, L., Gary, G.W., & Kissling, R. A hospital outbreak of Lassa fever in Zorzor, Liberia, March - April, 1972. Amer. J. Trop. Med. Hyg. 22: 773-784 (1973).

4. Fraser, D.W., Campbell, C.C., Monath, T.P., Goff, P.A. & Gregg, M.D. Lassa fever in the Eastern Province of Sierra Leone, 1970-1972. Amer. J. Trop. Hyg. 23: 1131-1139 (1974).

5. Frame, J.D. Surveillance of Lassa fever in missionaries stationed in West Africa. Bull. W.H.O. 52: 593-598 (1975).

6. Clayton, A.J. Lassa immune serum. Bull. W.H.O. 55: 453-439 (1977).

7. Frame, J.D., Verbrugge, C.P., Gill, R.G. & Pinneo, L. The use of Lassa fever convalescent plasma in Nigeria. Trans. Roy. Soc. Trop. Med. Hyg. 78: 319-324 (1984).

8. Leifer, E., Gocke, D.J., & Bourne, H. Lassa fever, a new virus

disease of man from West Africa. II. Report of a laboratory acquired infection treated with plasma from a person recently recovered from the disease. Am. J. Trop. Med. Hyg. 19: 677-679 (1970).

9. Frame, J.D., Casals, J. & Dennis, E.A. Lassa virus antibodies in hospital personnel in Western Liberia. Trans. Roy. Soc. Trop. Med. Hyg. 73: 219-224 (1979).

10. Jahrling, P.B., & Peters, C.J. Passive antibody therapy of Lassa fever in cynomologus monkeys: Importance of neutralizing antibodies and Lassa virus strain. Infec. and Immun. 44: 528-533 (1984).

11. Jahrling, P.B., Frame, J.D., Rhoderick, J.B., & Monson, M.H. Endemic Lassa fever in Liberia. IV. Selection of optimally effective plasma for treatment by passive immunization. Trans. Roy. Soc. Trop. Med. Hyg. 79: 380-384 (1985).

12. Frame, J.D., Annual Summary Report, Lassa Fever Immune Plasma, Division of Tropical Medicine, School of Public Health, College of Physicians and Surgeons, Columbia University, New York, N.Y. August, 1983.

13. Frame, J.D., Jahrling, P.B., Yalley-Ogunro, J.E., & Monson, M.H. Endemic Lassa fever in Liberia. II. Serological and virological findings in hospital patients. Trans. Roy. Soc. Trop. Med. Hyg. 78: 656-660 (1984).

14. Jahrling, P.B., Frame, J.D., Smith, S.B., & Monson, M.H. Endemic Lassa fever in Liberia. III. Characterization of Lassa virus isolates. Trans. Roy. Soc. Trop. Med. Hyg. 79: 374-379 (1985).

15. Yalley-Ogunro, J.E., Frame, J.D., and Hanson, A.P. Endemic Lassa fever in Liberia. VI. Village serological surveys for evidence of Lassa virus activity in Lofa County, Liberia. Trans. Roy. Soc. Trop. Med. Hyg. 78: 764-770 (1984).

16. Frame, J.D., Yalley-Ogunro, J.E., & Hanson, A.P. Endemic Lassa fever in Liberia. V. Distribution of Lassa virus activity in Liberia: Hospital staff surveys. Trans. Roy. Soc. Trop. Hyg. 78: 761-763 (1984).

17. Frame, J.D., Annual Summary Report, Lassa Fever Immune Plasma. Division of Tropical Medicine, School of Public Health, College of Physicians and Surgeons, Columbia University, New York, N.Y. August, 1984.

18. Frame, J.D., Annual Summary Report, Lassa Fever Immune Plasma. Division of Tropical Medicine, School of Public Health, College of Physicians and Surgeons, Columbia University, New York, N.Y. June, 1983.

19. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Lewis, S.C., & Maiztegui, J.I. Importance of doses of neutralizing antibodies in treatment of Argentine hemorrhagic fever with immune plasma. Lancet

2: 255-256 (1984).

VIII. Publications

Frame, J.D., Verbrugge, G.P., Gill, R.G. & Pinneo, L. The use of Lassa fever convalescent plasma in Nigeria. Trans. Roy. Soc. Trop. Med. Hyg. 78: 319-324 (1984).

Monson, M.H., Frame, J.D., Jahrling, P.B. & Alexander, K. Endemic Lassa fever in Liberia. I. Clinical and epidemiologic aspects at Curran Lutheran Hospital, Zorzor, Liberia. Trans. Roy. Soc. Trop. Med. Hyg. 78: 549-553 (1984).

Frame, J.D., Jahrling, P.B., Yalley-Ogunro, J.E. & Monson, M.H. Endemic Lassa fever in Liberia. II. Serological and virological findings in hospital patients. Trans. Roy. Soc. Trop. Med. Hyg. 78: 656-660 (1984).

Jahrling, P.B., Frame, J.D., Smith, S.B. & Monson, M.H. Endemic Lassa fever in Liberia. III. Characterization of Lassa virus isolates. Trans. Roy. Soc. Trop. Med. Hyg. 79: 374-379 (1985).

Jahrling, P.B., Frame, J.D., Rhoderick, J.B. & Monson, M.H. Endemic Lassa fever in Liberia. IV. Selection of optimally effective plasma for treatment by passive immunization. Trans. Roy. Soc. Trop. Med. Hyg. 79: 380-384 (1985).

Frame, J.D., Yalley-Ogunro, J.E. & Hanson, A.P. Endemic Lassa fever in Liberia. V. Distribution of Lassa virus activity in Liberia: Hospital staff surveys. Trans. Roy. Soc. Trop. Med. Hyg. 78: 761-763 (1984).

Frame, J.D., Yalley-Ogunro, J.E. & Hanson, A.P. Endemic Lassa fever in Liberia. VI. Village serological surveys for evidence of Lassa virus activity in Lofa County, Liberia. Trans. Roy. Soc. Trop. Med. Hyg. 78: 764 770 (1984).

IX. Personnel Receiving Contract Support

John D. Frame, M.D., Principal Investigator (17%). Adjunct Associate Professor of Public Health (Tropical Medicine) Columbia University School of Public Health, New York, N.Y.

Sylvia Terilli, (20%), Executive Secretary, Division of Tropical Medicine, Columbia University School of Public Health, New York, N.Y.

J.E. Yalley-Ogunro, B.S. (100%), Field Investigator; Resident Head, Lassa Fever Control Project, Liberian Institute for Biomedical Research, Charlesville, Liberia.

