



# ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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IMPORTANT NOTICE: This exchange is issued for the sole purpose of timely exchange of information among investigators of arthropodborne viruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions, or views in this exchange does not constitute formal publication. Any reference to or quotation of any part of this exchange must be authorized directly by the person or agency which submitted the text.

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Roy W. Chamberlain, Editor  
Margaret L. Hopping, Secretary

#### ERRATA

James G. Olson, LCDR MSC USN, has pointed out a miswording in the last report of the U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, which appeared on page 117 of Issue No. 38 (March 1980). The last sentence of paragraph 3 should be changed to read "Both the IAHA and the CF test shared one difficulty: many antigens prepared have shown some anti-complementary activity in both tests in spite of the use of only male mosquitoes."

SOMETHING NEW!

A SPECIAL SECTION ON VIRAL HEMORRHAGIC FEVERS

With increasing recognition of the public health importance of the viral hemorrhagic fevers, the leading workers in this field have seen the need to keep one another apprised of their findings on a current basis. They have considered distributing a "newsletter" of their own, patterned after our highly successful "Arthropod-borne Virus Information Exchange." However, in our judgement, there seems to be little need for this duplication of effort and expense, since the "Info-Exchange" has long embraced the hemorrhagic fever viruses, even the nonarboviral arenaviruses.

To assure this group of workers that their reports are truly welcome, we have set up a separate section for the hemorrhagic fevers, and if it meets with your favor, we'll continue to do so in the future.

Please Note: This new section will have its own special editor. If you have a report concerning viral hemorrhagic fevers which you wish to have included in this special section, it should be sent to:

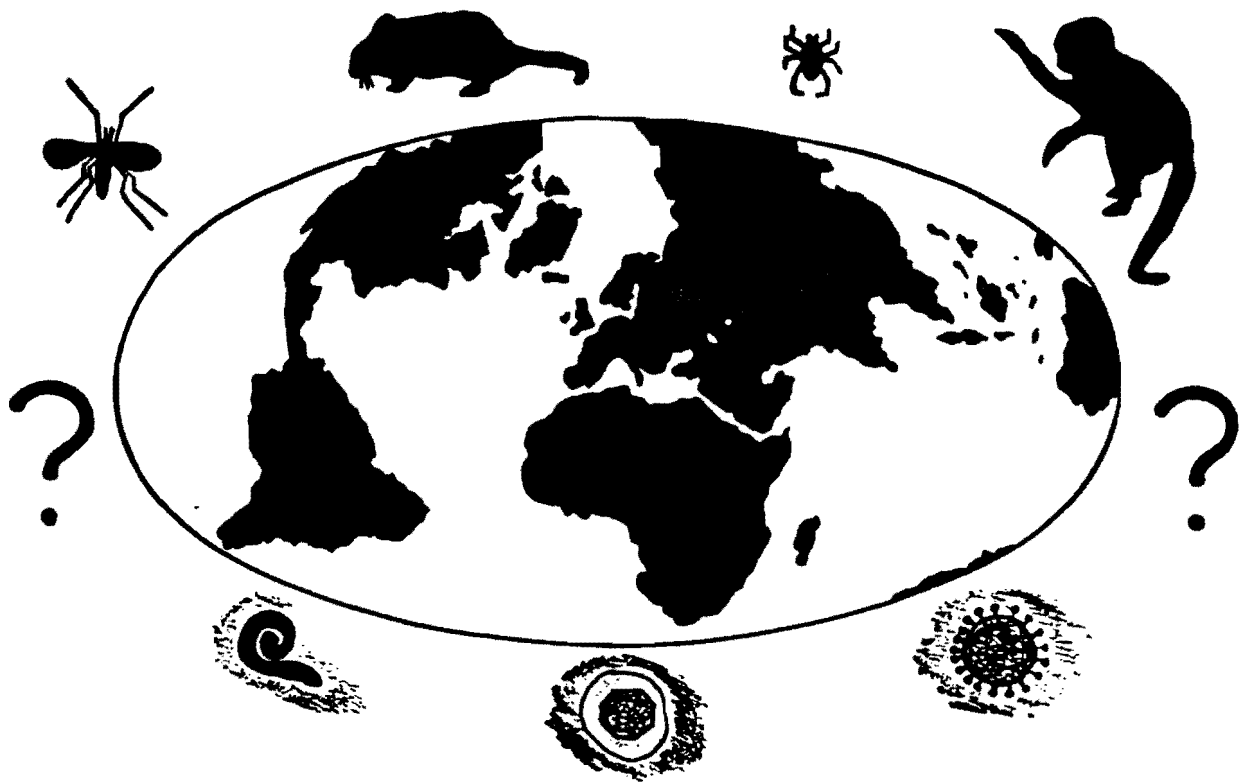
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Dr. Simpson's deadline for receipt of hemorrhagic fever reports will be February 1 and August 1, which will give him a month to edit and assemble the reports and send them to Atlanta to meet the Arthropod-borne Virus Information Exchange deadlines of March 1 and September 1.

Hemorrhagic fever reports which are not sent to Dr. Simpson, but which are sent directly to the editor of the Arthropod-borne Virus Information Exchange, will not be included in the Special Section. They will be placed with regular arbovirus reports in the usual manner.

Roy W. Chamberlain, Editor  
Arthropod-borne Virus Information  
Exchange

# VIRAL HAEMORRHAGIC FEVERS



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Report from Professor Margaretha Isaacson, South African Institute for Medical Research,  
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Report from the Instituto Nacional de Estudios sobre Virosis Hemorragicas  
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Report from the Special Pathogens Reference Laboratory, PHLS Centre for  
Applied Microbiology and Research,  
Porton Down, Salisbury, England

Report from the National Institute of Health,  
Tokyo 141, Japan

**INACTIVATION OF VIRUSES CAUSING HAEMORRHAGIC DISEASE**

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By G. VAN DER GROEN, A. EL MEKKI and S.R.PATTYN

It is absolutely necessary for viruses causing haemorrhagic diseases to find inactivating reagents, which keep both morphology and antigenicity intact. Safer handling of these viruses are then possible in less high containment laboratories.

CHIK, RVF, EBO and MBG viruses, incubated overnight with 2 % formaldehyde at 4°C, were mixed with their specific antisera for 30 min at 37°C. Pseudoreplica was performed on the antigen-antibody mixtures and negatively stained with 2 % uranylacetate. CHIK and RVF viruses were clumped. No clumping occurred with normal serum. With EBO some aggregated twins were observed, but in addition each particle was surrounded by a thick halo of antibodies, not seen when mixed with a normal serum. No halo was observed when EBO was mixed with MBG antiserum.

The degree of clumping and preservation of virus morphology was better with 2 % formaldehyde than with 1 % glutaraldehyde and 0.2 % beta-propiolactone treated viruses.

Experiments are going on to prove that the viruses after the indicated times are completely inactivated.

RVF infected Vero cells are completely inactivated after incubation during 1 hr at 4°C in the presence of 0.1 % or either 0.2 % beta-propionolactone, and additionally incubation at 37°C for 2 hrs.

The cells showed nice fluorescence in the indirect immunofluorescence test and the same RVF antibody titer was obtained compared with the non inactivated slides.

The same inactivation conditions were used to inactivate of RVF micebrain suspension.

Inactivated Ebola infected Vero cell slides were obtained after incubation of the infected cells for 4 times 10 minutes at 0°C with each time freshly prepared 0.1 % beta-propionolactone solution, and 10 minutes fixation in acetone.

Inactivated Lassa infected Vero cell slides were obtained after incubation of the infected cells at 0°C for 2 times 10 minutes with freshly prepared 0.2 % beta-propionolactone solution each time, and 10 minutes fixation in acetone.



REPORT FROM SPECIAL PATHOGENS BRANCH, VIROLOGY DIVISION,  
CENTER FOR DISEASE CONTROL, ATLANTA, GA 30333

K.M. Johnson, J.B. McCormick, P.A. Webb, M.P. Kiley, R.L. Regnery and D.M. Morens

LABORATORY INVESTIGATIONS

Quantitative Infectivity Assay for Ebola and Marburg Viruses

Since there is no reliable plaque assay for Ebola or Marburg viruses and because tube titration is quite time consuming, we have developed a rapid quantitative assay for these viruses. Dr. Allan Truant, now at the University of Texas in Galveston, was a guest researcher in our branch and was able to adapt the fluorescent focus assay to these viruses. Essentially, the test is a microscopic plaque assay where plaques are visualized by using an indirect fluorescence test.

In our system, HeLa cells are infected with virus, adsorbed, rinsed, and overlaid with 0.25% agar. Forty-eight hours later, agar is removed and cells are fixed with methanol, reacted with specific antibody, and finally stained with FITC-conjugated antiglobulin. Titer is determined by counting a statistically significant number of foci and multiplying by an empirically derived factor. Our first assays were performed in 10 X 35 mm petri dishes and currently we are using glass slides with eight growth chambers. The test should be adaptable to any similar system.

As a note of caution, it should be mentioned that the test is not currently accurate when the virus titer drops below  $10^4$  pfu/ml. It is hoped that a plaque assay for these viruses will soon be developed.

A more detailed description of the test may be obtained by contacting the CDC Special Pathogens Branch.

Ebola-Marburg Virion Proteins

In Johannesburg in 1979, we reported that when Ebola virions are grown in the presence of radioactive amino acids, and then purified, four proteins can consistently be detected by polyacrylamide gel electrophoresis. We have designated the proteins VP1 through VP4. They have molecular weights of 125,000, 104,000, 40,000 and 26,000. The same four proteins were found in Marburg virions with slightly different molecular weight values of 140,000, 100,000, 36,000 and 23,000. In each case, VP1 was labelled with glucosamine and is therefore a glycoprotein. Both VP2 and VP3 are associated with a detergent-released nucleocapsid structure.

We have recently extended our findings and are examining the relationship between structure and function in the virions, especially regarding antigenic determinants. As further proof that VP1 is the only virion glycoprotein, it is the only one labelled with  $^3\text{H}$ -fucose. That it is the major or only protein present in the virus surface projections, or spikes, is demonstrated by the fact that VP1 is lost from virions when spikes are removed by bromelain treatment.

Virus proteins have also been examined by staining gels with Coomassie Blue. Fig. 1 shows gel profiles of three Zaire strains (EZ) and one Sudan

strain (ES), all from the 1976 outbreak. It can be seen that the four profiles are essentially identical and all contain the four previously described proteins. In addition, the more concentrated preparations (EZ1211479, EZ3580) contain several additional protein bands. We are now determining which of these are virion proteins and which are host proteins.

Some of these results will appear shortly in the Journal of General Virology.

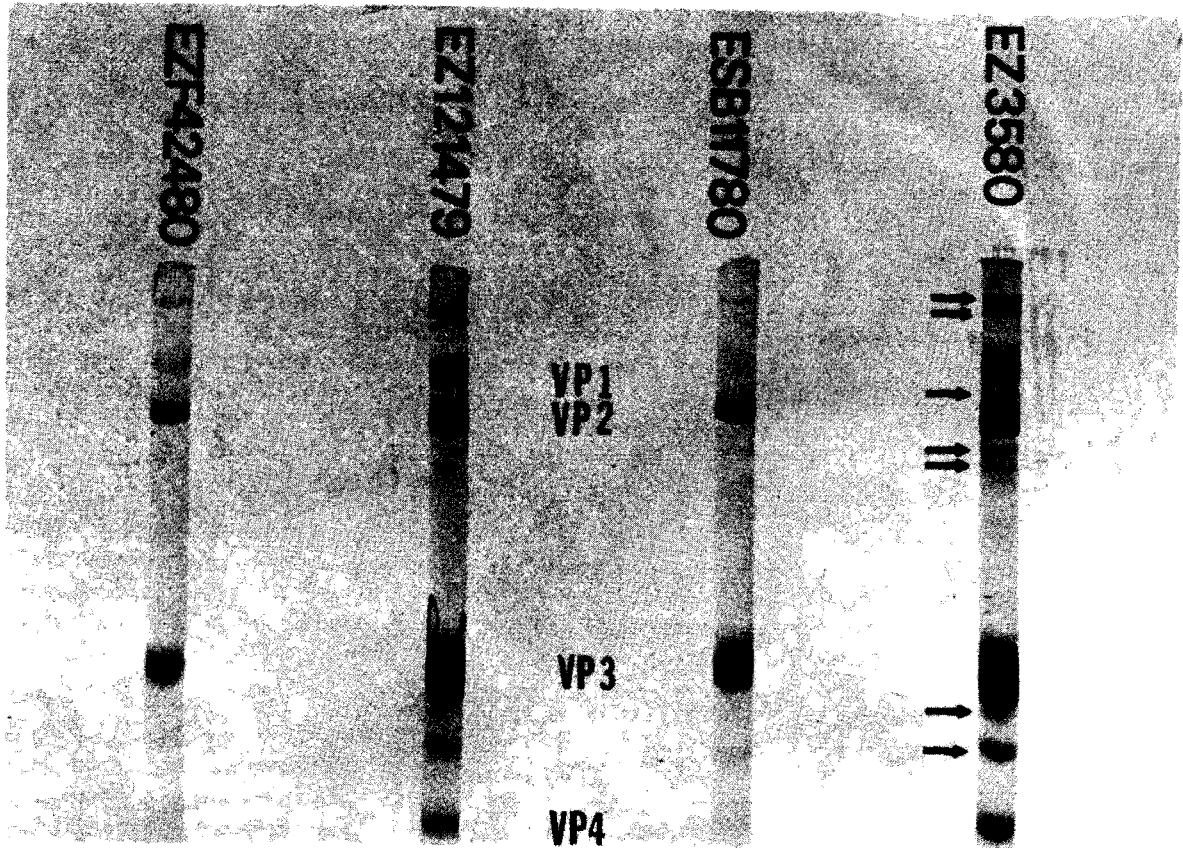


Figure 1. Several preparations of purified Ebola virus were disrupted with SDS and mercaptoethanol and electrophoresed in 7.5% cylindrical polyacrylamide gels. Gels were stained with Coomassie Blue and destained with a solution of acetic acid-methanol. Previously described proteins are designated VP1 through VP4 and are present in each gel. The arrows adjacent to gel EZ3580 indicate protein bands not previously reported for this virus. These are candidates for consideration as additional viral structural proteins.

### Virion RNA of Ebola Virus

To better understand the general taxonomic character of Marburg and Ebola viruses, the virion nucleic acid of Ebola virus has been studied in detail. The virion nucleic acid is RNA and single stranded, as determined by sensitivity to digestion with NaOH and ribonuclease. The genome consists of a single RNA species with a molecular weight of approximately  $4.0-4.2 \times 10^6$ , as determined by electrophoretic mobility on agarose gels under denaturing conditions. The RNA does not adhere to oligo(dT) cellulose under conditions favoring the binding of poly(A) containing RNA. Infectivity of the RNA could not be detected, although Sindbis virus RNA infectivity was demonstrated in the same experiments. We tentatively conclude that the genome of Ebola virus is a negatively stranded RNA with properties analogous to the genomes of Rhabdoviruses.

Current studies on the intracellular virus-specific RNAs should lead to a better understanding of the replicative strategies employed by these agents and how these strategies compare with those of other virus groups.

Comparative studies of the virion RNAs of the various isolates of Marburg and Ebola viruses are also underway to help understand the extent of relatedness and evolution of these viruses.

### Comparison of Marburg, Ebola-Zaire and Ebola-Sudan Viruses

Several recent experiences strengthen the growing impression that there are significant biological differences between Marburg virus and the different strains of Ebola virus.

Although comparison of case fatality ratios between different outbreaks can be treacherous, the differences for the three viruses appear to be large enough to suggest that they are real (Table 3). Thus Ebola-Zaire case fatality ratio was nearly 90% in 1976, while the original Marburg epidemic ratio was 23%, and the two Sudan epidemic ratios were in between.

We have now confirmed the difficulty in primary isolation of Ebola-Sudan virus which was reported in 1976. We were able to isolate virus from six patients during the 1979 epidemic. One grew in Vero cells from the primary inoculation. Two others grew only after a blind passage. A fourth grew in SW-13 cells, but not in Vero. Two others were recovered only from inoculation of guinea pigs. Specimen titers were very low in all instances. We did not encounter such problems with isolation of Marburg virus in the recent Kenya case.

Finally, we were interested in pursuing Dr. van der Groen's report that Ebola-Zaire virus was lethal to suckling mice, and to compare the two other viruses with Ebola-Zaire. Titrations of the three agents in suckling mice by IP or IC route revealed that about five TCID<sub>50</sub> of Ebola-Zaire strain represented the LD<sub>50</sub> in these animals with AST of 7 days. Larger doses killed mice in 5-6 days. In contrast, no amount of Ebola-Sudan or Marburg virus produced death in suckling mice.

We performed an LNI test against Ebola-Zaire virus using Zaire human plasma with an antibody titer by IFA of 512 and Sudan plasma with a titer of

>256. No protection of mice was observed for any combination of virus and plasma concentrations.

More detailed genetic and biochemical studies are presently in progress to better define the differences between these viruses.

TABLE 1

A General Comparison of Some Characteristics of Marburg, and Ebola-Zaire and Ebola-Sudan Viruses

<u>Virus</u>	<u>Human Case Fatality Ratio</u>	<u>Lethal to Suckling Mice</u>	<u>Ease in Vero Cell Isolation</u>
Ebola-Zaire	0.87	++	++
Ebola-Sudan	0.5-0.7	—	Difficult
Marburg	0.23	—	++

FIELD INVESTIGATIONS

African Serosurveys

Liberia. Sera from persons resident in different regions of the country were examined in our laboratory by Dr. Jorgen Knobloch, Institute for Tropical Diseases, Hamburg. IFA antibodies to Lassa virus were found in about 25% of persons from Lofa County in northwestern Liberia, a known Lassa-endemic area. Fewer positive sera were encountered in Bong County and in the coastal region near Monrovia. A few sera having 1:16 or greater titers for either Ebola or Marburg viruses were also found, all of them from persons living in central or northern rain forest regions of the country.

Central African Republic. In collaboration with Drs. Saluzzo, Gonzalez, and Georges at the Pasteur Institute, Bangui, 499 sera from persons resident in the southeast of the country near Bangasson (rain forest ecology) were examined for IFA antibodies to Lassa, Marburg and Ebola viruses. No reactors were found for Lassa virus, a finding confirmatory of results we obtained previously after testing more than 500 sera from persons in northern Zaire living in the same ecological zone. Eleven of the CAR sera (2%) had Ebola virus titers of at least 1:16. Two other persons had antibodies for Marburg virus only with titers of at least 1:64. We now suspect that Ebola virus may be present in the rain forests of West and Central Africa, and Marburg in Central and (more commonly) East and Southern Africa. A great deal remains to be done, however, to substantiate this important geographic hypothesis. In this connection, we now have prepared inactivated cell antigens and made polyvalent screening slides in which each well contains Lassa, Ebola, Marburg, Congo-CHF, and Rift Valley fever antigens. This approach promises to maximize information obtained with any good geographic serum collections which become available. We are prepared to send slides, positive sera, and monovalent antigens to persons having such serum collections and the desire to test them.