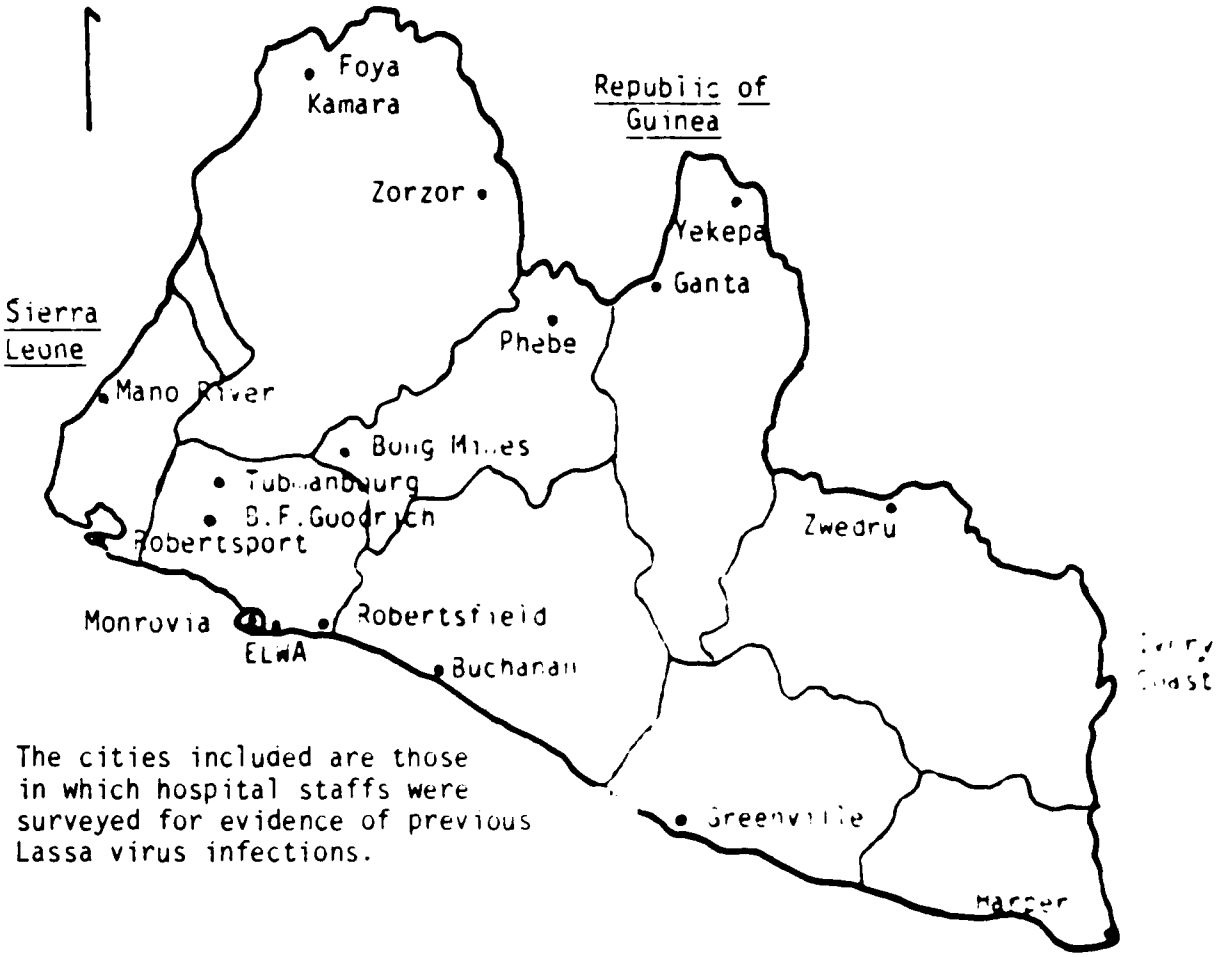
Andrew Cole, M.D. (50%), Clinical Investigator, Lassa Field Control Project, Kolahun, Lofa County, Liberia.

James Norman, B.S. (50%), Laboratory Technologist, Lassa Field

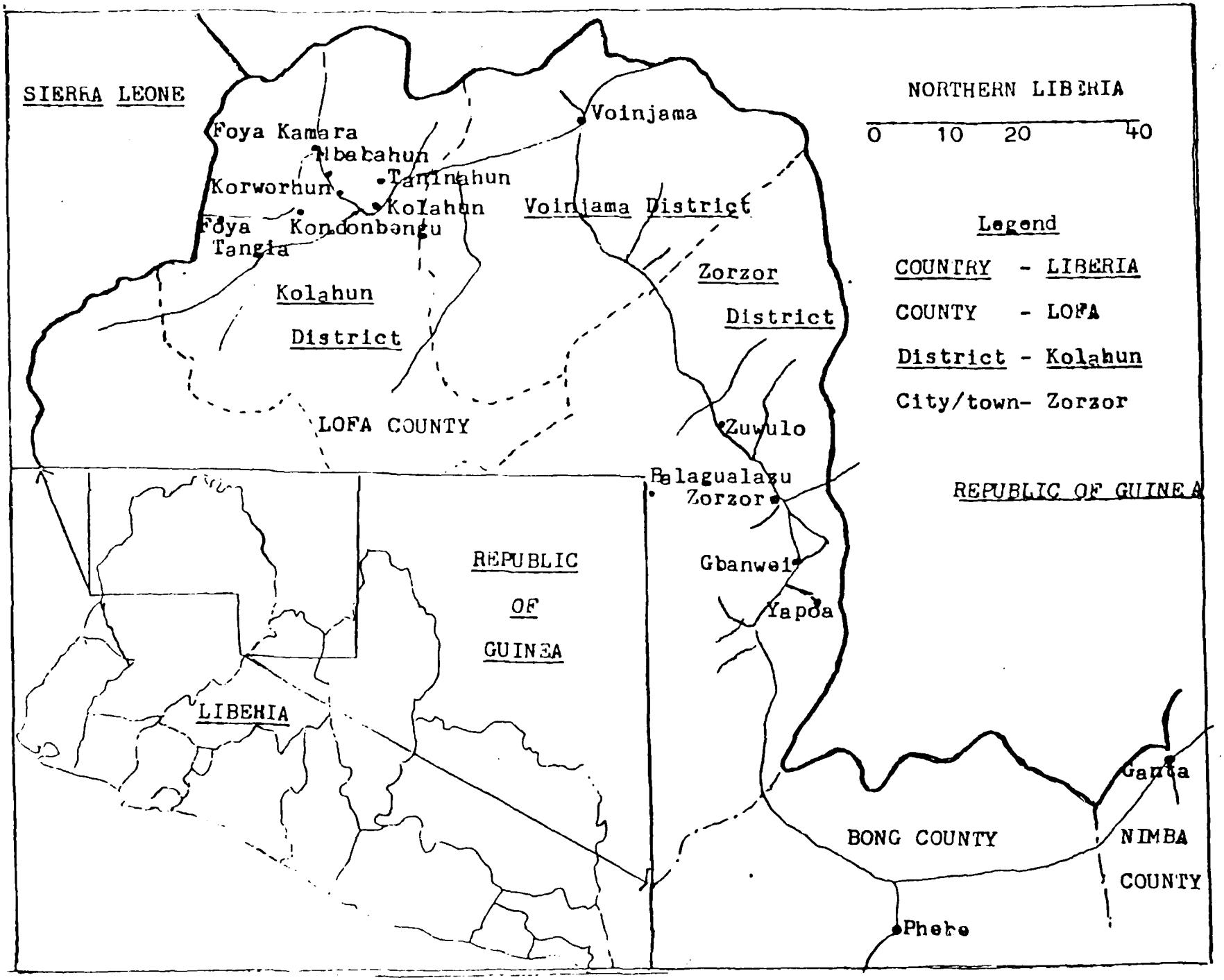
Control Project, Phebe Hospital, Bong County, Liberia.