Guinea. A serosurvey of several geographic areas of Guinea was carried out in 1978; 24 villages were visited and blood samples were taken from about 100 persons in each village, of which 10 were forest, 9 savannah, 4 coastal, and 1 mountainous. In addition, seven hospital staffs were sampled.

In the forest area, 1011 persons from 10 villages were tested, of whom 59 (5.8%) had anti-Lassa antibody titers of  $\geq 16$ . The highest levels were 16% in a single village, the lowest was 0. In the savannah area, 818 specimens were collected from 9 villages. Thirty-three persons had antibody (4.0%). The prevalence varied from 0% in two villages up to 26% in one village.

In the coastal area of Guinea, 356 specimens were collected from four villages. Only two specimens were positive (0.6%). Only a small sample (seven sera) was obtained from the mountainous areas and none were positive.

Staffs from seven hospitals in the different geographic areas were sampled; highest prevalences were found in the forest, where one staff had a prevalence rate of 10%. Other prevalence rates were from 0 to 5%.

As has been observed in Sierra Leone, Lassa fever is primarily a disease of the forest and savannah areas. It is a focal disease, presumably following the basic distribution of the rodent reservoir Mastomys.

#### Lassa Fever Research Project—Sierra Leone

Human Disease. Work on human disease is continuing. At present, there are two major goals. First, the investigation of therapeutic regimens which may be useful for therapy of acute Lassa fever. Second, the study of Lassa fever at a village level. Both studies are in progress, but a few interesting points are now apparent.

In our efforts to assay the value of particular therapeutic regimens, we examined several laboratory variables in addition to the usual clinical parameters of length of fever and survival. The most promising of these appears to be viremia on admission; and, more practically, SGOT and SGPT values on admission (Table 2). The difference in the mean chemistry values of those persons whose illness is fatal is significantly larger than in those who survive. The same is true for the levels of viremia, which are 100-fold higher in those who die compared to those who survive. Moreover, the enzyme levels and viremia tend to increase after admission in those patients who die, while they tend to decrease in those who survive.

Studies to date suggest that neither oral ribavirin in a dose of 15 mg/kg/day, nor one unit of Lassa immune plasma, have uniformly positive effects on clinical or laboratory measures of treatment efficacy. Neither has altered disease mortality as compared to untreated patients. Oral therapy has had no effect on viremia. Plasma therapy appears to have reduced viremia in certain individuals but the effect has not been consistent.

Incomplete studies on village transmission of Lassa fever have established the following:

- (1) Mild disease is the rule rather than the exception, and 10-20 infections occur for each hospitalized severely ill patient.
- (2) As much as 10% of febrile illnesses in some populations is due to Lassa virus.
- (3) Lassa is transmitted year around and across all age groups.

TABLE 2

Mean Values of Lassa Viremia and SGOT on Hospital Admission by Outcome

Outcome	N	Mean Admission Viremia	N	Mean Admission SGOT	N	Mean Admission SGPT
Died	28	3.7 dex	19	777 IU	17	176 IU
Survived	87	1.7 dex	58	169 IU	55	69 IU
Probability of Equal Means (Wilcoxon Test)		.0001		.002		.020

Rodent Infection

Study of the relationships between Mastomys rodents and transmission of Lassa virus to humans in Sierra Leone was begun in 1978 in collaboration with NIH and Colorado State University and is continuing under an interagency agreement with NIAID and Fogarty Center, NIH. During the initial program year, five fixed grids were trapped at 90-day intervals and rodents captured were examined for presence of Lassa virus and antibodies and for karyotype by direct examination and by hemoglobin electrophoresis. Grids were established within and just adjacent to two villages, one with high Lassa transmission and one with little activity. A fifth grid was sited in unmodified rain forest in eastern Sierra Leone. Results were as follows:

- (1) Mastomys were most numerous on the grid in the "high Lassa" village.
- (2) Almost no Mastomys were captured in any of the three grids outside villages.
- (3) Essentially all Mastomys captured were the 32-chromosome species.
- (4) Mastomys half-life was less than 3 months, so that recaptures over this interval were too infrequent to construct life tables.
- (5) Rattus rattus were found in association with Mastomys in both villages.
- (6) Lassa virus was isolated only from Mastomys in the "high Lassa" village. About 15% had viremia throughout the year, even during the dry-season month of December when populations were lowest. Antibody rates varied from about 20 to 30%.

(7) Neither virus nor antibody was detected among other rodent species.

Current work is focused on the questions of seasonal Mastomys breeding, attempted rodent control in a "high Lassa" village, and the issue of whether 38 chromosome Mastomys, found largely in bush habitat in the savannah ecosystem in northern Sierra Leone, are also Lassa virus reservoirs. Data are incomplete, but preliminary evidence suggests that:

- (1) Mastomys breeding (32 chromosome) in villages of man-modified rain forest in eastern Sierra Leone may be continuous. The percentage of pregnant or lactating females during wet, dry, and mixed trimesters of the year varied between 14 to 22%. Juvenile Mastomys were trapped continuously, as well.
- (2) Intensive trapping of a "high Lassa" village resulted in an 80% reduction of Mastomys 6 months later and there was no increase in population after a further 3 months. Serial serum specimens from a large human cohort sampled in this village are being tested to determine whether virus transmission was interrupted.
- (3) Both 32 and 38 chromosome Mastomys have been captured in villages and bush in northern Sierra Leone. The former is more numerous in houses and the latter predominates in bush. We found evidence of current or past Lassa virus infection in 4 of 28 rodents with 32 chromosomes and 19 of 113 animals with 38 chromosomes. Both species were infected in each habitat.

Our tentative conclusions are that both Mastomys species are virus reservoirs in their natural savannah habitats, that the 32-chromosome species is better adapted to commensal life and accompanies man into modified ecological zones (i.e., rain forest), and that rodent control therefore holds promise for disease control only in the latter situation where there is little evidence of existence of a Mastomys population outside the villages ready to rapidly reinvade houses. We have confirmed that hemoglobin electrophoresis gives reliable separation of 32 and 38 chromosome forms. Experimental infection of colonized Mastomys has been initiated in order to determine whether important differences in biology of infection occur. In view of the presence of viremia in both species in the north, we expect little difference in response. Age at time of infection may be the important variable in determining outcome.

#### Patterns of Viremia and Viruria in Laboratory-Bred 36K Mastomys from Zimbabwe

We have begun a study of the patterns of Lassa viremia and viruria in different karyotypes of Mastomys infected as adults and newborns. Four colonies have been established: 32- and 36-chromosome from Zimbabwe, and 32- and 38-chromosome from Senegal. Data presented here are from the 36-chromosome rodents.

Nine days after inoculation with 2.0 dex of Lassa virus, 3 of 6 adult Mastomys had  $>2.0$  dex/ml viremia. The viremia disappeared and all six developed Lassa antibody. None had viruria.

Twenty-nine newborn Mastomys were each inoculated with 30 infectious particles. After 9 days, 28 were viremic. Eight of eight animals

tested 23 days after inoculation were viremic. Three of five had 3-3.5 dex/ml of viruria. Six of six tested at day 23 had antibody of 1:80 or more. Nine of 11 animals tested at day 101 were viremic up to 5 dex/ml. Ten of 11 were viruric up to 6.5 dex/ml.

Breeding of chronically infected rodents infected noninfected mates, as measured by antibody to Lassa virus. There was no difference in fertility or fecundity between infected females or males and noninfected rodents. Infection in offspring appears to be related to viremia in the mother at the time of parturition. Those females with  $\geq 2$  dex of viremia had offspring which developed viremia within 1 week of birth.

Tentative interpretation is that viremia at the time of breeding does not affect the outcome of pregnancy (unlike Machupo virus in *Calomys*). It seems possible that infection of newborns in viremic dams occurs in utero, unlike American arenavirus infections. This pattern of intraspecific infection, if confirmed, could account for endemic transmission of Lassa virus to man.

#### Ebola Reservoir Survey, Zaire, 1979

During June and July 1979, serum samples and viscera for virus isolation were obtained from more than 1300 wild animals captured in the Yalosemba and Tandala areas of Equateur Province of northern Zaire, where cases of both monkeypox and Ebola disease had been previously recorded. This study was sponsored by the WHO Smallpox Unit in collaboration with CDC; the Institute for Tropical Medicine, Antwerp; the Geneva, Smithsonian, and Carnegie Museums; and the Zaire Ministry of Health.

Animals were obtained mainly by shooting or hand capture by local residents. Serologic testing for Ebola virus antibodies has been hampered by lack of species-genus specific antiglobulin conjugates. To date, the 246 monkeys have been tested, with negative results. A polyvalent conjugate prepared against squirrels and the African porcupine has been used to examine sera from these 132 animals, also with negative results. Other conjugates are being prepared. Isolation attempts with bat and rodent tissues are in progress at Antwerp. To date, more than 150 processed are negative. We shall persevere.

#### EPIDEMIC INVESTIGATIONS

##### Ebola Hemorrhagic Fever in Sudan, 1979

Thirty-four cases of hemorrhagic fever due to Ebola virus occurred in the Yambio-Nzoia District in southern Sudan between July 31 and October 6, 1979. The affected area was the same area where the Ebola epidemic of 1976 originated.

Twenty-two cases (65%) died and were defined by fever of at least 4 days' duration, diarrhea, or hemorrhagic manifestations. Twelve cases (35%) survived and were defined by fever of 1 or more days' duration with proven viremia or a convalescent antibody of 1:64 or greater.



Illness was characterized by sudden onset of fever, severe frontal headache, general myalgia and arthralgia, and retrosternal, nonpleuritic chest pain. Epigastric pain with abdominal tenderness was prominent. Severe gastrointestinal symptoms occurred on day 3 or 4 of fever. This resulted in frequent vomiting and watery diarrhea. Dehydration usually resulted. Sore throat was also a frequent finding. Hemorrhagic manifestation almost invariably heralded death and occurred on day 5 to 7 of illness.

As in previous epidemics, we found a strong relationship between illness and degree of physical contact (Table 3). Close physical contact, such as nursing the patient or preparing the body for burial, carried a 40% risk of falling ill.

We also looked at contacts exposed to cases with hemorrhagic manifestations and compared disease frequency in persons in contact with bleeding and non-bleeding patients. No clear risk could be defined, probably due to multiplicity of types of close contact, as well as the small number of affected persons who had no bleeding. However, this bears further investigation in any future epidemics.

Critical to the spread of disease in this epidemic was the nosocomial spread of disease from the index case to two other persons in the hospital. Similar circumstances occurred in 1976 in Zaire and Sudan, resulting in hospital amplified epidemics. This epidemic was no exception. Although the index case was a worker in the local cotton factory, we could not implicate cotton factory employment, using epidemiologic methods, as carrying an unusual risk of acquiring the disease. Use of the fluorescent antibody test in the field during the epidemic was helpful in sorting out many of these factors.

Finally, we observed a mortality of 89% during the first four generations of cases compared to 38% in the last three, suggestive of attenuation of virulence. This needs further investigation.

TABLE 3

Ebola Hemorrhagic Fever in 138\* Known Contacts of Active Cases  
by Degree of Exposure, Sudan, 1979

Degree of Exposure	Case	Not a Case	Total	Cases per 100 Contacts from July 31-October 8
No exposure	0	7	7	0
Exposure, no physical contact	1	36	37	2.7
Mild physical contact	2	22	24	8.3
Close physical contact	26	44	70	37.1

p<0.001 (test for a linear trend in proportions)

\*Index case not included. Also, four other cases and two asymptomatic contacts excluded (degree of exposure not ascertained).

## Marburg Disease—Kenya, 1980

On January 15, 1980, a French engineer working at a sugar mill at Nzoia in western Kenya was admitted to Nairobi general hospital with severe gastrointestinal hemorrhage and shock. He had been sick for about a week. This patient died within 6 hours of admission, and subsequent histologic examination of liver tissue revealed hepatocellular necrosis compatible with infection by a hemorrhagic fever virus. Electron microscopic examination of liver and kidney tissue fixed in formalin disclosed a few virions and many intracytoplasmic inclusion bodies pathognomonic for Marburg or Ebola virus infection.

Nine days after the death of this index case, a physician and a nurse who attended the patient developed fever, myalgia, and leukopenia. The nurse experienced a maculopapular rash, but recovered after several days of nonspecific symptoms. Repeated examination of serum specimens failed to disclose evidence for infection with Lassa, Marburg, Ebola, Congo-CHF, or Rift Valley fever (RVF) viruses. The physician, in contrast, developed jaundice and the diagnosis of biliary obstruction was entertained. On February 5, a laparotomy was performed and a liver biopsy was obtained. Focal, inflammatory lesions compatible with bacterial sepsis were observed. A blood sample obtained on January 30 was inoculated into four cell culture lines for virus isolation. Portions of serum from this specimen and another sample obtained 6 days later were sent to our laboratory for serological examination. We found a rising IFA titer specific for Marburg virus (less than 1:4 to 1:256) in these samples. At this time, blind harvests of the cell cultures were made in Nairobi and sent to Atlanta, where after passage in Vero cells the original harvest from Kenyan Vero cell cultures yielded Marburg virus. Identity of the agent was confirmed by IFA staining, by electron microscopic visualization of virions, and by characterization of viral proteins by polyacrylamide gel electrophoresis. The physician recovered after a stormy clinical course which fortunately did not include overt hemorrhagic manifestations. In view of the atypical liver pathology, it was concluded that he might have suffered both viral and bacterial (typhoid?) infection, although no bacterial pathogen was ever identified. Marburg virus was, however, isolated from a semen sample 63 days after onset of symptoms.

The source of infection of the fatal index case has not been determined, although this man's housekeeper was found to have IFA antibodies against the virus.

Serologic surveys were done among personnel of the Nairobi hospital, staff members and case contacts in Kisumu where the index case was first hospitalized, and in workers at the sugar mill and personal contacts in the Nzoia area. Results of IFA tests are given in Table 4. Taking a titer of 1:16 or greater as evidence of infection, it was apparent that both Marburg and Ebola viruses are active in Kenya. Two persons had antibodies for RVF virus. Further studies on prevalence and geographic distribution of antibodies in Kenya are in progress in collaboration with the Ministry of Health and the University of Nairobi.

TABLE 4

Marburg and Ebola IFA Antibodies ( $\geq 1:16$ ) in Kenya Human Sera, 1980

Group	Tested	Positive to:	
		Marburg	Ebola
Nzoia contacts of index case	16	3	0
Kisumu contacts of index case	45	3	3
Nairobi hospital staff and contacts of 2 <sup>o</sup> case	113	3	5*
Nzoia sugar mill workers	<u>63</u>	<u>2</u>	<u>12</u>
Total	237	11(4.6%)	20(8.4%)

\*One serum had a titer of 1:1024 for Ebola and 1:16 for Marburg antigen. All other positive sera were monospecific.

#### OPERATION OF THE CDC MAXIMUM CONTAINMENT LABORATORY (MCL)

On May 26, 1980, the CDC MCL completed its second year of operation. The laboratory is a self-contained unit housed in a separate building which contains the needed support systems (air handling, waste decontamination, etc.). The laboratory module itself is divided into two areas—a cabinet laboratory and a suit laboratory. In the cabinet line area work is conducted in a negative-pressure, airtight cabinet line through glove portals. The suit area is basically a standard virology laboratory which is sealed from the outside and also under negative pressure, where scientists work in totally encapsulating plastic suits to which breathing air is supplied and which are under positive pressure. The suit area also has an animal room which contains portable laminar flow rooms to house animals.

During our 2 years of operation we have had some very interesting times and have gained much experience in the operation of such a laboratory. In the MCL we have engaged in routine diagnostic work, reagent development and preparation, animal studies (mouse-guinea pig-monkey), and even some moderately sophisticated molecular virology. To this point, members of the Special Pathogens Branch and guests have logged close to 7,000 man hours in the module. The ratio of time worked in the suit laboratory to that in the cabinet laboratory is approximately 3 to 1.

There have been four planned shutdowns for routine maintenance and modifications. The down time for these shutdowns was programmed for 3 weeks and has varied from 3 to 6 weeks. The only major modifications to our system have been to (1) install a backup diesel generator, (2) replace the electric freeze with a liquid nitrogen tank, (3) install interior pneumatic

doors, and (4) modify the cabinet line to take a new binocular FA microscope.

We are now on our third generation of suits, with each generation having good and bad features. The ideal suit has not yet been manufactured, and we, along with others, are constantly looking for ways to improve the suits.

Our major findings, in general terms, are as follows:

- (1) The concept of using differential air pressure as a primary safety barrier appears to be realistic and practical (and it works).
- (2) In our system it is possible to perform a variety of experiments with hazardous agents in relative safety.
- (3) The suit laboratory concept has allowed us to apply many techniques in our studies which cannot be done in the cabinet line system.
- (4) No overt psychological problems have as yet developed due to the long hours of work in the laboratory, but we feel that it can be a stressful situation at times.
- (5) We have shown that it is possible to conduct a broad Class 4 virus program in our system, but it has required extensive cooperation among our staff, the Office of Biosafety, and the Engineering Services Office.

Limited editions of an MCL Operations and Safety Manual have recently been printed and are available to interested parties on request.

REPORT FROM THE SPECIAL PATHOGENS UNIT,  
SANDRINGHAM, SOUTH AFRICA.

by

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1. As to be expected, the unit has had teething problems, the most serious of which concerns unsatisfactory doors to the suit laboratory which we hope to replace with purpose-built metal doors and frames with inflatable seals. Consequently, we have limited ourselves to serology and "cold" virology (electron-microscopy and immunofluorescence) except for a few instances in which we were obliged to culture specimens.

2. KENYA

It appears that neither ourselves nor the Atlanta laboratory have the full picture concerning the Marburg incident in Kenya and we take the opportunity of presenting our fragmentary information here for the sake of clarification.

- (a) On the 13th of February, 1980, we received in formalin a liver biopsy from Dr. M of the Nairobi Hospital and Liver and kidney from the ostensible index case, Mr. C. These were subjected to electron microscopy and on the following day Dr. M. Lecatsas of the Institute for Pathology in Pretoria, reported finding Marburg-type inclusions in the kidney of Mr. C; a finding confirmed later that day by Dr. I. Spence of our Institute. Neither liver specimens showed virus particles or inclusions and the most marked histopathological feature of Dr. M's liver was some bile stasis.

We subsequently performed indirect immunofluorescence tests on these specimens with Marburg and Ebola sera and found Marburg fluorescence in Mr. C's kidney.