None of the above received support toward the earning of a graduate degree as a result of research done under this contract.

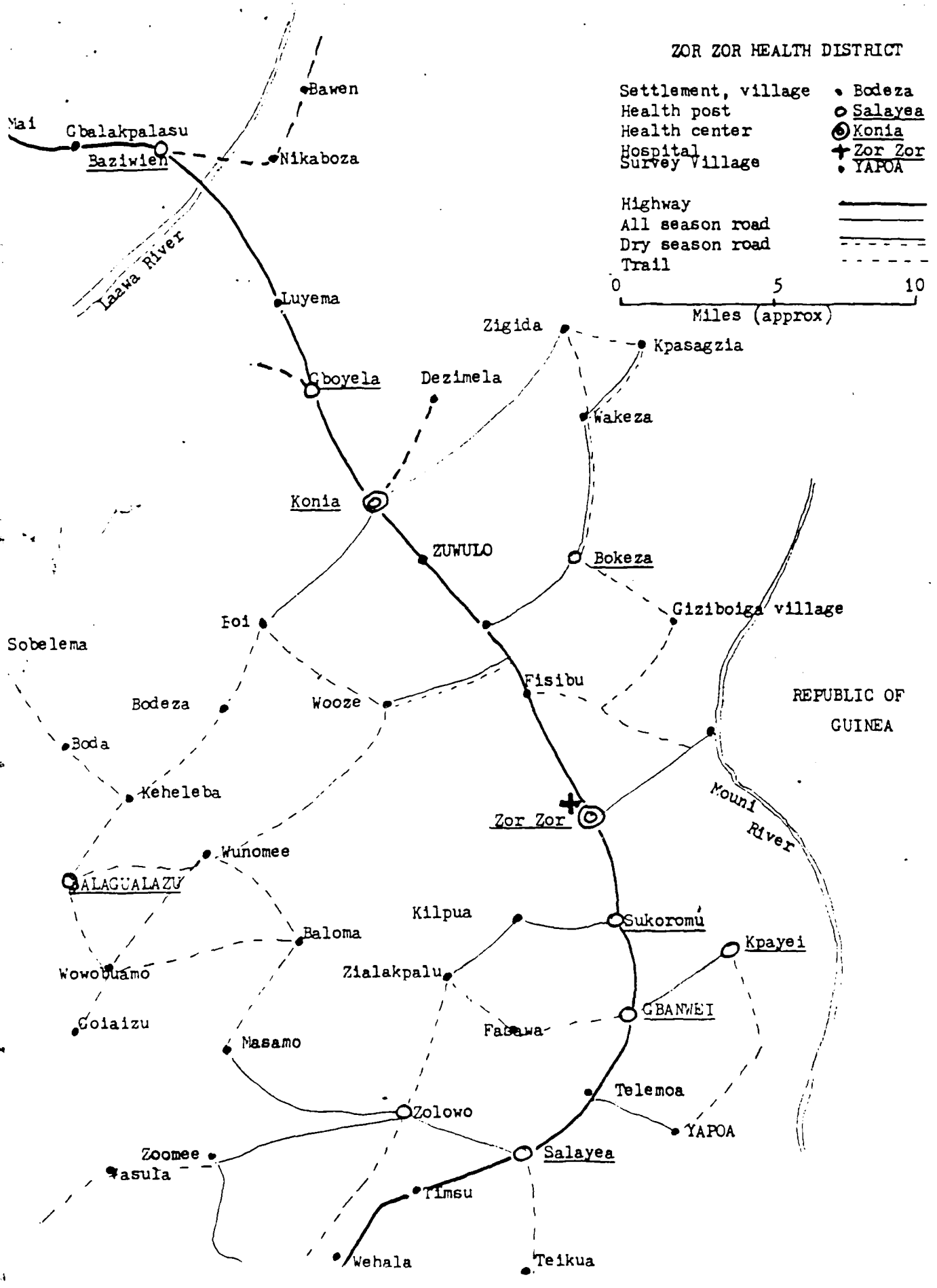
LIBERIA



The cities included are those in which hospital staffs were surveyed for evidence of previous Lassa virus infections.



MAP -- NORTHERN LIBERIA


ZOR ZOR HEALTH DISTRICT
Settlement, village
Health post
Health center
Hospital
Survey Village
Bodeza
Salayea
Konia
Zor Zor
YAPOA
Highway
All season road
Dry season road
Trail
0
5
10
Miles (approx)
Bawen
Mai
Gbalakpalasu
Baziwien
Nikaboza
Laawa River
Luyema
Zigida
Kpasagzia
Gboyela
Dezimela
Wakeza
Konia
ZUWULO
Bokeza
Giziboiga village
Foi
Sobelema
Fisibu
REPUBLIC OF GUINEA
Bodeza
Wooze
Boda
Keheleba
Zor Zor
Mouni River
Wunomee
SALAGUALAZU
Kilpua
Sukoromú
Kpayei
Baloma
Wowoduamo
Zialakpalu
GBANWEI
Goiaizu
Fasawa
Masamo
Telemoa
Zolowo
YAPOA
Zoomee
Salayea
Yasula
Timsu
Wehala
Teikua

Appendix A. Lassa Fever Immune Plasma Units

Table A-1. Lassa Fever Immune Plasma Units Collected in Liberia July, 1984 - July 1, 1985.