- (b) At the same time we received serum from Dr. M and from a nurse H who was in the acute stage of an illness. We found a Marburg indirect immunofluorescence (IF) titre of 1:256 in Dr. M's serum, which apparently coincided with a titre demonstrated earlier in Atlanta. The nurse's serum was negative for antibodies and was cultured in Vero cells without positive result.
- (c) On the 17th of February we received serum and urine from a Miss M, also apparently from the hospital staff and ill. Electron-microscopy and cultures were negative.
- (d) On the 27th of February, we received 14 sera from apparently healthy personal contacts of Mr. C at the Nzoia Sugar Estates and one of these, Miss IM, we found to have a Marburg titre of 1:16. Possibly we were a little conservative in this estimate since there was fluorescence at 1:32 and 1:64, and we subsequently learnt that she had been found to have a titre of 1:64 in Atlanta.
- (e) At the same time we received a number of further sera from Dr. M, the latest taken on the 25th of February, plus sera from Dr. R and nurse E from Nairobi Hospital. The sera of Dr. M had constant Marburg titres of 1:256 and the other two sera were negative.
- (f) On the 7th of March, we received liver, fresh and in formalin, plus serum from a Mr. MS from Mombasa who had died shortly after a visit to Uganda. Cultures and electron microscopy were negative

and the serum lacked antibodies to Marburg, Ebola, Lassa, Yellow fever and Rift Valley fever.

(g) On the 29th of April we received further serum, urine and a conjunctival swab from Dr. M who had apparently had a relapse of illness. The Marburg IF titre of the serum was again 1:256 and the urine and swab cultures were negative.

(h) At the same time we received acute phase serum from a Mr. W who had become ill after moving into the house of the late Mr. C. The serum lacked antibodies and cultures were negative.

(i) On the 27th of May we received 15 sera from Nairobi Hospital staff who had apparently been found positive on a previous occasion in Atlanta for Marburg and/or Ebola antibodies. We do not have details of the previous results and only have the tri-valent ELM slides from Atlanta (for which we are truly grateful). Hence we contemplated performing blocking tests to determine the specificity of antibodies. In the event, there was only one definite reactor and we found from control sera (nurse C of the 1975 Marburg outbreak and Lassa and Ebola control sera from Atlanta) that there is no difficulty in distinguishing Lassa, Marburg and Ebola fluorescence on the excellent ELM slides. We present our results in full so that comparisons can be made in Atlanta:-

<u>Kenya No.</u>	<u>Marburg</u>	<u>Ebola</u>	<u>Lassa</u>
K1001	-	-	-
K1002	-	-	-
K1003	Trace*	-	-
K1004	-	Trace	-
K1005	-	-	-
K1006	-	-	-
K1007	-	-	-
K1008	-	Trace	-
K1009	-	-	-
K1010	-	-	-
K1011	-	-	-
K1012	-	-	-
K1013	-	-	-
K1014	-	-	-
K1015	8**	-	-

\*Trace = less than 1:4

\*\*Weak fluorescence at 1:16 and 1:32.

(j) We received 10 sera from 7 sundry patients in Kenya at the same time as the above specimens, including two sera from Mr. W., with a request that these be tested for hepatitis A and B antigens and antibodies. The W sera of 30th April and 9th May contained HBsAg, HBcAb, HBeAg plus HBeAb and HBsAb by radio-immunoassay and at least the riddle of his illness would now seem to be resolved. Whether this will lift the curse that must cling to the residence of the late Mr. C, we cannot say! Two other patients were also positive for hepatitis B.

(k) At the same time we received further sera and semen from Dr. M. We understand that successive semen samples from him have been found to be negative for virus in Atlanta, so we have accorded these specimens low priority and they have still to be tested.

3. We have also had a number of local patients with query smallpox, Rift Valley fever and haemorrhagic fever, one of the latter proving to be a meningococcal septicaemia.



REPORT FROM SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH

Hospital Street, Johannesburg, South Africa

Since 1975 a 'Viral Haemorrhagic Fever Team' (VHFT) has been in existence in the Republic of South Africa. The team comprises specialists in various aspects of patient care, infection containment, virology, laboratory and hospital safety as well as engineers, administrators, nurses and other sources of expertise needed in the total onslaught against the haemorrhagic fever viruses and similar hazardous disease agents.

The team, though primarily South African in its composition, includes a number of overseas members and has thus the potential for coordinated action with organizations such as the World Health Organization, the Center for Disease Control (USA) and the Centre for Applied Microbiology and Research (UK).

From its inception the VHFT has adopted a policy of constant preparedness to render immediate assistance in emergencies anywhere in Africa or elsewhere. Thus it was instrumental inter alia in the investigation of Marburg Virus Disease (MVD) in South Africa and Zimbabwe in 1975, the evacuation of a US Peace Corps member suspected of having Ebola Virus Disease (EVD) from Zaire to South Africa for medical care, the investigation and control of EVD in Zaire in 1976 and the investigation and control of MVD in Kenya in 1980.

The VHFT is also engaged in an ongoing program of training which concentrates on such areas as aeromedical evacuation, isolator as well as open care of highly infectious patients, laboratory safety and other aspects and welcomes participation in its program from other countries.

(Professor Margaretha Isaacson)

## EPIDEMIOLOGY AND SPECIFIC TREATMENT OF ARGENTINE HEMORRHAGIC FEVER

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### Epidemiology

The principal epidemiologic characteristics of Argentine Hemorrhagic Fever (AHF) are:

A progressive extension of the endemepidemic area. It has increased 5 to 6 times during a period of 20 years. In 1958, the cases of AHF originated from a relatively small area located in the north-west of the Province of Buenos Aires. In successive epidemic years a slow but steady extension towards the north and the west has been observed, and the disease is now endemic in other regions of the humid pampa, such as the south of the Provinces of Córdoba and Santa Fe, and the north-east of the Province of La Pampa,

Seasonal distribution. Although cases of AHF are notified throughout the year, the disease has a seasonal distribution extending from the end of summer to mid-winter (March to August), with a peak incidence in the month of May.

Prevalence of AHF among males. Approximately 85% of all confirmed cases studied in Pergamino from 1965 to 1979 are males. The disease is prevalent in persons of between 15 and 60 years of age, and the majority of cases are observed in the groups of 20 to 40 years of age.

Prevalence among rural inhabitants. Almost without exception, AHF occurs in residents of rural areas or in persons that give a clear history of contacts with the rural environment within the incubation period of the disease.

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The foregoing characteristics are intimately related to the natural cycle of Junin virus, the etiologic agent of AHF, in the species of rodents acting as the main reservoir of this virus. The relevant factors are:

- a) the rural habits and population dynamics of these cricotine rodents.
- b) their chronic infection, with persistent viremia and continuous virus shedding in the saliva and urine.
- c) the coincidental fact that corn, sorghum and sunflower are harvested during the season when there is also an increased number of rodents in the fields.

These factors combined readily explain the main epidemiologic features of AHF.

Transmission of Junin virus from rodents to man. Although several forms of transmission have been postulated, the precise mechanism(s) and portal(s) of entry of the human infection remain unknown. Nevertheless, careful studies indicate that participation of arthropod vectors is highly unlikely. On the other hand, the persistent shedding of Junin virus by the saliva and urine of chronically infected rodents, with the consequent contamination of the environment strongly suggests a direct mechanism of transmission.

Transmission from man to man. AHF is not a contagious disease. However, there are a few well documented instances of interhuman transmission. Viremia is a constant feature during the acute febrile period of AHF, and virus in bloody secretions, in mucous membranes, or in the urine of some patients may occasionally result in transmission from person to person. For this a close and intimate contact with the patient would be generally required.

Activity of the virus of lymphocytic choriomeningitis (LCM) in rodents and humans of the endemic area of AHF. LCM virus infection of *Mus musculus* captured in Pergamino and in the south-east of the Province of Córdoba was demonstrated several years ago. Thereafter, infection with the virus of LCM has been documented in patients with presumptive clinical diagnosis of AHF.

The simultaneous activity of Junin and LCM viruses in the same geogra-

area represents a unique setting of natural events, that offer an opportunity to study the ecology, genetics and molecular biology of these arenaviruses. In addition, they could provide a better understanding of the pathogenesis of Junin and LCM virus infections in their natural rodent hosts, as well as in man.

Subclinical human infection. Evidences obtained from limited surveys indicate the occurrence of subclinical infections with Junin virus.

The preliminary results also suggest that the prevalence of subclinical human infection is not the same in different places of the endemic area of AHF. This interesting observation could be of considerable importance, if confirmed by more extensive seroepidemiologic surveys. In that case, the study of rodents captured in areas of high prevalence of subclinical human infection could lead to the isolation of naturally attenuated strains of Junin virus that might be used for the development of an effective vaccine against AHF.

#### Specific treatment of AHF

Efficacy of immune plasma. Categorical evidence of the efficacy of immune plasma has recently been obtained from a randomized therapeutic trial.

The results shown in Table 1 indicate that treatment with immune plasma determines a marked reduction of the mortality of AHF, when given before the 9th day of evolution of the disease. On the other hand, the mortality of AHF is not modified when treatment with immune plasma is given to patients with 9 or more days of evolution.

In Table 2 are shown the numbers of Junin virus isolations obtained from blood samples taken immediately before, and 24 hours after the administration of immune and normal plasma. These results demonstrate that viremia is neutralized 24 hours after treatment with immune plasma. In relation to this study, it is pertinent to comment that no significant differences were observed in the titers of Junin virus of the 46 blood samples shown in Table 2. These results are summarized in Table 3.

In another study, a good correlation between the detection of circulating Junin antibodies and clinical improvement was found, as

indicated in Table 4. This suggests a rapid clearance of viremia, in agreement with the results of the randomized therapeutic trial. In addition to their therapeutic significance, these studies contribute further evidences to support the hypothesis of a direct mechanism of viral damage in the pathogenesis of AHF.

Association between treatment with immune plasma and a late neurologic syndrome. As a result of the randomized therapeutic study, an association between treatment with immune plasma and a "relapse" with neurologic manifestations was discovered. This association is shown in Table 5.

Although the great majority of these "relapses" subside uneventfully in a few days, occasional cases with severe neurologic alterations and even death, have been observed. Usually, these episodes consist of fever, headache and a cerebellar syndrome which appear 4 to 6 weeks after the onset of AHF. Without exception, there is a latent period of 2 to 3 weeks between the recovery from the acute phase of the disease and the occurrence of the "relapse" with a neurologic syndrome. No correlation has been found between the severity of AHF and/or the neurologic manifestations of the acute phase, and the incidence of the "relapses". Several possibilities can be postulated to interpret the association between treatment with immune plasma and the late neurologic syndrome. However, until they are elucidated immune plasma is the only effective treatment available for AHF. The significant reduction of the mortality in AHF suggests a potential beneficial effect of this form of treatment in similar severe, life threatening viral diseases.

TABLE 1  
CONTROLLED THERAPEUTIC STUDY IN PATIENTS WITH AHF

TREATMENT	NUMBER OF PATIENTS			MORTALITY
	TOTAL	DIED	IMPROVED	
IMMUNE PLASMA	91	1	90	1 %
NORMAL PLASMA	97	16	81	16 %
TOTAL	188	17	171	

$\chi^2 = 13.53$      $p < 0.01$

TABLE 2

ISOLATIONS OF JUNIN VIRUS FROM THE BLOOD OF PATIENTS  
TREATED WITH IMMUNE PLASMA AND WITH NORMAL PLASMA

TREATMENT	ISOLATION OF JUNIN VIRUS <sup>*</sup>	
	BEFORE TREATMENT	AFTER TREATMENT
IMMUNE PLASMA	18	0
NORMAL PLASMA	17	11

$$\chi^2 = 7.26 \quad p < 0.01$$

\* In Vero cell cultures and in new\_born mice

TABLE 3

MEAN TITERS AND RANGES OF VIREMIA <sup>(1)</sup>  
 IN AHF PATIENTS TREATED WITH IMMUNE  
 PLASMA AND WITH NORMAL PLASMA

TREATMENT	BEFORE TREATMENT	AFTER TREATMENT
IMMUNE PLASMA	$\bar{X} = 2.30$ <sup>(2)</sup> ( 1.34 - 3.24 )	-
NORMAL PLASMA	$\bar{X} = 2.08$ <sup>(3)</sup> ( 1.24 - 3.24 )	$\bar{X} = 2.21$ <sup>(4)</sup> ( 1.24 - 3.50 )

(1) Titrations performed in Vero cell cultures, expressed in  
 TCID<sub>50</sub> per ml. of blood (cpe)

(2) 18 blood samples

(3) 17 " "

(4) 11 " "



TABLE 4

CORRELATION BETWEEN CLINICAL IMPROVEMENT AND DETECTION  
OF IF JUNIN ANTIBODIES IN 15 PATIENTS WITH AHF

PATIENT NUMBER	DAY OF CLINICAL IMPROVE- MENT	DAY OF DETECTION IF Ab	ANTIBODIES IN		
			IgG	IgM	IgA
1	11	12	ND*	ND	ND
2	13	14	ND	ND	ND
3	13	14	ND	ND	ND
4	13	15	ND	ND	ND
5	12	12	+	-	-
6	11	18	+	-	-
7	10	18	+	-	-
8	9	17	-	-	+
9	13	15	+	-	-
10	11	15	-	-	+
11	11	14	+	+	-
12	15	18	-	+	+
13	12	16	-	+	+
14	14	15	-	+	+
15	12	15	+	-	-

\* Not done

TABLE 5

RELAPSES WITH A NEUROLOGIC SYNDROME IN PATIENTS WITH ABF

TREATMENT	NUMBER OF PATIENTS		RELAPSES
	WITHOUT RELAPSE	WITH RELAPSE	
IMMUNE PLASMA	80	10	11 6
NORMAL PLASMA	81	0	0 8
TOTAL	161	10	171

$\chi^2 = 7.65$   $P < 0.01$

REPORT FROM THE SPECIAL PATHOGENS REFERENCE LABORATORY, PHLS CENTRE  
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WILTSHIRE, ENGLAND

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T. Southee

Comparative study of strains of Ebola viruses from Sudan and Zaire

Investigators in Sudan during the Ebola epidemic of 1976 gained an impression that fewer haemorrhagic manifestations and fewer fatalities occurred during the later stages of the epidemic when the virus had undergone several generations in man. Similar observations were obtained in guinea pigs and monkeys when Sudanese and Zairean strains were compared.

Studies in guinea pigs

Dunkin-Hartley strain guinea pigs were immunized with the Zaire prototype strain E718 of Ebola and with the Sudan strain Boneface. Fluorescent antibody studies suggested a very close similarity between E718 and Boneface. This close relationship was confirmed by cross-protection studies (Table 1) which also confirmed the antigenic differences between Marburg and Ebola viruses.

Primary isolation studies in guinea pigs revealed a considerable difference in the expression of virulence between E718 and Boneface strains of Ebola, the Sudan strain producing a much less intense illness and very few deaths. The differences are clearly illustrated in Tables 2, 3, 4 and 5. First passage of E718 caused only one death in 10 infant pigs. In the third passage (Table 2) just over 60% died and by the fourth passage (Table 3) all infected guinea pigs died. The Boneface strain (Table 4; Table 5) produced no deaths at the fourth passage and less than 50% at the sixth passage.

Studies in monkeys

Similar differences in virulence were found in rhesus monkeys; E718 produced severe illness, rash and death, whereas Boneface produced a similar but

less intense illness but only occasional deaths. Figure 1 shows a graphical example of the response of a rhesus monkey to infection with strain Boneface. The monkey became febrile on the third day which persisted until the 14th day; viraemia was apparent by day 4 reaching a maximum on day 6 and persisting until day 12; antibody was first detected on day 8 increasing to a titre of 1 : 1024 on day 21.

To determine whether we could demonstrate a similar type of cross protection shown in guinea pigs, this monkey was challenged on day 35 with the third guinea pig pass material of the Zaire prototype E718. The challenge dose was  $10^4$  GPIU intraperitoneally.

#### Results of challenge

On the fourth day post challenge the monkey became quiet and was off its food and water. Blood collected on day 4 was tested for virus and antibody. The fluorescent antibody titre against both strains of Ebola virus was 1/512-1/1024. Despite this level of antibody, the monkey was viraemic and was circulating  $10^{3.5}$  GPIU/ml of virus in the blood. On day 5 the monkey developed a maculopapular skin rash and was found dead on day 7.

The question was raised whether this monkey may have been challenged too soon. A second monkey was infected with the Sudanese strain of Ebola virus (Boneface). The course of the disease was very similar to that of the first monkey and the monkey appeared to be almost recovered by the fourth week post challenge.

This monkey was then challenged on day 63, first with the homologous Sudanese strain of Ebola virus, dose  $10^4$  GPIU I.P. There was no evidence of infection (the monkey did not develop a temperature and did not develop a detectable viraemia) giving every indication of a solid immunity. The monkey

was then re-challenged on day 111 with the heterologous Zaire strain E718, actual dose  $10^4$  GPIU intraperitoneally. The monkey developed a febrile illness and also an extensive maculopapular skin rash on day 7. The viraemia on day 7 was  $10^{6.5}$  GPIU/ml of blood. The monkey died on day 8.

In order to establish that the convalescent immunity demonstrated within the Sudanese homologous system was in fact a true phenomenon, a third monkey was infected with the Sudanese strain, Boneface. The monkey recovered and was challenged eight months later with the same homologous Ebola strain, Boneface. There was every indication that the immunity was in fact solid.

#### Immunotherapy experiments with Ebola plasma

Experience in the use of convalescent Ebola antibody for therapy is very limited and has only been successfully reported on one occasion, and this only in combination with human leucocyte interferon (Emond et al., 1977). The results of previous investigations by us on the use of convalescent plasma as specific treatment in the management of Ebola virus infection in non-human primates have been equivocal and in need of clarification. The following investigations were carried out in an effort to shed some light on some of the mechanisms involved.

Animals. Cynomolgus monkeys weighing 4.20 kg - 5.50 kg were used.

Immune plasma. Human convalescent plasma (IFA titre 1/256).

Challenging virus. The original source of virus was human acute-phase blood, E718 from the Zaire outbreak. This was passaged three times in guinea pigs. The virus inoculum was a suspension of guinea pig liver taken during the late febrile stage of the disease.

Challenge dose. Monkeys were inoculated on day 0 with either a 100 or 1000 guinea pig infectious units intraperitoneally.

### Immune plasma plasma treatment schedule

Group A. A total of four monkeys (two infected with 100 GPIU and two with 1000 GPIU) were used. 25 ml of convalescent plasma was administered within 30 minutes of infection.

Group B. A total of four monkeys (two infected with a 100 GPIU and two with a 1000 GPIU) were used. 25 ml of convalescent plasma was administered upon onset of fever.

Group C. Two plasma control monkeys.

Group D. Two virus control monkeys.

Blood samples were obtained daily by femoral venepuncture and estimations made of their virus and passive antibody (IFA) levels. Temperatures were recorded daily.

### Results

The results are set out in the accompanying Tables 6, 7, and 8 . These show that in the group receiving immune plasma within 30 minutes of infection, viraemia was delayed until day four in three out of the four monkeys infected. The mean survival time in this group was slightly prolonged with one of the monkeys having an inapparent (aborted) infection.

Passive antibody at a level of 1/8 was detected during the first 48 hours. This tended to fall off to undetectable levels from day three onwards.

In the group receiving immune plasma upon onset of fever, the virus was detected in the blood on day two reaching peak titres of  $10^6$  GPIU/0.2 ml of blood by day four. No passive antibody was detected in the blood of these four monkeys. This was not surprising with the level of viraemia and the time of administration of the plasma and the low level of passive antibody achieved in Group A. Further studies are now under way and will be directed towards maintaining the level of passive antibody for a longer period.

### Mozambique - Lassa Relationships

Studies directed at a better understanding of the taxonomic characteristics of Mozambique and Lassa isolates have been undertaken. Preliminary studies have suggested differences in their respective growth kinetics within different cell lines, Mozambique viruses growing more rapidly than Lassa isolates. Electron microscopical observations of the Mozambique viruses show that they are consistent with arenavirus development and morphology except that they do not display Lassa's pleomorphism.

The development and establishment of a plaque reduction tissue culture neutralization system has pointed to a close relationship between the viruses (Table 9) supporting the results achieved by immunofluorescence and complement fixation tests but has not yet been sufficiently developed to determine the immunotypical specificity of each strain. The usefulness of the neutralization system is dependent upon the standardization of quality, age and immunization protocol used in producing a reliable and reproducible series of neutralizing sera. Development of the immunological relationships in vivo and in vitro is at present being studied with a range of Lassa and Mozambique isolates. The use of monoclonal antibodies (supplied by Dr Buchmeier, Scripps Clinic) has proved an interesting and useful tool in differentiating between Lassa and Mozambique viruses. Some of these antibodies have also indicated possible differences between various Lassa isolates, an observation under further investigation.

More detailed biophysical, biochemical and immunological studies are presently in progress directed at differentiating between these viruses.

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Emond, R.T.D., Evans, Brandon, Bowen, E.T.W. and Lloyd, G. (1977). British Medical Journal, 2, 541-544.

TABLE 1.

Cross protection studies of Marburg and Ebola virus in guinea pigs

Immunizing virus	Challenging virus		
	Marburg 67	Ebola Zaire E718	Ebola Sudan Boneface
Marburg 67	3/3 <sup>a</sup>	0/3	0/3
Ebola Zaire (E718)	0/3	3/3	3/3
Ebola Sudan (Boneface)	0/3	3/3	3/3

<sup>a</sup>  $\frac{\text{No. of guinea pigs protected}}{\text{No. of guinea pigs challenged}}$



Table 2  
 Titration of Ebola virus in guinea pigs  
 (3rd guinea pig passage)  
 Rectal Temperatures (°C)

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
10 <sup>-1</sup>	38.5	38.9	39.6	39.6	39.5	<u>40.6</u>	<u>41.1</u>	<u>40.6</u>	39.7	37.3	36.5	DEAD				
	38.3	38.8	39	38.9	39.3	<u>40.2</u>	<u>40.7</u>	<u>40.7</u>	39.8	38.6	39.5	38.7	38.7	38	38.6	
10 <sup>-2</sup>	38.9	38.6	38.9	<u>40.6</u>	<u>40.9</u>	<u>40.5</u>	<u>40</u>	DEAD								
	38.2	38.3	38.5	<u>40.1</u>	<u>40.6</u>	<u>40.3</u>	<u>40</u>	DEAD								
10 <sup>-3</sup>	38.9	39.1	38.9	39.5	39.1	39	39.6	<u>41</u>	<u>41.3</u>	<u>40.8</u>	39.6	DEAD				
	38.5	38.8	38.8	39	39	39	39.4	39.7	<u>40.7</u>	<u>40.5</u>	39.8	39.2	39.2	38.3	38.7	
10 <sup>-4</sup>	38.5	38.9	38.8	38.8	38.9	<u>40</u>	<u>40.2</u>	<u>40.8</u>	<u>41.2</u>	<u>40.6</u>	<u>41.1</u>	<u>40.3</u>	39.5	39.2	39.5	39.2
	38.9	39	38.7	38.3	39.7	<u>40.2</u>	<u>40.6</u>	<u>41.1</u>	DEAD							
10 <sup>-5</sup>	39.3	39.1	39.3	39.5	39.1	39	<u>40.6</u>	<u>42.1</u>	<u>40.6</u>	DEAD						
	39.2	29.2	28.9	39.2	38.9	38.8	39.1	39.1	39	<u>40.5</u>	<u>41.7</u>	<u>41.2</u>	<u>41.1</u>	39.1	DEAD	
10 <sup>-6</sup>	39.1	39.5	39	39.1	38.7	38.5	39.2	39.1	39.2	38.9	39.3	38.7	39.8	39	38.8	38.5
	38.9	39.1	38.8	38.9	38.8	38.5	38.6	39.2	38.9	39.1	39.9	40.3	41.0	41.2	40.9	40.5

Table 3  
 Titration of Ebola virus in guinea pigs  
 (4th guinea pig passage)  
 Rectal Temperatures (°C)

DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
10 <sup>-1</sup>	39	39.5	38.8	39.5	<u>40.5</u>	<u>40.3</u>	<u>40.3</u>	<u>41.3</u>	<u>40</u>	39.7	<u>40.1</u>	39.2	DEAD			
	39.5	39.8	38.7	<u>40</u>	<u>41</u>	<u>40</u>	DEAD									
10 <sup>-2</sup>	39.1	39.7	39.2	39.7	<u>40.3</u>	<u>40.5</u>	<u>40.5</u>	<u>40.5</u>	DEAD							
	39.5	39.4	38.9	39.8	<u>40.5</u>	<u>40.5</u>	<u>40.1</u>	<u>40.5</u>	<u>40.2</u>	39.7	<u>40.5</u>	<u>40.1</u>	38	DEAD		
10 <sup>-3</sup>	38.9	39.4	38.6	39	39.1	<u>40.2</u>	<u>41.3</u>	<u>40.8</u>	DEAD							
	38.9	39.1	38.4	38.4	38.4	<u>40</u>	<u>40.5</u>	<u>40.5</u>	39.4	DEAD						
10 <sup>-4</sup>	38.9	38.9	38.3	38.4	38.5	<u>40</u>	<u>40</u>	DEAD								
	38.3	39.5	38.5	38.8	39.1	38.3	38.7	<u>41.1</u>	<u>40.8</u>	<u>40</u>	35.6	DEAD				
10 <sup>-5</sup>	39	39	38.4	39	38.5	<u>40</u>	<u>40.5</u>	<u>40.5</u>	<u>40</u>	37.3	DEAD					
	39.4	39.7	38.9	39	38.8	<u>40.9</u>	<u>40.9</u>	<u>40.5</u>	39.5	DEAD						
10 <sup>-6</sup>	39.1	38.9	39.5	39.1	39	39.9	<u>41.1</u>	<u>41.3</u>	39.7	37.6	DEAD					
	39.1	39.5	39.1	39	38.9	39.1	38.9	39.5	39	39	38.9	39.2	39.2	38.9	38.6	38.8

Table 4  
Serial passage of Ebola virus, Sudan strain (Boneface)  
in guinea pigs  
Rectal Temperatures (°C)

Day	1	2	3	4	5	6	7	8	9	10	11	12	21	
Guinea pig Pass 1	39.2	39.0	39.1	<u>39.5</u>	<u>40.5</u>	40.6	39.8	39.3	39.1	38.7	ND	38.9	Survived	
Guinea pig a Pass 2 b	38.9 38.5	39.0 38.6	39.0 38.9	39.2 38.8	39.4 39.1	<u>40.0</u> <u>40.1</u>	39.8 40.4	<u>40</u> <u>40.6</u>	<u>40.8</u> <u>40.6</u>	39.4 39.5	39.1 38.9	39.2 38.9	Survived Survived	
Guinea pig Pass 3	39.2	39.1	39.4	<u>40.2</u>	<u>40.9</u>	<u>40.9</u>	<u>40.8</u>	39.5	39.4	39.0	39.1	39.0	Survived	
Titration of Guinea pig Pass 4	10-1	38.8 39.2	39.4 39.7	38.4 38.4	39.7 39	<u>40.2</u> <u>39.4</u>	<u>40.7</u> <u>40.1</u>	40.6 40.4	<u>41.1</u> <u>40.1</u>	<u>40.1</u> <u>40.5</u>	39.8 40.3	<u>39.4</u> <u>40.0</u>	38.8 39.8	Survived Survived
	10-2	38.8 38.6	39.0 39.7	38.7 38.4	38.9 39.2	38.9 39.4	38.9 39.7	39.8 40.4	<u>40.3</u> <u>40.7</u>	<u>40.6</u> <u>40.6</u>	<u>40.7</u> <u>40.6</u>	<u>39.9</u> <u>40.0</u>	38.9 38.9	Survived Survived
	10-3	39.0 39.4	39.2 38.9	38.9 39.4	39 39.5	39 39.8	38.9 39.4	<u>40</u> <u>40.7</u>	<u>40.6</u> <u>40.8</u>	<u>40.6</u> <u>40.6</u>	<u>40.8</u> <u>40.8</u>	<u>40.1</u> <u>39.4</u>	39.2 39.2	Survived Survived
	10-4	38.8 39.0	38.3 38.9	39.7 38.9	39.5 39.4	38.8 38.8	39.2 38.9	39 39	39.4 39	39.4 39.3	39.4 38.9	39.5 39.7	39.0 39.2	Survived Survived

N.D. Not done.

Table 5  
Titration of Ebola virus, Sudan strain (Boneface) Pass 6  
in guinea pigs  
Rectal temperatures (°C)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21
Dilution	10 <sup>-1</sup>	38.9 39.0	39.4 39.6	39.4 39.6	39.4 40.0	38.9 40.4	40.5 40.2	40.3 39.1	39.4 39.4	38.3 39.4	DEAD	38.8	38.3	38.9	39.3	38.8 Survived
	10 <sup>-2</sup>	38.9 39.0	38.9 39.3	38.3 38.8	39.1 40.1	39.4 40.4	39.0 40.3	40.5 39.6	40.5 40.1	40.3 39.4	38.9 38.9	38.8 38.7	DEAD 38.5	39.0	38.7	38.7 Survived
	10 <sup>-3</sup>	38.9 38.9	39.1 38.8	39.1 38.9	38.9 39.0	40.1 39.1	39.8 39.7	40.2 39.6	40.3 40.2	40.5 40.5	40.0 40.3	40.0 39.5	39.4 38.9	40.2 39.2	39.3 38.7	38.8 38.7 Survived
	10 <sup>-4</sup>	38.6 38.9	38.4 39.1	38.3 39.1	38.3 39.1	38.6 39.0	39.0 39.2	38.9 39.1	39.1 40.2	39.2 40.5	39.0 40.5	38.9 40.3	39.4 40.7	38.7 40.1	38.5 DEAD	38.4 Survived
	10 <sup>-5</sup>	39.0 38.9	39.5 39.2	39.2 38.9	39.2 39.5	39.0 38.9	39.2 38.6	39.0 38.6	39.4 38.9	39.2 36.9	38.9 38.8	39.0 38.8	39.1 38.8	39.1 38.9	38.9 38.6	38.8 38.5

N.I. = Not infected

TABLE 6. Daily temperature °C

Group	Virus Dose GPLU	Monkey No.	0	1	2	3	4	5	6	7	8	9	10	11	12	Weight Kg
A 25 ml of plasma infused within 30 mins. Ab. titre = 1/256	1000	1	39*	39	39.1	39.4	40.5	39.9	Dead	)						5.200
		2	38.3*	38	38.5	39.7	39.4	39.5	39.5	Dead)	)					5.200
	100	5	38.5*	38.7	38.3	39.1	40.7	40	39.4	36.6	Dead					4.250
		6	37.9*	37.8	37.9	38.3	38.3	37.8	37.4	38	37.8	37.9	37.8			4.350
B 25 ml of plasma infused upon onset of fever Ab. titre = 1/256	1000	3	38.9	38.8	39.1	39.1	40.3*	Dead	)							4.550
		4	38.6	38.1	38.7	40.2*	39.2	39	Dead)	)					4.750	
	100	7	38.7	38.6	38.7	38.6	39.4*	39.0	Dead	)					4.500	
		8	38.3	38.7	38.9	38.8	39.4*	39.7	36.1	Dead)	)				4.300	
Plasma control	0	9	38.8*	38.9	39.1	38.9	38.8	38.6	38.3	38.9	38.8	38.7	38.9			5.500
	0	10	38.3*	38.7	38.7	38.3	38.2	38.1	37.2	38.3	38.2	38.3	38.3			5.400
Virus control	1000	11	38.2	38.3	38.4	38.4	39.4	38.0	Dead)	)						4.350
	100	12	38.6	38.2	38.0	38.7	39.2	37.4	Dead)	)						4.700

\* Day plasma administered

TABLE 7.  
Level of viraemia log 10 (days)

Group	Virus Dose GPIU	Monkey No.	1	2	3	4	5	6	7	8
A 25 ml of plasma infused within 30 mins of infection Ab. titre = 1/256	1000	1	< 0.5	< 0.5	< 0.5	<u>3.0</u>	<u>5.0</u>	<u>4.0</u> <sup>+</sup>		
		2	< 0.5	< 0.5	<u>2.0</u>	<u>5.0</u>	<u>5.0</u>	<u>5.0</u>	≥ <u>6.0</u> <sup>+</sup>	
	100	5	< 0.5	< 0.5	< 0.5	<u>3.0</u>	≥ <u>6.0</u>	≥ <u>6.0</u>	≥ <u>6.0</u>	≥ <u>6.0</u> <sup>+</sup>
		6	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
B 25 ml of plasma infused upon onset of fever Ab. titre = 1/256	1000	3	< 0.5	<u>1.0</u>	<u>4.0</u>	≥ <u>6.0</u> <sup>*</sup>	≥ <u>6.0</u> <sup>+</sup>			
		4	< 0.5	<u>2.0</u>	<u>5.0</u> <sup>*</sup>	≥ <u>6.0</u>	≥ <u>6.0</u>	<u>5.0</u>		
	100	7	< 0.5	<u>0.5</u>	<u>4.0</u>	≥ <u>6.0</u> <sup>*</sup>	<u>5.0</u>	≥ <u>6.0</u>		
		8	< 0.5	<u>0.5</u>	<u>3.0</u>	≥ <u>6.0</u> <sup>*</sup>	≥ <u>6.0</u>	≥ <u>6.0</u>	≥ <u>6.0</u> <sup>+</sup>	
Virus control group	1000	11	< 0.5	<u>1.0</u>	<u>2.0</u>	≥ <u>6.0</u>	<u>5.0</u>	≥ <u>6.0</u> <sup>+</sup>		
	100	12	< 0.5	<u>2.0</u>	<u>4.0</u>	≥ <u>6.0</u>	≥ <u>6.0</u>	≥ <u>6.0</u> <sup>+</sup>		

+ Dead

\* Day plasma administered

TABLE 8

Level of passive antibody (days) reciprocal of dilution

Group	Virus Dose GPIU	Monkey No.	0	1	2	3	4	5	6	7	8
A 25 ml of plasma infused within 30 mins. Ab. titre = 1/256	1000	1	<4*	8	4	<4	<4	<4			
		2	<4*	8	8	<4	<4	<4	<4		
	100	5	<4*	<4	8	<4	<4	<4	<4	<4	<4
		6	<4*	8	3	<4	<4	<4	<4	<4	<4
B 25 ml of plasma infused upon onset of fever Ab. titre = 1/256	1000	3	<4	<4	<4	<4	<4*	<4			
		4	<4	<4	<4	<4*	<4	<4			
	100	7	<4	<4	<4	<4	<4*	<4			
		8	<4	<4	<4	<4	<4*	<4	<4		
Plasma control group		9	<4*	8	8	<4	<4	<4	<4	<4	<4
		10	<4*	<4	<4	<4	<4	<4	<4	<4	<4

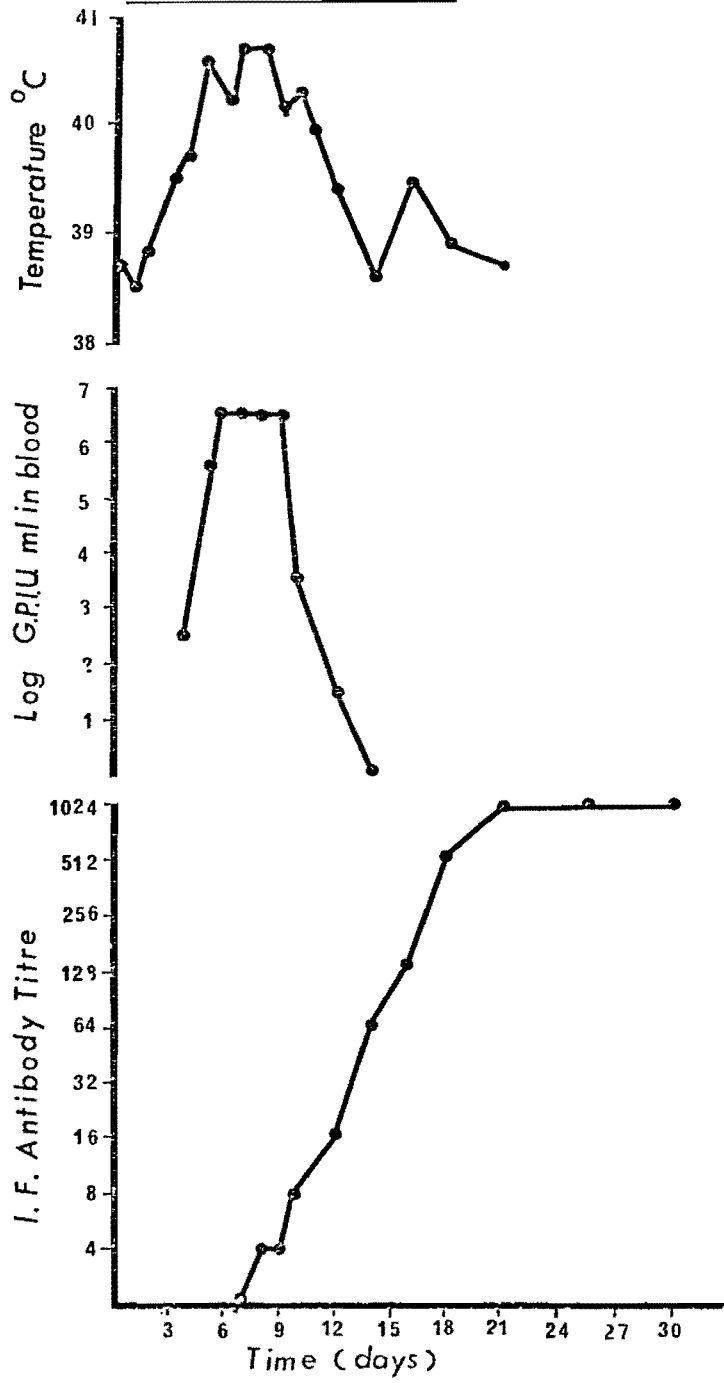
\* Day plasma administered

TABLE 9. A general serological comparison between Lassa and Mozambique viruses

Antibody	Technique	Viruses		
		Lassa (L.P.)	Lassa (G.A.)	Mozambique
Convalescent Human L.F. (G.A.)	Indirect I.F.	+	+	-
	Neutralization	+	+	-
Hyperimmune Guinea pig anti-Mozambique	Indirect I.F.	+	+	+
	Neutralization	+	+	+
LCMV hybridoma (1-1-3)	Indirect I.F.	+	+	+
LCMV hybridoma (9-2-5)	Indirect I.F.	+	-	+



Figure 1. Rhesus monkey 43. Rectal temperature, virus and antibody levels in the blood.