Donor	Date of Illness	Date of Donation	IFA Titer*	LNI (Neutralization) Josiah	Macenta	# No. of Units Collected	USAMRIID**
MiAm		7/24/84	-	0.9		2	1
DaBa	11/82	11/06/84	?			2	
		12/13/84	+	2.1		2	1
		03/24/85	?			2	
		06/20/85	?			1	
NaBa	02/84	07/31/84	8	3.3+		2	1
		10/10/84	8	2.3+		2	1
ArCo		05/10/85	?	2.2+		2	2
JaDa		07/25/84		1.4		2	1
DaDo	04/77	07/31/84	?			2	1
		11/06/84	?	1.6		2	
		03/25/85	-			2	
		07/01/85	?			2	2
LoFl	01/82	12/11/84	4	2.4+		2	1
		03/24/85	4			2	
PaFl	07/83	12/12/84	8	2.4+		2	1
KlGa		02/22/85	8			2	1
JaGh		05/09/85	32			2	2
DaJa		03/24/85	?	0.4		2	1
		07/01/85	4			2	2
IrJo	02/83	08/01/84	32	3.3+		2	1
BoKa	10/82	07/30/84	?			2	
		10/11/84	-			2	
		12/11/84	?	1.0		2	1
		02/25/85	?	2.2+		2	
		03/26/85	?			2	
		05/10/85	?			2	
		07/01/85	4			2	2
KeKa	10/82	11/07/84	8			2	
		12/12/84	?	2.4+		2	1
		03/25/85	4	1.7		1	1

JoKe		07/25/84	-	0.0	2	1
JohKe		06/14/84@		2.0		1
		11/06/84	4	2.3+	2	
		07/01/85			2	
DaKo	10/81	07/31/84	32	3.3+	2	1
		10/10/84	32		2	
		11/11/84			2	
		12/11/84	8	2.4+	2	1
		02/26/85	16			
		05/10/85	8		2	
DaKo		07/30/84		3.3+	2	2
GaKoII	06/83	08/01/84	?	0.1	2	1
		02/25/85	-	0.4	2	1
		07/01/85	-		2	2
JoKc		07/24/84	-	0.5	2	1
KoKo		06/30/85	8		2	1
		12/12/85			2	1
YaKo	10/81	06/12/84@	4	2.3		1
		10/10/84	-	2.3	2	
		12/12/84	4	1.6	2	1
		02/25/85	8		2	1
		06/30/85	8		2	
GaLa		02/26/85			2	1
FlMa	09/81	10/11/84	-		2	
		02/26/85	?		2	1
KaMa	03/83	08/01/84	8	3.3+	2	2
		11/05/84	8		2	
		02/20/85	8		2	
		03/24/85	8		2	
		06/30/85	8		2	2
NoMa	03/82	06/13/84@	?	3.3+		1
		10/11/84	?	3.3+	2	
		05/10/85	16		2	
JaMo	?	06/13/84@	?	0.8		1
		07/31/84	?		2	
		11/06/84	-	0.7	2	
		12/11/84	?	0.4	2	1
		03/24/85	4	0.6	2	1
		05/10/85	?	0.5	2	2
CeMu	04/83	08/01/84	32	3.3+	2	2

DaMu		07/24/84	-	1.2	2	1
SaPa		07/24/84	-	1.4	2	1
JoPe		07/25/84	?	0.9	2	1
ErRi		11/05/84	32	2.3	2	
		02/22/85	32	2.2+	2	1
		05/ /85			2	
		07/01/85	32		2	2
DaSu	01/83	10/12/84	?		2	
		12/11/84	?	0.5	2	1
		06/18/85	4		2	
MuSu	11/81	06/13/84@	8	3.3+		2
		12/12/84	-	2.4+	2	1
		03/25/84	4		2	
		07/01/85	8		2	1
YaTa		03/24/85	?	2.2+	2	1
BaTo	03/83	06/13/84@	4	1.4		1
		07/31/84	4		2	
		11/05/84	-	1.7, 2.3+	2	
		12/11/84	?	1.1	2	1
		02/25/85	-	1.1	2	1
		05/09/85	?		2	
		07/01/85	4		2	2
DeTo		06/14/84@	8	2.4, 3.3+		2
		11/05/84	8	2.3+	2	
		05/10/85			2	
JoTo		03/24/85	?		2	
NoTo		05/10/85	32	2.2+	2	2
GeTu		07/25/84	-	2.4	2	1
BeVa	?	07/31/84	-		2	
		12/11/84	-	0.5	2	1
		03/25/85	-	0.7	2	1
		07/01/85	-		2	2
GoVa		02/26/85	4	2.2+	2	1
		05/10/85	8		2	
YaVa	02/82	06/13/84@	?	1.9	2	1
		10/11/84	-		2	
		02/26/84	?	2.2+	2	1
		05/10/85	?		2	

KlVe	07/83	11/07/84	4			2	1
		12/13/84	+	2.0		2	1
		02/22/85	8	2.6+		2	
		03/25/85	4	2.2+		2	2
		05/09/85	8			2	
		07/02/85	4			2	1
RaVe	12/82	11/07/84	-			2	
		12/14/84	?	0.2		2	1
		03/25/85	-	0.3		2	1
		05/09/85	?			2	
		07/02/85	?			2	2
JoVes		10/10/84	-	2.3+		2	
		12/11/84	4	1.6		2	1
		03/25/85	?	2.2+		2	1
		06/30/85	?			2	2
JoVet		07/26/84	4	0.9		2	1
MoWo		10/10/84	8			2	
		02/25/85	32	2.2+		2	2
		05/11/85	36	2.2+		2	2
		06/30/85	16			2	2
ElYa		07/24/84	?	1.9		2	1
SuYa		07/31/84	8	1.5, 1.8		2	2
MaZa	07/82	06/14/84@	4	1.4			1
DaZe		11/07/84	4	0.6	0.3	2	
						246	108

* Reciprocal of titer of indirect fluorescent antibodies against Lassa virus, tested at the Liberian Institute for Biomedical Research. ? = questionable reactivity at screening titer of 1:4.

\# Log Neutralization Index of neutralizing antibodies to Josiah and Macenta strains of the Lassa virus, tested at USAMRIID

** Plasma units forwarded to USAMRIID.

@ Units collected and listed in prior report forwarded to USAMRIID during this report year.

Appendix A.

Table A-2. Summary of LFIP obtained by plasmapheresis during the present investigation.

Date of Collection	Total LFIP	LFIP Units Sent to USAMRIID	
		Total	Acceptable (LNI > 0.3)
Oct 1980 - Nov 1982	123	98	59
Dec 1982 - June 1983	79	52	48
July 1983 - June 1984	141	59	59
July 1984 - June 1985	246	108	106
Total	589	317	272

Appendix B. Lassa Fever among febrile patients in selected Liberian Hospitals.