Report from Takashi Kitamura, National Institute of Health, Tokyo, Japan

1. Construction of Maximum Security Laboratory Started in N.I.H., Japan :

A Maximum Security Laboratory (MSL) complex is now under construction under the following conditions:

- 1) Site of construction: Murayama Branch of N.I.H., Japan. (Fig.1)
- 2) Construction schedule:
  - Building construction: April - October, 1980.
  - Interior and cabinet line installation: Nov. 1980 - Jan. 1981.
  - Physical checks and adjustments: Feb. - March, 1981.
  - Biological checks and formulation of operational manual; April - June, 1981.
  - Start of experimental works; July, 1981.
- 3) Budget allotment: Total ¥ 850 million (ca. \$ 4 million)
  - Building with secondary barrier system: ¥ 500 million
  - Glove box lines (GBL: Primary barrier system): ¥ 280 "
  - Equipments (including those for P2 and P3 laboratories): ¥ 70 "
- 4) Main components:
  - a. P4 GBL laboratory (B1) ... for biological<sup>(works,)</sup> with fl. microscope, inverted microscope, incubator, refrigerator and cryostat cabinets.
  - b. P4 GBL laboratory (B2) ... for analytical works, with incubator, refrigerator, microscope, ultracentrifuge and spectrophotometer cabinets.
  - c. P4 animal laboratory (C) ... GBL type animal isolators for monkeys and mice, each 2 units, may be connected with an autopsy cabinet, which is bound to the B1-GBL, by the use of a transfer box.
  - d. P3 laboratory ... ca. 40 m<sup>2</sup>, with 3 glass-II cabinets.
  - e. Cell culture laboratory ... ca. 40 m<sup>2</sup>, with 2 clean benches.
- 5) Floor plan: Fig.2.
- 6) Design features:
  - a. Negative air pressure ... Pressure difference is maintained in the order of: outside - air lock(outer) - shower - air lock(inner) - laboratory - glove box.
  - b. Waste water disposal ... to be sterilized at 120°C, 60 min., by a dual tank system.
  - c. Exhaust air ... to be sterilized by filtration through 2 layers of HEPA filter.
  - d. Supply air ... filtered through single layer of HEPA filter.
  - e. Exhaust ducts of the laboratory and GBL are merged after HEPA filter to secure the pressure balance between GBL and the laboratory.

7) Design and construction:

- a. <sup>Building:</sup> Chiyoda Chemical Engineering and Construction, Ltd., under the supervision of Ministry of Construction, Government of Japan.  
b. GBL: Hitachi, Ltd., Department of Bioclean Engineering.

8) Future Administration: "Special Pathogens Reference Laboratory" (Chief. Dr. T. Kitamura) will start at the completion of MSL construction with the following functions:

- a. WHO Variola Reference Center.  
b. Diagnosis of and basic studies on class-4 HF viruses.  
c. Regular check of herpes-B antibody in cooperation with Tsukuba Medical Primate Center ( a branch of N.I.H., capacity 3000 monkeys).  
d. Animal experiments with class-3b HF viruses. - T. Kitamura -

2. Check of Korean Haemorrhagic Fever (KHF) Antibody among Rodents Captured at Quarantine Stations of Kobe and Yokohama Port Areas.

Rodents were captured at monthly intervals from international port areas of Kobe and Yokohama during the years of 1978 through 1980. Nearly 400 serum samples were collected and checked of the KHF antibody, in parallel with those against other virus groups, in the following manner:

- 1) Method: Indirect immunofluorescence staining with fluorescein conjugates against Mus and Rattus IgG.  
2) Antigen: KHF-infected Apodemus lung sections, supplied by Dr. H. W. Lee, Korean University, Seoul.  
3) Technical standardization: The workers (T.K., H.T., & A.O.) stayed for a week in March, 1980, in Dr. Lee's laboratory with several serum samples and confirmed the effectiveness of Dr. Lee's original procedure with several control materials.

The tests are now under way. So far, the results with 18 Mus musculus, 10 Rattus norvegicus and 72 Rattus rattus sera were all negative.

- T. Kitamura, Hisao Takeda, & Akira Oya -

FIG.1

Location of the Maximum Safety Laboratory

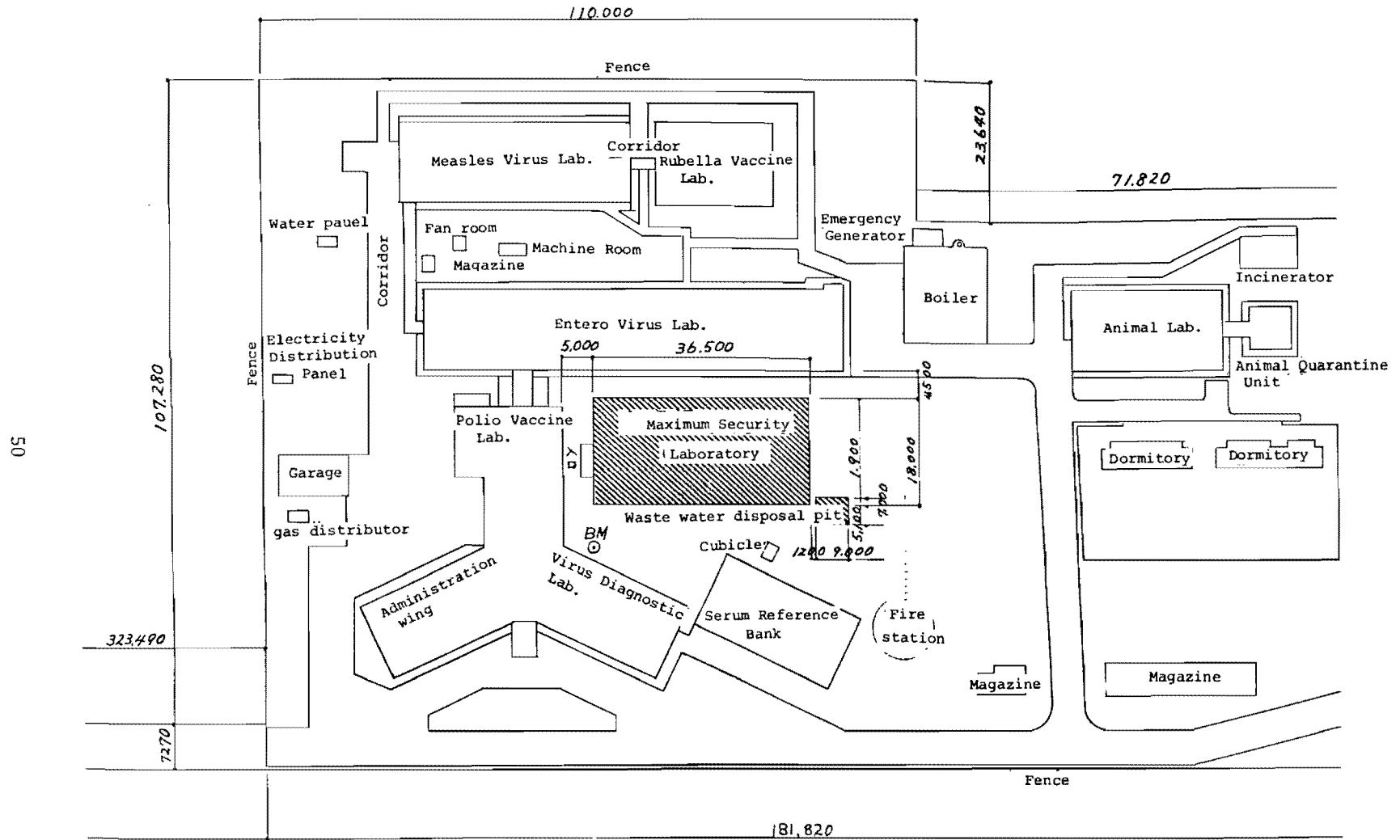
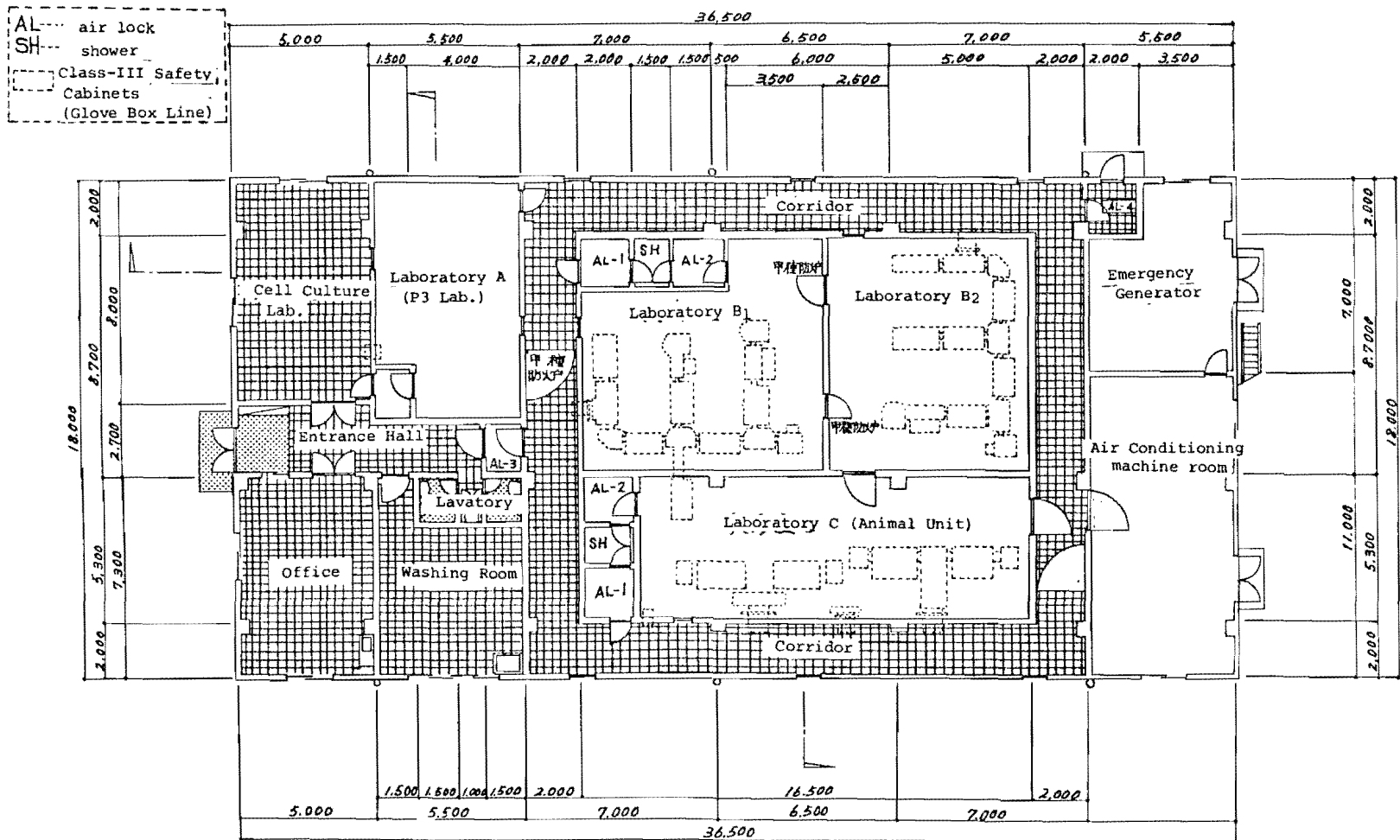


FIG. 2

Floor Plan of the Maximum Security Laboratory



SI

REPORT FROM THE ARBOVIRUS LABORATORY  
INSTITUT PASTEUR DE NOUMEA-NEW CALEDONIA

Human infections caused by dengue type 4 virus

In New Caledonia, the last case caused by dengue 1 virus was seen in December 1978 ; three months later, dengue 4 virus was isolated from an imported case and soon after, from indigenous. Transmission was ramping till October 1979 ; outbreaks occured in Thio, at the fall of November 1979, and in Noumea from March 1980 ; 12 strains were identified as dengue type 4 by CF test. Numerous other virus isolations are on the way either by intracerebral inoculation to baby mice or by intra-thoracic route in Toxorhynchites mosquitoes. HI tests on paired sera were positive in 538 cases, from January to July 1980.

Patients affected by dengue 4 virus exhibit in most cases the typical symptoms of dengue illness but the frequency of signs observed in type 4 and in type 1 infections are slightly different. In Thio, hepatalgia was noted in 54,6 % of cases ; hair fall in 5 % ; epistaxis in 3,5 % ; a severe haemorrhagic form with recovery, was observed in a child of 10.

Strain NC-H-178 was obtained in a case of dysimmunitary adenopathy.

In Vanuatu (New Hebrides), dengue 4 was discovered in June 1979, by virus isolation from the serum of a young man with fever and swollen knees ; three other men of the same village, in the N.W of Espiritu Santo island, exhibited fluxionary arthritis of the knee ; HI tests on sera showed a significant rise of anti-Flavivirus antibodies.

Dengue 4 was imported into Wallis island, probably from New Caledonia and was responsible of an outbreak in October 1979. Later, cases were seen in Horne islands (Futuna) and confirmed by laboratory tests.

Mosquito infection by dengue 4 virus

During an entomological survey of La Tontouta (New Caledonia) airport, 60 Culex annulirostris female mosquitoes were collected in a light trap operating in front of the dispensary between 18th and 19th February 1979 ; intra-cerebral inoculation to baby mice resulted in isolation of NC-AR 281 strain ; at the third passage, HA titer was 1/80 (optimum pH 6,6) ; HI test performed with immune ascitic fluids from Queensland Institute of Medical Research (Dr Aaskov) gave the following results:

<u>Alfuy</u>	<u>Stratford</u>	<u>Edge hill</u>	<u>MVE</u>	<u>Kokobera</u>	<u>Kunjin</u>	<u>DEN.1</u>	<u>DEN.2</u>	<u>DEN.3</u>	<u>DEN.4</u>
20	20	20	40	20	20	40	40	80	160

.../...

Provisory identification is dengue type 4. Infectious mosquitoes were caught 6 days after the lone case (imported) observed in La Tontouta ; this locality is free of Aedes aegypti.

Alphavirus infections in the south-west Pacific area

In November 1979, 12 paired sera and 4 unique specimens were received from Horne islands (Futuna) ; dengue was suspected in patients with fever, headache and arthralgia ; i.c. inoculations to baby mice resulted in 7 virus isolations ; the strains proved to be Alphaviruses by CF test with grouping ascitic fluid NIAID G-209-701-567. HI tests with rabbit antisera, kindly provided by Queensland Institute of Medical Research gave the following results with VK strain (4698) :

	<u>Bebaru</u>	<u>Getah</u>	<u>Ross River</u>	<u>Sindbis</u>
HI Titer	<u>20</u>	20	40 960	80

Saccharose acetone antigen prepared from VK strain (4598) was used to test paired sera ; a significant rise of anti-Alphavirus HI antibodies was found in 9 patients.

Another strain was recovered from the serum of a girl of 11 living in Falaleu, Wallis island, during the dengue outbreak and a positive serology (10 / 20) was seen in a boy of 17.

In New Caledonia, the first Alphavirus isolation was obtained from the blood of the writer (P.F.), one day after the onset of the illness : moderate fever during two days, lassitude and severe polyarthrititis were the major symptoms ; acute phase lasted 5 days. Monthly record of cases with positive HI test on paired sera was as following :

<u>Febr.</u>	<u>March</u>	<u>April</u>	<u>May</u>	<u>June</u>	<u>July</u>	<u>TOTAL</u>
7	6	0	2	8	2	25

Five strains were isolated from blood of patients in acute phase.

First identification tests indicate that Ross River virus is very probably responsible of human infections observed in New Caledonia, Wallis and Futuna. Importation of R.R. virus in these islands occurs after a large outbreak in Fiji islands where about 30 000 cases were reported from April 1979. Australian tourists are numerous in Fiji and New Caledonia. Probable vectors are Aedes vigilax and Culex annulirostris. In Wallis and Futuna, Ae.polynesiensis is also suspected.

P. FAURAN

G. LE GONIDEC

REPORT FROM CSIRO DIVISION OF ANIMAL HEALTH

PRIVATE BAG NO. 1, P.O., PARKVILLE, VICTORIA, 3052, AUSTRALIA

1. Serotyping of Additional Australian Bluetongue Virus Isolates

Attempts were made during 1979 to isolate viruses causing bluetongue serological responses in cattle, as detected using a group-antigen test, the agar gel diffusion precipitin test (AGDP). There was one isolate of a virus (CSIRO154) and eight isolates of another virus (CSIRO156) made from the blood of healthy cattle in the Northern Territory. These viruses could not be distinguished from bluetongue virus (BTV) serotype 20 by AGDP, complement-fixation, or fluorescent antibody tests and hence were designated members of the BTV serogroup. Serotyping was carried out using the plaque-inhibition test (Tables 1 and 2). CSIRO154 virus (Table 1) was found to be similar but not identical to BTV6, hence it is probably not one of the known 20 BTV serotypes. CSIRO156 virus could not be distinguished from BTV1 by plaque-inhibition (Table 2) or plaque reduction neutralization tests and it was concluded that it was an Australian isolate of BTV serotype 1. None of the Australian BTV isolates have been associated with disease in the field and only mild to moderate signs of disease have been produced in experimentally infected sheep, similar to that reported for the first Australian bluetongue isolate BTV20 (St. George and McCaughan, Aust.vet.J., 55 : 198-199, 1979).

(A.J. Della-Porta and D.A. McPhee in collaboration with T.D. St. George and D.H. Cybinski of CSIRO Division of Animal Health, Long Pocket Laboratories and M.C. Wark of Commonwealth Serum Laboratories, Parkville).

2. Intra-uterine and Intravenous Inoculation of Cows During Estrus with Akabane virus

Previous studies had shown that Akabane virus inoculated into the uterus of susceptible heifers during insemination at estrus did not interfere with subsequent pregnancy. Two more groups of cows were either inseminated (Group 1) or mated naturally to a bull (Group 2). At the time of service, Akabane virus was inoculated into the uterus (Group 1) or intravenously (Group 2). The animals in both groups were slaughtered at 2 day intervals and tissues and blood were taken for virus isolation and tissues for histopathological examination. Akabane virus was isolated from the blood and from a number of tissues to day 8 after inoculation, no further isolations were made after that time. Histopathology showed that lesions were present in the corpus luteum of the ovary of all cows inoculated with virus but in no other tissues. No virus isolations were made, or histopathological lesions seen, in the control cows.

It should be noted that we have not been able to demonstrate excretion of Akabane virus in the semen of viremic bulls. Semen was collected over a period of 10 days from 8 bulls, that were infected intravenously, and which had viremias of from 3 to 5 days duration. The spermatozoa from the infected bulls were of normal morphology and virus was not recovered from any of the semen collections. Semen samples injected intravenously into cows did not produce infection with Akabane virus or was there any evidence of seroconversion.

(I.M. Parsonson, A.J. Della-Porta, W.A. Snowdon and M.L. O'Halloran).



### 3. Akabane Virus Infection in Pregnant Ewes

Studies suggested that Akabane isolates may differ in virulence, either because of the origin of the original isolate or as a result of passage in laboratory systems. An experiment was designed to test the effect of different laboratory virus passage systems on the virulence of an Akabane isolate CSIRO16, originally isolated from a biting midge, Culicoides brevitarsis. The 3 different pools of virus were each inoculated into a separate group of ewes at 33 to 36 days of pregnancy. The ewes were slaughtered between 70 and 105 days of pregnancy and the fetuses examined for gross abnormalities. Samples were taken for virus isolation, for histopathology and blood was collected for serological examination.

From the 39 ewes inoculated with Akabane virus, 55 (141%) fetuses were examined. As there was no significant difference between the virus pools the results were treated as a single group. Of the 55 fetuses, 44 (80%) had congenital defects of arthrogryposis (AG), hydranencephaly (HE) and porencephaly (PE). Akabane virus was isolated from only 4 of the fetuses from the placentomes and fetal fluids. Serum neutralizing antibodies were present to Akabane virus in the fetal bloods from day 75 of gestation and correlated well with the appearance of IgG<sub>1</sub> immunoglobulin in the fetal sera.

(I.M. Parsonson, A.J. Della-Porta, W.A. Snowdon and K.J. Fahey in collaboration with H.A. Standfast of CSIRO Division of Animal Health, Long Pocket Laboratories).

Table 1. Plaque-inhibition (PI) neutralization comparison of CSIRO154 virus and BTV6

Antiserum <sup>(1)</sup>	PI zone (mean ± S.E. in mm) against <sup>(2)</sup>	
	CSIRO154 <sup>(3)</sup>	BTV6
Anti-CSIRO154, ovine (E957)	17.9 ± 1.7	7.0 ± 2.0
Anti-CSIRO154, ovine (E958)	17.4 ± 1.3	1.6 ± 3.6
Anti-CSIRO154, rabbit	12.4 ± 1.3	0 ± 0
Anti-BTV6, guinea pig	15.4 ± 0.8	23.0 ± 1.9

(1) All antisera treated with 0.4% β-propranolol to inactivate any residual virus in the serum. The sera were then dialyzed and adjusted to a dilution of 1:2 with respect to original serum volume.

(2) Mean and standard error of 5 to 7 determinations done on 2 to 5 separate occasions. PI method similar to Porterfield (Bull.W.H.O., 22 : 373-380, 1960).

(3) CSIRO154 virus was tested against guinea pig reference sera for BTV1 to BTV19, ovine sera for BTV20 and CSIRO156 virus and there was no zone of inhibition produced with any of these antisera except anti-BTV6 serum.

Table 2. Plaque-inhibition (PI) neutralization comparison of CSIRO156 virus and BTV1

Antiserum <sup>(1)</sup>	PI zone (mean ± S.E. in mm) against <sup>(2)</sup>	
	CSIRO156 <sup>(3)</sup>	BTV1
Anti-CSIRO156 ovine (E960)	16.6 ± 1.0	17.3 ± 1.1
Anti-CSIRO156 ovine (E961)	16.9 ± 1.5	18.9 ± 2.5
Anti-BTV1, Guinea pig	16.3 ± 1.1	17.3 ± 1.1

(1) Footnote as for Table 1

(2) Footnote as for Table 1

(3) CSIRO156 virus was tested against guinea pig reference sera for BTV1 to BTV19, ovine sera for BTV20 and CSIRO154 virus and there was no zone of inhibition produced with any of these antisera except anti-BTV1 serum.

REPORT FROM THE VIROLOGY SECTION

DEPARTMENT OF MICROBIOLOGY

PRINCE HENRY HOSPITAL, LITTLE BAY, SYDNEY, AUSTRALIA, 2036

Detection of Antibodies to Ross River virus by Enzyme-Linked  
Immunosorbent Assay

During the last few years, enzyme-linked immunosorbent assay (ELISA) has been used in the detection of a wide range of antigens and antibodies. For the detection of antibody, the advantages of ELISA over conventional tests (complement fixation, haemagglutination-inhibition and neutralization) include the use of serum without prior treatment, increased sensitivity and rapidity with no loss in specificity, greater stability of reagents, the possibility of detecting immunoglobulin type-specific antibodies, and the potential for objective reading of results and for automation. With arboviruses, ELISA has been successfully used for the detection of antibodies to alphaviruses (Frazier and Shope, 1979), dengue virus (Dittmar *et al.*, 1979) and tick-borne encephalitis virus (Hofmann *et al.*, 1979). This report describes our experience with ELISA in the detection of antibodies to Ross River virus (RRV).

The method used was essentially that previously described for alphaviruses (Frazier and Shope, 1979) but differed in several respects. First, RRV (T48 strain) and control antigens were prepared from BHK-21 cells. Secondly, the solution of phosphate-buffered saline-Tween 20, when used as test and reagent diluent, contained 4% bovine serum albumin (fraction V). The conjugate employed was rabbit anti-human IgG (Y-chain specific) conjugated to horseradish peroxidase (DAKO-immunoglobulins, Denmark) and the substrate was O-phenylene-diamine dihydrochloride (0.4mg/ml) and urea peroxide (0.2mg/ml) in phosphate-citrate buffer pH 5.0 (0.1M citric acid, 0.2M phosphate). The reaction was stopped with 8N H<sub>2</sub>SO<sub>4</sub>. The colour development was recorded visually on a 0-4 + scale and absorbance values were read through the plate at 490nm (Dynatech Microelisa Reader MR590). The visual end-point was taken as the highest serum dilution showing 3-4 + colour, and reactions were only recorded as specific when the corresponding wells with control antigen showed only 0-1 + colour. When the results (titres) were expressed spectrophotometrically, the mean absorbance of 5 negative control sera was used to calculate a positive/negative (P/N) ratio for each test serum. The titre was calculated at the highest dilution giving a P/N ratio  $\geq 2.1$ .

Sixty-two specimens previously tested by haemagglutination-inhibition (HI) were tested by ELISA. Nineteen sera negative by HI (<20) were also negative by ELISA (<10). Furthermore, all 43 sera with HI antibody were positive by ELISA. The ELISA titres (visual reading) were either the same as or two to four times higher than the corresponding HI titres. When the results obtained visually by ELISA were compared with those determined spectrophotometrically, in 41 sera (95%) the titres were two eight times higher when the plate reader was used. In addition, three sera which were antibody negative when tested by HI and ELISA (visual reading) were positive (titres 10 - 20) when read objectively. Provided that an appropriate spectrophotometer is available, results may be based on one dilution only and the significance of a given absorbance value assessed against positive and negative reference sera. In addition, the latter can be used to set appropriate confidence limits. In the present study, the absorbance values at 490nm of sera at the 1:320 dilution were compared with ELISA titres and there was good agreement between the two measurements.

This study has shown that RRV IgG-specific antibodies in whole serum can be detected by ELISA. Further studies will be carried out which aim at developing an ELISA for IgM-specific RRV antibodies.

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## EPIDEMIOLOGY

We continue our focus on three mosquito-borne viruses of medical importance in Australia:- Murray Valley encephalitis (MVE), Kunjin (KUN, and Ross River (RRV). Three cases of MVE occurred in 1979 whereas cases of RRV have been prolific throughout Australia and the Pacific.

Our studies are directed towards (1) the vectors (2) vertebrate hosts and (3) the viruses themselves to gain a greater appreciation of how these systems operate and hence, to aid in planning strategies to minimize human infection.

### The Vector

We know that the chief vector of arboviruses in Australia is the freshwater breeding Culex annulirostris. Accordingly, investigations have centred around this mosquito although other species, e.g. the saltmarsh mosquito, Aedes vigilax and some of the temporary ground pool breeding Ae normanensis, Ae lineatopennis and Ae theobaldi would repay further investigation. Culex quinquefasciatus (= fatigans), an introduced domestic breeding species, once the major vector of Bancroftian filariasis in Australia, is currently being evaluated as a vector of MVE and RRV because of previous circumstantial evidence.

A study of regional vector competence of Cx annulirostris and Cx quinquefasciatus nears completion. Samples of Cx annulirostris from Brisbane, Charleville, Cairns, Townsville (Qld), Darwin, (N.T.), Mildura (Vic), and Port Hedland, Karratha and Mt. Tom Price (W.A.) have proved most efficient vectors of MVE with 50% infection achieved after feeding log  $10^{1.9-3.9}$  suckling mouse infective doses per mosquito, total susceptibility and upwards of 50% transmission 10 days after ingestion. This means that most Cx annulirostris are capable of infecting susceptible vertebrates from ingestion of the third and subsequent blood meals. The vector competence of these populations of Cx annulirostris with KUN showed greater heterogeneity in response but nevertheless was reasonably efficient.

By contrast, Cx quinquefasciatus from Brisbane, Charleville, Kowanyama, Cairns (Qld), Darwin (N.T.), Mildura (Vic) were either unsusceptible or poor vectors of MVE, KUN and RRV. In 1978, one MVE case from Port Hedland was thought to be transmitted by Cx quinquefasciatus: accordingly, a colony was raised and evaluated. It proved as poorly susceptible as the other populations tested. Similarly, because of the great abundance of Cx fatigans during the height of the RRV epidemic in Fiji, this species was suspected as a vector. Our Australian data, from six localities, suggests that this is unlikely (Table 1).

A study of host-feeding patterns (in collaboration with Dr P.F.L. Boreham, formerly Imperial College at Silwood Park, U.K.) at Charleville nears completion. The blood meals of 8133 Cx annulirostris have been processed from five major collections, three in summer following the major annual rainfall and two at the end of the dry seasons 1978 and 1979. A further 1961 blood meals were smeared from another 13 species. Data from precipitin test studies at both Kowanyama and Charleville indicate that Cx annulirostris feeds on a wide range of, mainly mammalian, hosts which suggests that many of these vertebrate species could be involved in, particularly MVE, virus transmission cycles.

### The Vertebrate

Orally-infected Cx annulirostris are induced to bite selected vertebrates (chosen on the basis of the host-feeding results) to study the development of viraemia and subsequent serological response. When available, colonized Cx annulirostris are induced to feed off these infected animals when they are thought to be viraemic.

The following species have been infected: piglets (7), lambs (12), chickens (8), domestic rabbits (5), wild rabbits (16), galahs (10) sulphur crested cockatoos (14), black duck (1), grey kangaroos (4) and dogs (11). Most species developed a low titre viraemia lasting 3-4d although viraemia in wild rabbits, as yet untitrated, lasted 6 days and was sufficient to infect 50% of Cx annulirostris that fed on them. In lambs, MVE virus evoked no viraemia and only 5 of 12 developed detectable serum antibodies.

### The Virus

In the insectary at 27<sup>o</sup>, MVE virus was transmitted transovarially to approximately 1.5% of progeny of Ae aegypti. However, the Jim Hardy *et al.* studies of SLE virus have shown that infection of progeny increases considerably when larvae are reared at 18<sup>o</sup>C. Temperatures such as this occur in the Murray Valley in Spring. Our studies of transovarial transmission will be extended to investigate relevant Aedes species at more permissive temperatures.

Other methods for virus survival in temperate zones, e.g., overwintering in the vector or host, continuous transmission in warmer localities, have not been discounted. Whatever the mechanism, there is mounting evidence for survival of these viruses in areas other than tropical ones. This knowledge is crucial to predictive methodology.

(B.H. Kay, J.G. Carley, P.L. Young, P. Mottram, I.D. Fanning, G.J. Barrow)

TABLE 1

VECTOR COMPETENCE OF CULEX QUINQUEFASCIATUS AFTER FEEDING ROSS RIVER VIRUS

LOG DOSE/MOSQUITO	PERCENTAGE INFECTION RATE OF DIFFERENT POPULATIONS					
	BRISBANE	CHARLEVILLE	CAIRNS	KOWANYAMA	MILDURA	DARWIN
5.6	0 (5)*	20 (5)		100 (1)	16 (18)	44 (11)
4.7	0 (32)	0 (44)	0 (25)	0 (33)	3 (61)	0 (31)
3.7	11 (17)	11 (17)		0 (31)	0 (51)	0 (30)
2.7				0 (9)		0 (36)

\* Number tested in parenthesis

## GENETIC INTERACTIONS BETWEEN VIRUSES

In mixed infections of cells with two serologically related orbiviruses, a reassortment of the ten genes of each virus occurs so that new virus particles are assembled which contain genes from each parent. We had established this phenomenon by isolating the recombinant viruses and comparing their genetic material with each parent strain. On electrophoresis in polyacrylamide gels the RNA of the viruses produce a characteristic pattern of separation due mainly to the different sizes of the 10 double-stranded molecules. By comparing the electrophoretic patterns of RNA of viruses and their recombinants, the parental origin of most of the genes could be determined. Some are indistinguishable by this method but digestion of the RNA with ribonuclease T<sub>1</sub> before electrophoresis produces a characteristic fingerprint for each gene. Comparison of the fingerprint of a gene of a recombinant virus with the fingerprints of the genes of the parent virus, allows unambiguous allocation of gene origin.

By a combination of these methods we have established the genotypes of a number of recombinants of two serotypes of the Wallal serological group of orbiviruses (TABLE 2). Work in progress seeks to assign function to certain genes.

Recombination provides a method for the production of a large variety of viruses. We have found that viruses of the Wallal serological group vary greatly in the electrophoretic patterns of their RNA and at least 6 different genotypes have been found from viruses isolated in one area near Charleville in 11 days. In an environment where the distribution of different antigenic types is widespread in insect populations and in vertebrate hosts, genetic recombination provides a mechanism for the production of great genetic diversity within populations of viruses.

### Variation in the Wallal serological group of orbiviruses

Wallal virus was first isolated from a pool of 100 biting midges (*Culicoides dycei*) which were collected in south-west Queensland in 1970. The virus has morphological and biochemical characteristics consistent with its classification as an orbivirus. In the course of our study we isolated plaque clones in tissue culture from the original virus pool passaged in mouse brain. Examination of the RNA isolated from two of these clones showed the characteristic ten discrete pieces but a slight difference in electrophoretic mobility in one RNA gene distinguished the two clones. More detailed analysis was undertaken by fingerprinting RNA segments. By this technique two genes of the Wallal virus clones were distinct.

In the course of verification of a virus isolation, it is usual to reisolate the virus from the original insect pool. This procedure had been followed after isolation of Wallal virus and the identity of the two isolates was considered confirmed by their identity in a serological (complement-fixation) test. However the electrophoretic pattern of separation of RNA from a clone of the reisolated virus was easily distinguishable from either of the two clones obtained from the original isolate.

An investigation of the composition of the original pool of Wallal virus is continuing. Eight well separated plaques from the earliest (third) mouse brain passage have produced six distinct patterns on electrophoresis of the genome RNA. Analysis of plaque clones selected from the fifth mouse brain passage revealed additional RNA patterns. It is possible then that isolates of orbiviruses contain a heterogeneous population with distinct genotypes and that further generation of diversity is obtained by reassortment of genes during passage of the virus pools in mice or in tissue culture.

TABLE 2. Genotypes of ts<sup>+</sup> recombinants derived from mixed infection with ts mutants

Recombinant (ts <sup>+</sup> )	Parental mutants		Derivation of segments									
	Wallal	Mudjinbarry	1	2	3	4	5	6	7	8	9	10
R7	ts101 (group II)	ts3 (group I)	M	W	W	M	W	M	W	W	W	-
R27	ts101	ts3	-	W	W	W	M	W	-	-	-	W
R6	ts101	ts3	M	W	W	W	W	W	W	W	W	W
R28A	ts101	ts3	M	W	W	W	W	W	W	W	W	W
R28C	ts101	ts3	-	W	W	W	W	W	-	-	-	M
R201C	ts30 (group I)	ts5 (group II)	W	M	M	W	M	W	M	W	W	W
R202	ts30	ts5	W	M	W	W	M	W	W	M/W <sup>1</sup>	W	M
R202C <sup>2</sup>	ts30	ts5	-	M	W	W	M	W	-	M	W	M
R208	ts30	ts5	-	M	M	W	M	W	-	-	-	M
R209	ts30	ts5	-	M	M	W	M	W	-	-	-	M
R205	ts30	ts5	-	M	W	W	M	W	-	-	-	W
R206	ts30	ts5	-	M	-	W	M	W	-	-	-	M
R210	ts30	ts5	-	M	M	W	M	M	-	-	-	W

<sup>1</sup> Fingerprint analysis revealed segments from each parent.

<sup>2</sup> Clone derived from R202 virus.



REPORT FROM THE DEPARTMENT OF VIROLOGY  
SCHOOL OF TROPICAL MEDICINE  
CALCUTTA, INDIA.

Studies on inapparent Japanese encephalitis infection in  
West Bengal, India.

A sudden epidemic of Japanese encephalitis (JE) broke out in a number of districts in the state of West Bengal, India during the monsoon of 1973. Thereafter, recurrent epidemics of JE were observed in 1976, 1978 and in 1979. The last two epidemics were widescale in nature involving a large number of districts. These recurrences of JE prompted us to undertake a seroinvestigation in some selected areas comprising both affected and unaffected places in a bid to find out the ratios of apparent to inapparent infection of JE in humans.

Two JE affected villages in Burdwan district and one affected village in Hooghly district, adjacent to Burdwan were selected for this study from where laboratory confirmed cases were reported for the first time. Another village Ajhapur, from where no case was reported was chosen as control.

A limited number of blood samples were collected from both affected and unaffected villages from healthy individuals one month after the last reported case in the affected villages. Blood were collected from individuals from members of 'affected', adjoining houses and also from different corners of the villages. On an average, about 4% of the total population of the villages were bled for seroinvestigation. All the sera were tested for the presence of haemagglutination-inhibiting (HI) antibodies against dengue-2 (DEN-2), JE and West Nile (WN) antigens. All the HI positive JE sera were also tested for the presence of complement-fixing (CF) antibodies against these three flavivirus antigens.