Because of the limited capacity of the containment laboratory at USAMRIID and the need to use it in other investigations virus isolation was not attempted routinely in all febrile patients during this past year. In Table B-1 are listed the results of serological tests performed in CLH and the Tellewoyan Memorial Hospital in Lofa County, Phebe Hospital in Bong County, and the G.W. Harley Memorial Hospital in Ganta, Nimba County.

In Table B-2 the results of investigations since the start of the program in Liberia are summarized. The greatest amount of work has been done at CLH. The results in the hospital show the results of complement fixation tests (7/70-6/80), and with IFA serological testing together with virus isolation when an ordinary (-20°C) freezer (7/80-11/83) and an ultra-freezer (-70°C) (12/83-4/84) were used. Since May, 1984, virus isolation has not been attempted routinely on all patients.

The case fatality rates for LF and presumptive LF were determined for 182 patients at CLH whose clinical courses were adequately documented (Table B-3). The highest rate, .321, was for women pregnant or in the first post-partum month; the rate among children five years of age and under was also high. Overall, 10.4% of LF and presumptive LF patients died.

In Table B-4 the effectiveness of virus isolation and serodiagnosis in the diagnosis of LF are compared. Virus isolation is the more effective technique if an ultra-freezer is available to permit persistent virus activity and increased recovery of virus by cell culture techniques.

Table B-1. Results of testing of febrile patients in selected Liberian Hospitals for evidence of LV infections, Spring 1984-June 1985.

Hospital and Dates	No. Tested	Lassa fever*			Presumptive LF - LVA 1:64 & over	Total (rate)	Other LVA positives
		VI	SC	Total (Rate)			
CLH							
5/84-6/85	426	55	10	65(.153)	8	73(.171)	40
PH							
4/84-6/85	188		8	8(.042)	5	13(.069)	16
GWH	28		3	3(.107)	1	4(.143)	3
Total	642	55	21	76(.118)	14	90(.140)	59

* VI - virus isolation; SC - seroconversion or 4-fold rise in IFA titers.

Table B-2. Summary of investigations for the diagnosis of Lassa Fever among febrile patients in selected Liberian Hospitals, 1979-1985.

Hospital/ Dates	No. Tested	Lassa fever			Presumptive LF	Total (rate)
		Virus Isolation	Sero-Conversion	Total (rate)		
Curran Lutheran						
1/79-6/80*	35	#	1	1 (.029)	5	6 (.121)
7/80-11/83	675	43	37	80 (.119)	27	107 (.159)
12/83-4/84	229	34	6	40 (.175)	5	45 (.197)
5/84-6/85	426	55	10	65 (.153)	8	73 (.171)
Total	1365	132	54	186 (.136)	45	231 (.169)
Phebe						
5/81-2/83	165	7	7	14 (.085)	10	24 (.145)
3/83-6/85	283	#	10	10 (.035)	13	23 (.081)
Total	448	7	17	24 (.054)	23	47 (.096)
G.W. Harley	33	#	3	3 (.091)	3	6 (.182)
Swedish Mission Clinic	7	#	-	-	-	-
Tellewoyan	12	#	-	-	-	-
ELWA	24	#	-	-	-	-

* Testing done by complement fixation; all other tests by the IFA technique.

\# Virus isolation not attempted.

Table B-3. Mortality of 182 cases of LF and presumptive LF at CLH.

Sex/Age	Number	Deaths	Rate
Adult males	43	3	0.070
Adult females	117	13	0.111
Pregnant and post-partum*	28	9	0.321
Not pregnant	89	4	0.045
Children	22	3	0.136
Fetus	1	1	1.000
0-5 years	7	2	0.286
6-12 years	4	0	-
13-18 years	9	0	-
"Child"#	1	0	-
Total	182	19	0.104

* Including women within one month after delivery

\# Age not specified

Table B-4. Comparison of virological and serological techniques in the diagnosis of LF at the Curran Lutheran Hospital, Zorzor, Liberia, October, 1983 through April, 1984.

	Lassa fever			Presumptive LF: high IFA titers	Total
	Virus Isolation	Seroconversion or rising IFA titers	Total cases		
Diagnosed by:					
Virus isolation only	12		12		12
Virus isolation & IFA	22	14	22	8	22
Serology only		6	6	5	11
Total	34	20	40		45

Total LF: 40 cases. Total LF + presumptive LF: 45 cases

Virus isolation: 34/40 or 85% of LF cases; 34/45 of 76% of LF + presumptive LF cases

Serological diagnosis: 20/40 or 50.3% of LF cases; 33/45 or 73% of LF + presumptive LF cases.

Appendix C. Survey of residents in the Ganta Rehabilitation Center, Nimba County, for prevalence of LVA determined by the IFA technique.

In June 1984 the residents of the Ganta Rehabilitation Center were examined for the presence of antibodies to LV. The Center is primarily engaged in the treatment of cases of leprosy, and it was felt that comparison of patients with differing forms of this disease might cast light on the epidemiology of LF.

Table C-1 indicates the number of sera positive by titer, by the presence or absence of leprosy, and by type of leprosy. Only lepromatous, borderline and unclassified leprosy patients are known to be in the Center. The few known cases of tuberculoid leprosy in Liberia are not institutionalized. The presence of cases with relatively high titers of antibodies to LV suggest that LV activity is a recurring matter here.

Table C-2 demonstrates no difference in the presence of LVA between males and females, nor among age groups.

Table C-3 presents the prevalence of LV antibodies by age groups and diagnosis. There are no differences in prevalence among the various diagnostic categories. There is evidence of LV activity in all age groups.

Table C-1. Distribution of LVA positives among inhabitants of the Ganta Rehabilitation Center, by IFA titers and diagnosis.

Diagnosis	No. Tested	Number positive by antibody titer 1:8	1:16	1:32	1:64	1:128	Total Positive No.	Total Positive Rate	Questionable Reactions
Leprosy									
Lepromatous	98	4		1	1		6	0.061	6
Borderline	168	4	3	1	3	2	13	0.077	14
Unclassified	22		2	1			3	0.136	2
Total Leprosy	288	8	5	3	4	2	22	0.076	22
Not Leprosy									
Family of Patients	52	2	1		1		4	0.077	1
Non-patients	41	3	2				5	0.122	1
Total not leprosy	93	5	3		1		9	0.097	2

Table C-2. Distribution of LV positives among leprosy patients at the Ganta Rehabilitation Center, by age and sex.

Sex	Adults	Ages 13-18	Ages 5-12	Total	Rate
Males	13/153*	0/11	1/14	14/178	0.079
Females	6/89	1/7	1/14	8/110	0.076
Total	19/242	1/18	2/28	22/288	0.076

* Positive reactors/number tested

Table C-3. Prevalence of LV positives among residents at the Ganta Rehabilitation Center by age and diagnosis.