In the present study, sera showing both HI and CF antibodies exclusively to JE or having 4-fold or higher HI and CF antibody titre against JE than DEN-2 or WN antigens were interpreted as JE positives and such persons were considered as inapparently infected.

Table-I depicts the details of seropositivity of individuals in the followings villages.

Table-I

<u>Village</u>	<u>No. of JE overt cases</u>	<u>Household contacts</u>	<u>Others</u>	<u>Total</u>
<u>BURDWAN DISTRICT:</u>				
Panchra	4	10/27* (37%)	53/151(35.1%)	63/178(35.4%)
Adampur	1	2/6 (33.3%)	8/26 (30.8%)	10/32(31.2%)
<u>HOOGHLY DISTRICT:</u>				
Bhupur	4	3/12 (25%)	15/24 (27.7%)	18/66(27%)
<u>Control village:</u>				
Ajhapur (Burdwan district)	-	-	-	19/166(11.4%)

\*Numerator - No. of JE positive sera.  
Denominator - No. of sera tested.

The overall percentage of seropositivity in the respective JE affected villages did not differ among themselves when analysed statistically, but the percentage in the village Ajhapur(control) was significantly lower than any of the three villages even at 1% level of significance( $P < 0.01$ ).

The ratio of apparent : inapparent infection of a particular village was calculated by applying the percentage of sero positivity of that village to its entire population (assuming the rate of infection to be the same as the study group) and then calculating the ratio with the total number of overt cases of that village (Table-II).

Table-II

<u>Village</u>	<u>Population</u>	<u>No. of JE overt cases</u>	<u>% of sero positivity</u>	<u>No. of sero positives for whole population</u>	<u>Ratio Apparent : Inapparent</u>
Panchra	4375	4	35.4	1548	1:387
Adampur	750	1	31.2	234	1:234
Bhupur	1675	4	27.0	454	1:113

The ratios of apparent : inapparent infections in different villages ranged between 1:113 to 1:387 and these differences in the ratios are not significant.

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REPORT FROM THE VIRUS DIAGNOSTIC LABORATORY, SHIMOGA - 577 201 (KARNATAKA, INDIA)

DYNAMICS OF KYASANUR FOREST DISEASE

A notable feature of Kyasanur Forest Disease as observed during the last twenty years is its geographical shifts from one area to another. The shift is generally along the contiguous forests. However, it has occurred in some entirely unconnected areas also, in recent times.

To start with, the disease appeared in Sagar taluk of Shimoga district in 1957, and the virus was active in this taluk as well as the contiguous Sorab taluk, covering an area of about 250 square miles, for about 15 years. In 1972, it made its appearance in Hosanagar taluk which is also contiguous with Sagar taluk. Since then, there is a steady south-ward spread of the disease involving new areas of this taluk every season, so much so that, almost the entire taluk covering 1400 square miles is now involved. In 1974, two new foci towards the west, one in Sirsi taluk and another in Honavar taluk of North Kanara district, appeared. These areas, especially the latter, are well away from the original spots of virus activity. In fact, Honavar focus is distinctly separate with about 70 kms. of forest stretch in between, without any apparent virus activity. The infection persisted these two foci till 1976 and disappeared suddenly. However, it has made its appearance again in Honavar focus in 1980 with severe human involvement, after a silence of nearly four years.

In the meantime, the disease spread to the contiguous Thirthahalli taluk of Shimoga district in 1975 and since then it is slowly but relentlessly making its march in this taluk involving new areas every season.

The infection disappeared in Sorab taluk in 1975 and in Sagar taluk in 1978 and since then these areas of intense viral activity, have become very quiet. No reports of either monkey or human involvement have come from these villages, in spite of continued and effective surveillance. A recent study undertaken in these areas to find out the persistence of humoral immunity in decade old human KFD positive cases has revealed high titres of neutralising antibodies in these cases while control subjects of same age groups from the same area without history of infection, showed no neutralising activity at all, indicating the possible absence of the virus in this area. Several tick surveys conducted in these forests also showed no evidence of virus activity.

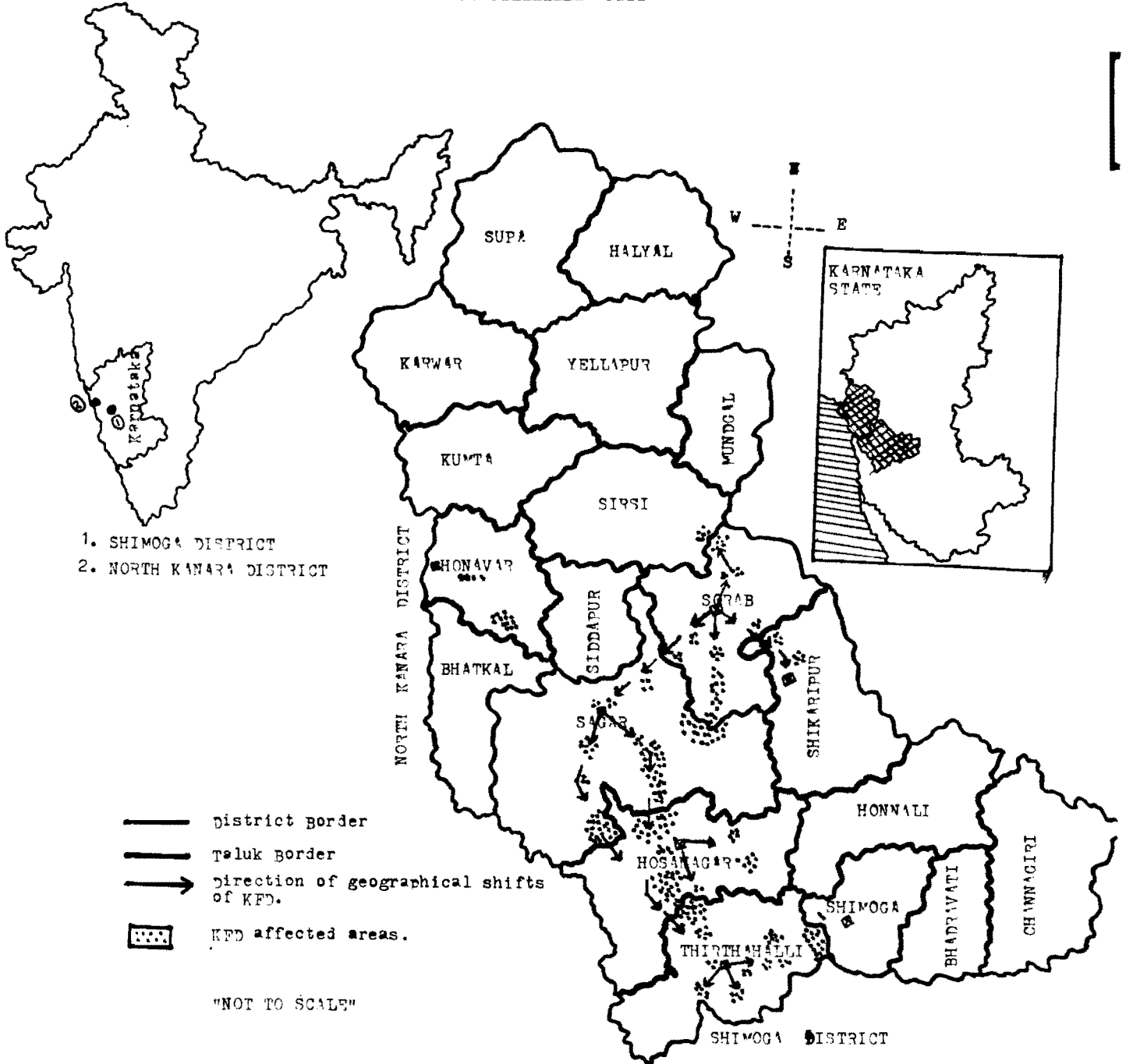
From the above, it could be assumed that KFD virus is migratory in nature. The migration occurs along the contiguous forests depending on the availability of its natural hosts which are the small mammals in the forests. The factors influencing the migration might be either exhaustion of susceptible mammalian hosts and/or immunity in the host population.

Further studies, both virological and epidemiological, are necessary to understand and evaluate the various factors involved in the migration of KFD virus.

(T. R. Achar, Deputy Director, Virus Diagnostic Laboratory)

MAP SHOWING THE GEOGRAPHICAL SHIFTS OF KYASANUR FOREST DISEASE  
IN SHIMOGA AND NORTH KANARA DISTRICTS  
OF KARNATAKA STATE

INDIA



REPORT FROM THE DEPARTMENT OF MEDICAL SCIENCES, BANGKOK, THAILAND AND WHO,  
SEARO, NEW DELHI, INDIA

A Prospective Study on Dengue Haemorrhagic Fever in Thailand:  
The Rayong Study. A First Report

A WHO-sponsored longitudinal study on DHF/DSS was begun in Thailand in January 1980. The purpose of the study is to measure transmission of dengue viruses in a defined population and relate these data to the occurrence in this population of carefully characterized cases of DHF/DSS. The study will be extended for five years so as to determine whether or not certain patterns of virus transmission are always associated with DHF/DSS. This study will also provide an opportunity to relate dengue infection to parameters of vector density and to collect dengue virus strains from infected human beings. The basic goals and design of this epidemiological study conform with the recommendations of the WHO Research Study Grant on DHF Epidemiology 26-29 March 1979 (SEA/RES/17, SEA/Haem. Fever/32).

Initially, it had been hoped to locate the study in Bangkok. A review of the situation revealed that there were more than 40 government and private hospitals as well as numerous private clinics which admit and treat DHF/DSS cases. This would have made it impossible to collect numerator data in any study catchment area.

Accordingly, Rayong was selected as site of the study. Rayong, a city of 40,896 persons (municipality) is in a district with a population of 90,170 and a province with 330,000 persons. The city is 230 kilometers southeast of Bangkok and is served by a single 500-bed provincial hospital.

Rayong is highly endemic for DHF/DSS as the following hospitalization statistics document:

Haemorrhagic fever cases and deaths, Rayong, 1970-79.

<u>Year</u>	<u>Cases</u>	<u>Deaths</u>	<u>Year</u>	<u>Cases</u>	<u>Deaths</u>
1970	5	-	1975	212	3
1971	298	-	1976	40	1
1972	533	8	1977	285	5
1973	277	2	1978	158	-
1974	57	1	1979	294	2

The municipality and surrounding suburban villages are designated as the catchment area which has an estimated population of 40,000. There are seven government and private primary schools in the municipality and thirteen additional primary schools in contiguous communities. Approximately 45% of the population of Rayong Province is 15 years or younger. It can be estimated that there are approximately 1000 children in each age group from 1-15 in the catchment area.

The study consists of the following elements:

I. Clinical-Virological Study

All patients admitted to Rayong Hospital with the diagnosis of haemorrhagic fever will be included in the study. The clinical features of all illnesses will be recorded on standardized protocol sheets. Blood samples will be obtained for virus isolation and serological diagnosis. In acute phase blood samples serum will be separated by centrifugation, transferred to sterile plastic vials, labeled and stored in liquid nitrogen.

Two liquid nitrogen containers will be rotated to and from Bangkok every 2-3 weeks. Virus isolation attempts will be made in several recovery systems.

## II. Serological Study

a. Age stratified survey. In January and February, 2,750 children, ages 4 - 14 were bled in primary schools. In addition, 421 infants and pre-school children were bled. Bleedings were from the finger-tip. Two Nabuko's filter papers were filled for each subject. Duplicate filter papers have been divided between the Virus Research Institute, Department of Medical Sciences and the Department of Tropical Medicine of the University of Hawaii.

Blood in filter papers will be eluted and treated with a sterile kaolin suspension and then tested for dengue HI antibodies using two cross-reactive dengue antigens. The remainder will be saved for neutralization testing in the future.

b. Cohort study. Children in kindergarten and first grade will comprise the cohort study. These children will be rebled in January 1981. Sera from those children who convert from no antibody in 1980 to detectable HI antibody in 1981 will be studied for specific neutralizing antibody using a micro-neutralization test. A technician from the Virus Research Institute will receive special training to learn the micro-neutralization method in Hawaii. The samples collected during the first year will be tested during the training period. Neutralization tests will make it possible to identify which dengue virus types infected children in the catchment area during the rainy season and to establish a rate of infection for each virus type. From these and age stratified serologic data it will



be possible to estimate total primary and secondary dengue infections which occurred in 1980 and relate these to primary and secondary infection DHF/DSS cases.

### III. Entomological Study

Careful measurements of vector density will be made in the study area. Since this is a mosquito abatement district, no attempt will be made to interfere with mosquito abatement programs during the course of the prospective study.

(Nadhirat Sangkawibha, Scott B. Halstead)

REPORT FROM THE DEPARTMENT OF PREVENTIVE MEDICINE  
 RESEARCH INSTITUTE FOR MICROBIAL DISEASES  
 OSAKA UNIVERSITY  
 YAMADA-KAMI, SUITA, OSAKA, JAPAN

A case report of primary dengue infection occurred in laboratory

On May 2, 1980, one of the authors of this report happened to prick his finger slightly with a syringe-needle containing dengue virus. At night on May 8, he developed symptoms of malaise and fever (38.0 C). Blood samples were collected from May 9 and the sera were examined for viremia and neutralizing (N) antibodies against dengue viruses. The infecting virus was a clone (C15/124:SA-9), which had been plaque isolated from strain 124:SA-9. The strain 124:SA-9 had been originally isolated from a Thai DHF patient in 1978, using a clone (C6/36) of Singh's *Aedes albopictus* cells and identified as dengue type 4 by CF tests.

By the infectivity assay of the sera with BHK21 cells employing the peroxidase-anti-peroxidase (PAP) staining method, viremia was detected on the 1st, 2nd and 3rd days of illness at titers of  $4.3 \times 10^3$ ,  $5.3 \times 10^5$  and  $3.0 \times 10^2$  FFU/ml, respectively. On the other hand, the C6/36 cells inoculated with 10-fold diluted serum of May 9 began to show CPE on the 3rd day after inoculation and developed more apparent CPE on the 4th day, but on the 5th day the CPE was not expanding in the culture and then the culture fluid was harvested and stored at -70 C. The infectivity titer of the isolate (SI-Y.O:SA-1) was found to be  $5.7 \times 10^7$  FFU/ml in BHK21 cell-PAP staining system.

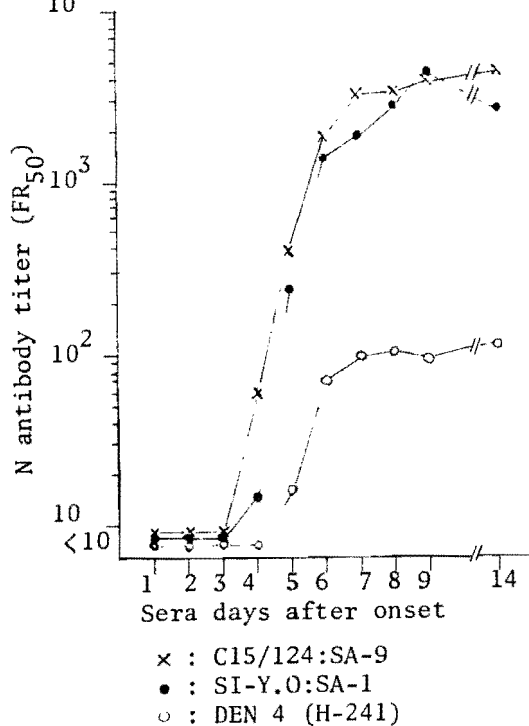
As shown in the figure, the serum specimens of the patient gave very similar and rather high N antibody titers against both of the infecting and isolated viruses, but the titer against the prototype of DEN 4 virus appeared to be much lower. Moreover, as shown in the table, the sera demonstrated N antibody titers against other 3 prototypes (DEN 1, 2 and 3) at levels similar to that against DEN 4.

Table. N antibody titers against dengue prototype viruses

	Sera (Days after onset)			
	2	7	14	30
DEN 1	20	80	330	320
DEN 2	20	110	220	230
DEN 3	20	70	80	70
DEN 4	20	100	110	ND

Toshihiko Fukunaga, Yshinobu Okuno  
 and Konosuke Fukai

Fig. N antibody titer after  $10^4$  onset of illness



REPORT FROM DEPARTMENT OF ARBOVIRUSES, OF THE  
IVANOVSKY INSTITUTE OF VIROLOGY, ACAD. MED.  
SCI. USSR, MOSCOW

Isfahan virus in Turkmenia (USSR)

Isfahan virus is a new representative of Rhabdoviridae family, isolated from *Phlebotomus papatasi* by R.Tesh et al./in 1975/ in Iran.

In our previous study antibody to Isfahan virus were found in residents of Turkmenia, Tajikistan and Uzbekistan, predominantly in Turkmenia (1). Investigation of 174 human blood samples, collected in rural area of the Kirovsky district, Turkmenia, by plaque-reduction neutralization, indirect immunofluorescence and passive hemagglutination inhibition revealed antibody to Isfahan virus in 70 per cent of the sera (2). The results of the three tests coincided. Antibody were found both in adolescents and adults with slight prevalence in adults. Neutralizing antibody to Isfahan virus were detected in the sera of wild rodents caught in the same area: in 7 from the 11 gerbils *Rhombomys opimus* Licht, and in 1 from 7 red-tailed gerbils *Meriones libicus* (3). These results suggest that Isfahan virus is ecologically linked with both rodents.

The data of serological survey evidenced the existence of natural foci of Isfahan virus in the area under study; attempts of virus isolation from sandflies were undertaken.

In June and August, 1979, several hundreds of *Phlebotomus papatasi* were collected by aspiration from the burrows of gerbils. For virus isolation newborn white mice were used. Two strains were isolated: T-200 from the pool of 100 engorged females, caught on June 6, and T-227 from the pool of 95 engorged females, collected on August 20-24. Both strains were isolated after the first inoculation of mice. The virus was successfully reisolated 3-4 months later from the same suspension of sandflies kept at  $-70^{\circ}\text{C}$ .

According to the data cross neutralization and complement fixation tests between T-200, T-227 strains and the Iranian strain 91026-167 of Isfahan virus (strain 91026-167 was kindly provided by dr.Tesh), the isolates were referred to Isfahan virus: The Isfahan virus is isolated in the USSR for the first time (4).

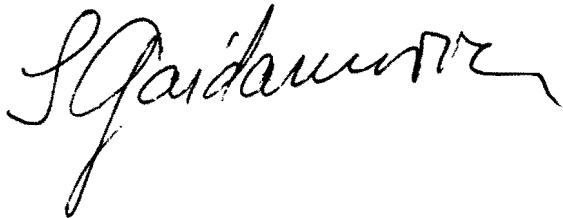
In the course of the study it was shown that newborn mice can be used for isolation of Isfahan virus instead of Vero cells, used by R.Tesh. Besides the routine neutralization test, indirect immunofluorescence and passive hemagglutination inhibition were used for the detection of antibody.