Diagnosis	Adults	Ages 13-18	Ages 5-12	Total	Rate
Leprosy					
Lepromatous	6/87*	0/8	0/3	6/98	0.061
Borderline	11/143	0/7	2/18	13/168	0.077
Unclassified	2/12	1/3	0/7	3/22	0.136
Total Leprosy	19/242	1/18	2/28	22/288	0.076
Not Leprosy					
Family of Patient	1/18	0/	3/37	4/52	0.076
Non-patients	4/19	1/7	0/15	5/41	0.122
Total, not Leprosy	5/27	1/14	3/52	9/93	0.097

* Positive reactors/number tested

Table C-4. Comparison of prevalence of LVA in Lofa County roadside villages with those in "bush" villages.

Location	IFA Positive	IFA Negative	Total	Rate
Roadside				
Zuwulo	12	281	293	0.041
Gabanwei	32	359	391	0.082
Mbabahun	15	355	370	0.042
Korworhun	13	217	230	0.060
Total	72	1212	1284	0.056*
"Bush"				
Yapoa	12	200	212	0.057
Balagualazu	12	380	392	0.031
Tanninahun	5	231	236	0.021
Kondonbengu	6	233	239	0.025
Total	35	1044	1079	0.032*

$\chi = 9.9$, $P < .001$

Table C-5. Prevalence of LVA in surveyed villages of Lofa County, Liberia, by age groups.

Age	No.	Observed Cases (O)	Rate	Expected Cases (E)	$(O-E)^2$	$(O-E)^{2/E}$
Adults	1107	61	0.055	47.1	193.2	4.10
5-18 yrs	794	18	0.023	33.8	249.6	7.10
Under 5	613	28	0.046	26.1	1.9	.14
Totals	2514	107	0.043	107.0	444.7	11.62

$\chi^2 = 11.62$, $P < .01$

Appendix D. Surveys of hospital staffs for the determination of the prevalence and incidence of Lassa Fever.

Table D-1. Prevalence of LVA in the staff of the Tellewoyan Memorial Hospital, Voinjama, Lofa County, Liberia, June, 1984.

No. tested	IFA titers*					Total postive#	
	?	4	8	16	32	No.	Rate
89	3	5	5		1	11(14)	.126 (.157)

* Expressed as reciprocals
\# Values in parentheses include borderline positive results at a titer of 1:4.

Table D-2. Prospective investigation of beginning midwifery and practical nursing students at Curran Lutheran Hospital, Lofa County, Liberia for evidence of LV infections during their period of instruction.

Class	Initial Testing		Follow-up Testing					
			First Year			Second Year		
	No. Tested	No. Pos.	No.* Tested	No. Pos.	Sero-Conversion	No.* Tested	No. Pos.	Sero-Conversion
1985	26	2	24	2	0	15	1	1#
1986	29	1	21	2	0			
1987	16	1						
Total	71	4	45	4		15	1	-

* Not all students found to be positive on initial examination submitted to retesting.

\# Clinical diagnosis of Lassa fever February 1984, IFA 1:256

Table D-3. Prevalence of LF in staffs of Liberian Hospitals.

Hospital	County	Year of Survey	No. Tested	No. Tested	Prevalence
Swedish Free Pentecostal Mission Clinic	Lofa	1977	35	9	.229
		1979	52	21	.404
		1981	40	9	.235
Curran Lutheran	Lofa	1977	95	12	.126
		1979	97	22	.223
Phebe Hospital	Bong	1977	236	24	.102
Bong Mining Company	Bong	1977	74	3	.004
G.W. Harley Memorial	Nimba	1977	59	4	.068
		1982	69	12	.174
LAMCO-Nimba	Nimba	1977	82	4	.049
National Iron Ore Co.	Cape Mount	1981	67	6	.090
Robertsport Government	Cape Mount	1981	52	3	.058
Bomi Territory Govt.	Bomi Terr.	1981	52	3	.058
B.F. Goodrich	Bomi Terr.	1980	48	6	.125
Martha Tubman	Grand Gedeh	1982	55	4	.058
ELWA	Montserrado	1976	92	5	.054
Maternity Center	Montserrado	1977	109	7	.064
LAMCO-Buchanan	Grand Bassa	1977	62	4	.065
J J Dossen	Maryland	1982	80	3	.038

Table D-4. Comparison of prevalences of LVA positives among adults in selected Liberian Villages with staff members of nearby hospitals with IFA titers of 1:8 and over.

Location	No. Tested	IFA titers over 1:8	Rate
Zorzor district			
Gbanwei	144	9	0.063
Yapoa	80	1	0.013
Zuwulo	126	8	0.063
Balagualazu	163	9	0.055
Totals	513	28	0.055
Curran Lutheran Hospital (1979)	97	18	0.186
Ratio of prevalences, hospital: community - 3.38			
Kolahun district			
Taninahun	126	4	0.032
Kondonbengu	111	5	0.045
Mbabahun	154	10	0.065
Korworhun	105	8	0.076
Totals	496	27	0.054
Tellewoyan Memorial Hospital (1984)	89	6	0.067
Ratio of prevalences, hospital: community - 1.24			
Foya Vicinity (Kolahun district)			
Foya village	30	3	0.100
Borlelo	68	4	0.059
Totals	98	7	0.071
Swedish Mission Clinic (1979)	52	19	0.365
Ratio of prevalences, hospital: community - 5.14			
Ganta district (Nimba County)			
Rehabilitation Center	242	19	0.079
G.W. Harley Memorial	69	9	0.130
Ratio of prevalences, hospital: community - 1.65			

Appendix E. Lassa fever in households of hospital cases.

The investigation of families of hospital cases of LF for evidence of LV infections among them was conducted by two American medical students under the supervision of the Field Investigator. The Principal Investigator designed the experiment and suppled the investigating team with documents including the scope of the survey, the protocol for the investigation, a form to be used in recording results, and instructions for obtaining blood specimens; these are included in the Annexes to this Appendix.

Nine patients at CLH were selected for the investigation; on subsequent testing at the LIBR and USAMRIID 6 were found to be viremic and one showed evidence of seroconversion (Table E-1). In 2 the diagnosis could not be confirmed (Table E-2). Ten control households were tested as well (Table E-3).

Among the confirmed cases of LF a sister of index case A was found to have LVA at high titers; she did not recall a significant fever. The husband of the index case had had a fever 4 days after the onset of her illness, but no LVA were found on testing. The husband of index case C was also found to have LVA in high titer, but did not report a significant febrile illness. There were 4 other seropositives at low titers among members of these households, and 6 who reported fevers, of 47 household members in all.

Among the 78 members of the control households of these 7 cases there was one with LVA at a high titer and 7 others at low titers. Fourteen reported fevers of whom 1 also had LVA at low titer.

The problems of this approach are examplified by the household of patient H,the wife and mother. About 6 days after the onset of her illness 7 members of her household developed a febrile illness, and when tested in the survey three weeks later 5 had LVA in titers ranging from 1:32 to 1:256. However, the index case herself did not have LVA when tested on the sixth, seventh and tenth day of her illness, and no virus was isolated on any of those days.

The number of persons with LVA positive sera was not significantly different in case households from controls (Table E-4). In both the categories of of fever and of serological findings the highest rates were found in the households of patients suspected of LF whose diagnosis could not be confirmed by laboratory tests.

Table E-1. Serological investigation for Lassa Fever in households of confirmed LF cases.

House	Dates of Illness, Index case (Diagnosis)*	Room of index case				Other rooms of house			
		No.	IFA pos.	IFA titers	Fever cases (dates)	No.	IFA pos.	IFA titers	Fever cases (dates)
A	12/31-1/15 (VI)	1	-	-	1 (1/4)	3	2	1:4, 1:128	-
B	1/19-1/20 (VI)	4	-	-	-	12	2	1:4,1:4	-
C	2/8-2/19 (VI)	3	1	1:256	1 (3/2)	4	-	-	1 (3/9)
D	2/25-3/23 (SC:IFA 0 to 1/256)	4	-	-	1 (4/1)	2	1	1:8	1 (3/11)
E	3/17-3/26 (VI)	1	-	-	1 (3/23)				
F	3/19-3/27 (VI) (Deceased)	1	-	-	-				
G	3/26-4/11 (VI)	5	-	-	-	7	-	-	-
Total		19	1		4	28	5		2

*Diagnosis -- VI = virus isolation; SC = seroconversion

Table E-2. Serological investigation for Lassa Fever in households of suspected LF cases, not confirmed.

House	Dates of Illness, Index case	Room of index case				Other rooms of house			
		No.	IFA pos.	IFA titers	Fever cases (dates)	No.	IFA pos.	IFA titers	Fever cases (dates)
H	2/23-3/13	1	1	1:16	1 (2/7)	8	5	1:16, 1:32 1:64 1:256(2)	5 (3/1,3/1, 3/1,3/1, 3/5)
J	3/24-4/11	5	-	-	1 (4/13)	3	-	-	-
Total		6	1		2	11	5		5

Table E-3. Serological investigation for Lassa fever in control households.

House	Dates of Illness, Index case	Room of head of household				Other rooms of house			
		No.	IFA pos.	IFA titers	Fever cases (dates)	No.	IFA pos.	IFA titers	Fever cases (dates)
A-1		2	-	-	1 (3/8)	8	-	-	3 (12/25, 3/8, 3/8)
B-1		3	1	1:32	-	3	-	-	-
B-2		3	-	-	-	2	-	-	-
C-1		3	-	-	2 (3/2, 3/2)	8	3	1:8(2) 1:32	1 (3/2)
D-1		3	-	-	-	5	1	1:8	-
E-1		2	-	-	-	10	-	-	1 (3/22)
F-1		14	2	1:16, 1:32	3 (3/20, 4/1, 4/11)				
F-2		2	-	-	-	8	1	1:256	3 (4/1-11, 4/5, 4/8)
H-1		1	1	1:64	-	8	1	1:128	2 (3/20, 3/25)
J-1		1	-	-	-	11	-	-	1 (4/19)
Total		34	5		6	63	6		11

* The initial letter of each household identification conforms to that of the case household for which it is a control.

Table E-4. Summary of serological investigations for Lassa Fever in household contacts of hospitalized LF patients.

	Room of index case or household head			Other rooms			Total		
	No.	IFA pos. (rate)	Fever (rate)	No.	IFA pos. (rate)	Fever (rate)	No.	IFA pos. (rate)	Fever (rate)
LF Confirmed	19	1 (.053)	4 (.211)	28	5 (.179)	2 (.071)	47	6 (.128)	6 (.128)
LF Suspected, not confirmed	6	1 (.167)	2 (.333)	11	5 (.454)	5 (.454)	17	6 (.353)	7 (.412)
Controls	34	5 (.147)	6 (.176)	63	6 (.095)	10 (.159)	97	11 (.113)	16 (.165)

Appendix E. Lassa Fever in Households of Hospital Cases.

Annex 1. Scope of the survey

A. It is hoped that at least 10 households of index cases and 20 control households will be investigated during this period.

B. The investigation to be conducted by Mr. and Mrs. Johnston will be constrained by their responsibilities for patient care, their ability to make good contacts with the patients' families, and the availability of transportation to the patients' homes.

C. It is suggested that patients be selected whom the clinical staff believes on clinical grounds to be highly suspicious of LF; these usually will be patients to whom the administration of Lassa Fever Immune Plasma is being considered. There are ordinarily about five such patients treated at Curran Lutheran Hospital each month.

D. From among these patients cases will be selected whose residence is within one hour's drive from CLH.

E. Surveys should be started near the end of the first month of experience at CLH, and two or three surveys should be performed each month for the first four months of service at CLH. Surveys in the last two months of service are not discouraged, but information about the results of serological testing may not be completed for these households before the end of the six months' work at CLH.

F. Whenever possible a neighboring household should be tested as control to that of the index case. The Community Health worker should be asked to discuss this with neighbors at the time of planning the initial home visit, or during its course. If permission for evaluating a near-by home is given, methods used should be those employed for the subject household.

Appendix E. Lassa Fever in Households of Hospital Cases.

Annex 2. Protocol for the Investigation of the Epidemiology of Lassa Virus infections in the Families of Lassa Fever Patients

1. Introduction:

The question, what proportion of Lassa fever cases are due to person-to-person from patients, is not clear for Liberia. The observation, that the prevalence of LV antibodies among hospital personnel is 3 to five times that in the surrounding community, suggests that in the hospital environment such transmission is common. In the outbreaks which brought LF to attention it was believed that most cases were due to infection by aerosol, personal contact, contact with body excretions or accidental parenteral infection. These means were postulated for the spread of infection among family members as well as among health workers in outbreaks in which the virulence of the agent was likely greater than is the case in Lofa County, Liberia, at present.

2. General measures and rationale for the present investigation:

A. Investigations of household members of cases of LF identified in the hospital may elucidate the epidemiology, and in particular, the likely mode of LV infection within the households.

B. Data will be collected to ascertain the dates of febrile illness affecting other members of the households, to determine what temporal relationship may exist between them and the illness of the index case.

C. A floor-plan of the house, with the number of rooms and the people sleeping in each, will help determine whether LV infections are related to the density of the "micropopulations" within the households during the hours of sleep.

D. Serum will be obtained to be tested for serological evidence of LV infection within the household, in accordance with Appendix 2.

E. In order to safeguard non-immune investigators from the acquisition of LF, on-site investigations of households will be conducted at least two weeks after discharge of a case from the hospital, or two weeks after the development of secondary cases, if any, determined by inquiry of the members of the households to be investigated.

3. Scope of the investigation:

It is hoped that least ten households will be investigated. See Annex 1.

4. Protocol

4.1. Preliminary steps in the Hospital.

4.1.1. From data on the patient's chart names of the patient, the husband

or father, town and quarter of town of residence at the time of the onset of illness will be obtained.

4.1.2. The patient's visitors will be met, and with the assistance of ward personnel arrangements will be made for a subsequent visit to the patient's home.

4.1.3. Hospital or Community Health personnel will be consulted about the feasibility of follow-up in the home to investigate the case contacts.

4.1.4. Consideration will be given to obtaining serum specimens from visitors who are members of the family living in the same households (See Appendix 2).

4.1.5. A date for a home visit will be made for a day at least two weeks after the discharge or death of the index case, and at a time when most family members will be present at home.

4.2. The Home Visit

4.2.1. The house will be visited and the information requested by the report form regarding the name of the patient, the village and the quarter in which the house is located recorded. A code number will be assigned to the household (See Annex 2).

4.2.2. Names of all family members will be recorded, together with the age, sex and relationhip of each to the patients.

4.2.3. Particular inquiry will be made for each household member of a history of a recent febrile illness; the nature and approximate date of the fever will be recorded.

4.2.4. Permission will be sought to inspect the house.

4.2.4.1. The outside dimensions of the house will be measured roughly, e.g., by pacing.

4.2.4.2. The position of the rooms will be asked; if a Lassa Immune Community Health worker such as Bokai Kamara is present, he may be able to determine the approximate size of the rooms.

4.3. Specimen collection (See Annex 3)

4.3.1. Sera will be obtained from as many as possible of household members.

4.3.2. Each specimen will be labeled with the date, code number of the household and the name of the subject; it is important to ascertain that each name corresponds to a name on the roster.

4.3.3. If some family members are absent, attempts will be made to set up a time when they can be tested.

4.3.4. Before the investigators depart, they will leave with the household Referral/Notification cards, asking that if a person develops a fever within a month, that person present the card at the hospital so that particular attention may be given to the discovery of LF.

4.4. Dispostion

4.4.1. Each family record will be reviewed for completeness.

4.4.2. A completed questionaire will be given to the Director of the Hospital.

4.4.3. Copies of the questionaire will be forwarded to the Principal Investigator, John D. Frame, M.D., and to Mr. J. E. Yalley-Ogunro, Resident Head, Lassa Fever Control Project, Liberian Institute for Biomedical Research, Box 31, Robertsfield.

4.4.4. Serological specimens will be left with the Hospital laboratory, to be deposited in the freezer there until picked up by or forwarded to Mr. Yalley-Ogunro.

4.4.5. Results of serological testing will be returned to the investigators as soon as possible.

4.4.6. The investigators may make whatever statistical correlations seem appropriate to them. They will forward copies of their correlations to the Principal Investigator.

5. Security

5.1. The Investigators will keep all information confidential, and if they refer to it in published communications, will refer to each family by code number, not by name.

5.2. They will keep all records in a safe place.

6. Supplies needed for the home visits will include:

Record forms
Referral/Notification cards
Labels
Vacutainer tubes
Vacutainer needles
Vacutainer holders/adapters
Filter paper discs
Glassine envelopes

Appendix E. Lassa Fever in Households of Hospital Cases.

Annex 3. Record of Household Epidemiology Study

Household Code #____Index Case: Name_____Father/_____

Husband_____

Town_____Quarter_____Survey Date_____

Family Members:

Name	Relationship to Index case	Sleeping Quarters*	Date of Febrile Illness (Approx)	IFA Titer

Floor Plan of House

Floor plan of house: indicate approximate dimensions, assign each room a number*, to be used above to indicate sleeping quarters for each.

Room No.	Number: Sleeping in it	IFA Pos.

Appendix E. Lassa Fever in Households of Hospital Cases.

Annex 4. Means of Obtaining Samples for IFA Testing.

A. 1. In general, venous blood will be obtained from adults, and children old enough to walk.

2. Capillary finger or heel blood will be obtained from babes in arms, and older children whose veins appear inadequate for easy venepuncture.

3. It may at times be necessary to obtain finger tip blood from all those tested. The representative of the Community Health service will be able to advise regarding this issue.

B. A label will be prepared for each donor before the specimen is obtained; the label will bear the name of the subject, the date and the code name of the household.

C. 1. Venepuncture specimens will be collected in Vacutainers, and the labels placed on the tubes.

2. Tubes will be delivered to the laboratory for separation of sera.

3. Sera will be placed in Falcon tubes, the labels transferred to the tubes, and the tubes stored in the freezer.

D. 1. Finger tip (or heel) capillary blood will be obtained by use of a lancet; a double- or triple-prick will ordinarily be needed to obtain enough blood for testing.

2. Blood will be collected on filter paper discs to the point of saturation of two discs.

3. The labels will be affixed to glassine envelopes provided to store the samples, and the discs placed on them to be air- and/or sun-dried, protected from marauding insects.

4. When partly dried, the discs will be placed in the glassine envelopes.

5. Laboratory personnel at the hospital will be asked to place the capillary blood specimens in the freezer together with the serum specimens of the same household.

Appendix F. Passive immunotherapy with LFIP in Curran Lutheran Hospital, Zorzor, 1981-1985.

To estimate the dose of neutralizing antibodies adminstered to each patient the volume of each administered plasma units was multiplied by the LNI to produce an arbitrary Therapeutic Unit (TU). The TU's of all the plasma units administered to the patient were added to indicate the dose received.

Early during the period some patients were administered plasma of doubtful value, and in a few the plasma donor was not identified. Patients receiving such units are classified as "Uncertain".

Table F-1. Summary of treatment of LF patients with LFIP, 1981-1985, by age of patient and TU's administered. (See Text).

	Therapeutic Units						
Age	Uncertain	Under 500	500-599	600-699	700-799	800-899	Over 900
Adults	8(2)*	7	2	2	3(1)*	1	14
Children		1	1				
Total	8(2)*	8	3	2	3(1)*	1	14

Less than 500 TU - 16 (2)*

500 and over - 23 (1)*

* Number of deaths in parentheses

DISTRIBUTION LIST

5 copies	Commander US Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, MD 21701-5011
1 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RM1-S Fort Detrick, Frederick, MD 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN; DTTIC-DDA Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100

END

6-87

DTIC