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S.Ya.Gaidamovich, V.R.Obukhova, L.M.Alkhutova

A handwritten signature in black ink, appearing to read 'S. Gaidamovich', with a stylized flourish at the end.

REPORT OF THE LABORATORY OF ECOLOGY OF VIRUSES, THE D.I. IVANOVSKY INSTITUTE  
OF VIROLOGY, THE USSR ACADEMY OF MEDICAL SCIENCES, MOSCOW

Paramushir virus, Sakhalin antigenic group, Bunyaviridae family

Paramushir virus, isolated in the Far East, USSR, in 1969-1974 (1,2), had been described earlier as an independent virus of the Bunyaviridae family (3,4). As a result of serological studies by the complement fixation test (CFT) and the biological neutralisation test (NT) with newborn white mice, we determined antigenic relationships between Paramushir virus and viruses of the Sakhalin group (Table 1). As an antigen in the CFT we used fluid culture medium of Vero cells 48 hours after infection, concentrated by polyethylene-glycol (mol. wt. 6000) and then worked up three times by ultrasonics. In the NT we used the immune ascitic fluid to the strain of Sakhalin virus obtained in the immunisation of white rats (5).

By CFT, the reaction of diffusion precipitation in agar and NT with newborn mice, the identity of Paramushir virus and Avalon virus isolated in Newfoundland, Canada, in 1971-1972 (6) (Table 2) was shown.

Thus, Paramushir and Avalon viruses we can consider as strains of the same virus, isolated in different continents. Since the first publication concerning Paramushir virus dates from 1975, and of Avalon virus from 1976, we suggest the name "Paramushir" be kept for this virus.

(D.K. Lvov, N.G. Kondrashina, L.K. Berezina, T.M. Skvortsova, V.L. Gromachevsky)

Table 1

ANTIGENIC BONDS BETWEEN PARAMUSHIR VIRUS AND VIRUSES OF  
SAKHALIN GROUP

S e r a	Viruses	CFT	NT	
		Paramushir LEIV-2268-C	Sakhalin LEIV-2004-Ka	Paramushir LEIV-2268-C
Paramushir LEIV-2268-C		1/1024	3,1 <sup>x)</sup>	5,5
Sakhalin LEIV-2004-Ka		1/16	3,3	2,9
Tagert		1/8		

x) index of neutralization (in lg)

Table 2

IDENTIFICATION OF PARAMUSHIR AND AVALON VIRUSES IN  
SEROLOGICAL REACTIONS

S e r a	Viruses	CFT	NT	RDFA		
		Avalon LEIV-2268C	Paramushir LEIV-2268C	Avalon Paramushir LEIV-2268 C		
Avalon	320 <sup>xx)</sup> /64 <sup>x)</sup>	320/64	2,0 <sup>xxx)</sup>	3,0	8 <sup>x)</sup>	4
Paramushir LEIV-2268 C	320/256	320/512	2,0	3,0	32	32

x) the meaning of antibodies titre (I.A.F)

xx) the meaning of antigen titre

xxx) index of neutralization (lg)

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REPORT FROM THE INSTITUTE OF PARASITOLOGY,  
CZECHOSLOVAK ACADEMY OF SCIENCES, PRAGUE,  
CZECHOSLOVAKIA

Sex-related and dose-related immune response of mice to the  
Tahyna virus (California group, Bunyaviridae) infection

Six-week-old SPF albino mice NMRI/ICR were inoculated intraperitoneally with Tahyna (TAH) virus strain T16 (cf. Bárdoš et al. 1975: Acta virol. 19, 447) in its 3rd suckling mouse brain passage. Two doses were used: 125 SMICLD50 (d) and 12 500 SMICLD50 (D), and each of both was inoculated (0.2 ml) into 22 males and 22 females (average wt 29.6 g and 24.1 g, respectively). In addition, 4 males and 4 females were inoculated by 0.2 ml of the diluent without virus (control group). The mice were bled 14 days p.i., the sera maintained at -20 C, and inactivated at 56 C for 30 min before use. Neutralizing (Nt) antibodies were detected by plaque-reduction neutralization microtest on XTC-2 cells at 28 C (Hubálek et al. 1979: J. gen. Virol. 42, 357), and the titres expressed as 50% plaque-reduction values. Each serum was examined twice; the variability of the titres did not exceed two- to threefold level, except for 2 sera (out of 88 examined) where the titre was changed by fourfold.

The experimental infection was asymptomatic in majority of mice, but marked symptoms of CNS disorders appeared in 3 males and one female, inoculated with D dose; 3 of those animals died on day 10 p.i., and in their brains high titres (more than 5 log SMICLD50 per ml) of TAH virus were found.

Specific Nt antibodies were detected either in low titres (1:32, or less: "weak reactors") or in high titres (1:128, or more: "strong reactors") in all experimental animals (except for the control group). Interestingly, no "intermediate" reactors (with titres between 1:32 and 1:128) appeared.

Variant	♂ <u>d</u>	♀ <u>d</u>	♂ <u>D</u>	♀ <u>D</u>	♂	♀	<u>d</u>	<u>D</u>
No. inoculated	22	22	22	22	44	44	44	44
No. "strong react."	10	7	22	18	32	25	17	40
% " " "	45.5	31.8	100	81.8	72.7	56.8	38.6	90.9
log <sub>2</sub> GMT ("strong")	8.50	9.07	8.32	8.67	8.37	8.78	8.73	8.47
t-value	2.19 <sup>+</sup> )		1.65		2.40 <sup>+</sup> )		1.37	

<sup>+</sup>) the difference is significant at P < 0.05.

The males reacted in a higher proportion than the females by the "strong" production of Nt antibodies to the infection with both d and D doses of TAH virus. However, the "strongly" responding females had higher mean titres of Nt antibodies than the "strongly" reacting males. The dose d, irrespective of sex, induced the strong immune response in 38.6% of the animals, in contrast to the dose D (90.6% strong reactors); this difference is significant at P < 0.01. On the other hand, there was no significant difference between the strong reactors inoculated with the dose d and D in the mean titre.

(Z. Hubálek)



REPORT FROM WHO COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE  
AND RESEARCH, INSTITUTE OF VIROLOGY, BRATISLAVA, CZECHOSLOVAKIA

In vitro cultivation studies with Skalica strain of tick-borne  
encephalitis (TBE) virus.

Organ cultures may be considered to be more similar to the organism of animals than are tissue cultures. Therefore we used mouse organ cultures to study the multiplication of Skalica strain of TBE virus. This strain was found to be a spontaneous variant of TBE virus with lowered virulence for juvenile white mice after subcutaneous inoculation (Grešíková and Sekeyová, 1980).

Also antibody production was studied in mice infected with Skalica strain using the explantation method.

Organ cultures have been prepared from the spleen, from the liver, from the inguinal lymph nodes, from the brain, from the spinal cord and from the muscles of juvenile mice. The tissues were dissected into pieces 1 - 2 mm<sup>3</sup> in size, washed with Parker's 199 medium, and then 6 - 9 pieces were put on plastic grids in Petri dishes containing Parker's 199 with 10 % fetal calf serum and antibiotics. The organ cultures were incubated at 37 °C in CO<sub>2</sub> atmosphere for 18 hours.

Organ cultures of juvenile white mice were inoculated with Skalica strain of tick-borne encephalitis virus (10<sup>5.5</sup> ic mice LD<sub>50</sub> per 0.01 ml). In the course of culturing, cell

proliferation was seen at the edge of explants from lymph nodes, spleen, liver, brain, spinal cord, and muscles. It was evident that Skalica strain of tick-borne encephalitis virus multiplied in organ cultures of lymph nodes, spleen, liver, brain, spinal cord, and muscles (Tab. 1). The maximum titre of 8.1 - 8.3 LD<sub>50</sub>/0.01 ml was obtained in the spleen, liver, brain, and spinal cord cultures from the 6th to 9th days after virus inoculation.

Interferon could not be detected in organ cultures media.

Haemagglutinin was not detected in organ culture media from the second up to 15 days after cultivation. Nor was complement-fixing antigen found in the fluid phase of explants from the 3rd up to 15 days in culture.

To study antibody production, white mice were inoculated with Skalica strain of TBE virus; organ cultures were prepared on 3rd, 6th, 9th, 12th and 15th days after inoculation of white mice.

HI antibodies were detected in the fluid phase of lymph nodes, spleen, and liver organ cultures of mice, 6 days p.i. and 15 days after seeding; the results of mercaptoethanol treatment indicated that the antibodies were IgM (Table 2). In organ cultures of lymph nodes, 9 days p.i. of mice, IgM antibodies of low titre were detected.

In organ cultures prepared 3, 6, 9 and 12 days p.i. of white mice the production of CF antibodies was practically

nil. 14 days p.i. of white mice, the fluid phase of organ cultures from the lymph nodes, spleen, lungs, liver, brain and spinal cord contained CF antibodies, 15 after seeding (Table 3).

In the present work it was shown that Skalica strain of TBE virus had a considerably wide affinity to mouse organ cultures. In spite of this finding, interferon, haemagglutinin, and complement-fixing antigen were not detected in the fluid phase of organ cultures.

The present studies demonstrated that organ cultures of lymph nodes, spleen, liver, lungs, brain and spinal cord of white mice inoculated 14 days prior cultivation in vitro with Skalica strain, produced complement-fixing antibodies on 15 days after seeding.

(Grešíková, M., and Sekeyová, M.: Acta virologica, In press)

Table 1

Multiplication of Skalica strain of TBE virus in mouse organ cultures.

Organ culture	Amount of virus detected in fluid phase postinfection (in days)						
	1	2	3	6	9	12	15
Spleen	2.5	3.0	4.3	8.1	4.8	2.8	2.5
Liver	2.5	2.8	4.3	8.1	8.3	3.6	3.1
Lymph nodes	2.5	3.5	4.1	4.2	3.4	2.5	2.5
Brain	2.5	4.1	4.8	8.1	8.3	3.5	3.0
Spinal cord	2.3	3.5	4.3	8.1	8.3	3.5	3.0
Muscle	2.5	3.0	3.6	6.5	5.8	5.1	4.1

Table 2

Detection of HI antibodies in organ cultures of mice infected with Skalica strain of tick-borne encephalitis virus (6 days p.i.)

Organ culture media	HI titre in fluid phase cultivation (in days)											
	3		6		9		12		15		15 <sup>x</sup>	
	A	ME	A	ME	A	ME	A	ME	A	ME	A	ME
Lymph nodes	0	0	0	0	0	0	0	0	2	0	2	0
Spleen	0	0	0	0	0	0	0	0	2	0	0	0
Liver	0	0	0	0	0	0	0	0	2	0	2	0
Lungs	0	0	0	0	0	0	2	0	0	0	2	0
Brain	0	0	0	0	0	0	0	0	0	0	0	0
Spinal cord	0	0	0	0	0	0	0	0	0	0	2	0

A = Sera treated by acetone

ME = Sera treated by mercaptoethanol and then by acetone

x = Suspension of organ cultures

Table 3

Detection of complement-fixing (CF) antibodies in organ cultures of mice infected with Skalica strain of tick-borne encephalitis virus (14 days p.i.)

Organ culture media of	CF titre in fluid phase after cultivation (in days)				
	3	6	9	12	15
Lymph nodes	0	0	0	0	16/4 <sup>x</sup>
Spleen	0	0	0	0	16/4
Liver	0	0	0	0	8/4
Lungs	0	0	0	0	8/4
Brain	0	0	0	0	32/4
Spinal cord	0	0	0	0	8/4

x = Titre of sera/titre of antigen

Infection and transmission of tick-borne encephalitis  
/TE/ virus with Haemaphysalis concinna tick

*H. concinna* tick is widely distributed species in the forests of temperate Eurasia.

*H. concinna* ticks originated from laboratory bred. The first bred has been derived from individuals collected in the locality P. Biskupice in Danube region, the second bred from individuals collected in Primorsky region.

The larvae were infected by feeding on viraemic 4-6 days old suckling white mice. The mice were infected with 10 % brain suspension of TE virus /strain 204/, isolated from *I. ricinus* tick,  $M_8$  passage/ diluted  $10^{-2}$  i.p.  $LD_{50}$  per 0.03 ml. The titre of virus in 10 % brain suspension reached  $10^{8.2}$  mouse i.c.  $LD_{50}$ . The suspensions from engorged larvae and nymphs were prepared individually in 1 ml of basal Eagle medium with 10 % inactivated cattle serum. Suckling mice 1-3 days old were used for isolations and titrations of positive samples. The transmission of TE virus by viruliferous nymphs of *H. concinna* to the white mice weighing 8-10 g was carried out in translucent feeding capsulae, each capsula contained 3 nymphs.

Six engorged larvae from each group were examined. All larvae contain virus in titres  $10^1 - 10^2$  mouse i.c.  $LD_{50}/0.01$  ml. Virophoric period in nymphs from the first bred lasted 56 days or in nymphs from the second bred of Dr. Churnikhin 97 days, respectively. *H. concinna* nymphs originated from Danubian swampy woods transmitted the TE virus to the adult white mouse. Similarly, the nymphs of *H. concinna* originated from Primorsky region transmitted this virus to the

adult white mouse. The mice showed clinical symptoms of illness and died on the 7<sup>th</sup> or 8<sup>th</sup> respectively. Infection rate in nymphs ranged 10%-20%, transmission rate amounted 12 %. The titre of virus in individually examined engorged nymphs ranged from  $10^1$  -  $10^3$  mouse i.c. LD<sub>50</sub>/0.01 ml.

Our previous studies on experimental infection and transmission of TE virus by viruliferous nymphs of *H. concinna* originated from Central Europe as well as from the Far East showed that *H. concinna* may be vector in wide area of its distribution. The transmission rate in both cases is equal. The relatively low transmission rate may be caused by age of laboratory mice.

*H. concinna* may be considered as complementary vector in the natural foci of TE.

/Kožuch, O., Nosek, J., Acta virol. in press/.

REPORT FROM THE NATIONAL INSTITUTE OF HYGIENE  
BUDAPEST, HUNGARY

Two arbovirus strains isolated in the Western part of Hungary in 1972 proved to belong to the CHF-CON group based on results of most recently performed identification experiments.

Virus isolation was carried out by intracerebral inoculation of suckling mice with homogenized groups of *Ixodes ricinus* L. nymphs, - identification by CF and AGP tests. Both virus strains proved identical with CHF-CON prototype strains Hodja and Congo.

In the same area 7 of 117 healthy forestry workers' blood samples proved to contain precipitating antibodies against CHF-CON prototype strain.

Antibody against CHF-CON prototype strain was also found elsewhere in blood samples of men, cows and sheep.

No clinical cases have, however, been detected, so far.

/E.Molnár, S.Horváth, M.Grešiková, T.K.Dzagurova, M.P.Chumakov/

STRUCTURAL AND NON-STRUCTURAL PROTEINS OF SINDBIS VIRUS

Purified Sindbis virus subjected to discontinuous Tris-SDS polyacrylamide gel electrophoresis is shown to consist of three structural proteins: E1, E2 and C. The pattern of slab gel electrophoresis of infected chicken embryo fibroblast extracts, labeled by a short pulse with amino acids, is qualitatively identical with respect to the time after infection but depends upon the length of the labeling period.

After a very short labeling (3 min) only C and, to a lesser extent, E1 proteins are present; after longer labelings (10 min, 15 min, 20 min) E2 protein does not yet appear, while a non-structural virus-specific protein (PE2), larger than E1, is now heavily labeled. Short pulse-experiments, followed by a long chase period, indicate that PE2 protein, thought to be a precursor to E2 protein, is not cleaved to the latter but rises during the chase interval.

Two additional non-structural proteins (NS1, NS2), larger than PE2, tentatively identified as virus-specific by comparison with uninfected cells and by using Actinomycin D as inhibitor of host protein synthesis, are also found. These proteins could undergo a cleavage to PE2 because they disappear, while PE2 rises during the chase period.



These findings suggest that C and E1 proteins are cleaved from a very instable precursor or from a growing polypeptide chain, or that they are synthesized by a translation from distinct messenger RNAs; PE2 could arise from larger polypeptide(s). The cleavage of PE2 to E2 may coincide with the complete budding of the virions and thus it seems to take place topologically outside of the infected cells.

A. SANNA, C. CHEZZI, F. LEONE, F. ARDITO, G. DETTORI, and A. NACCI

REPORT FROM THE VIRUS LABORATORY, FACULTY OF MEDICINE,  
BREST, FRANCE.

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*STUDIES ON ARBOVIRUSES ASSOCIATED WITH TICKS AND MARINE BIRDS ALONG  
THE COASTS OF FRANCE AND MOROCCO.*

Since 1977, a number of ornithological reserves located on the coasts of Brittany, France, and on Essaouira island near Essaouira (Mogador), Morocco, were investigated for tick-borne arboviruses associated with marine birds. At time, we isolated 23 strains of viruses belonging to the HUG, UUK and KEM groups of arboviruses.

*I. ISOLATION AND CHARACTERIZATION OF VIRUS STRAINS ISOLATED FROM COASTS OF FRANCE.*

Soldado and Soldado-like viruses were isolated repeatedly from Ornithodoros (A.) maritimus ticks caught in the nests of herring gulls (Larus argentatus) and shags (Phalacrocorax aristotelis) on Cap Fréhel, Côtes-du-Nord : more strains were obtained from female than male ticks, and from males than from nymphs.

One strain of Soldado-like virus (Brest/Ar/T 101), isolated in June 1978, appeared quite different of typical strains of Soldado virus by CF and ID tests ; it was sent to the Y.A.R.U. The results obtained by Dr J. Casals showed that this strain was closer by CF tests to Soldado than to the other viruses of the HUG group. There was, however, a definite 4-fold difference both ways between the homologous and heterologous serum titers. The results of NT tests, using IC route of inoculation to 1-day-old mice, were unfortunately not satisfactory due to the low titers of the viruses. In view of these results, Dr J. Casals thinks Brest/Ar/T 101 can be considered a serological subtype of Soldado virus.

In July 1979, from ornithological reserve of Cap Sizun, Finistère (Fig. 1), three strains of viruses were isolated :

- strain Brest/Ar/T 247, from O. (A.) maritimus, associated with herring gulls and belonging to the HUG group ;
- strain Brest/Ar/T 260, from Ixodes uriae, associated with kitiwakes (Rissa tridactyla) and belonging to the UUK group ;
- strain Brest/Ar/T 261, also from I. uriae, not yet classified and poorly adapted to suckling mice.

Interesting enough is the fact that two different specialized ticks can occupy the same ecological niche on Cap Sizun.

In order to study, the possible effects of these viral infections on marine birds, other wild animals and humans, we collected again in 1980 many specimens of O. (A.) maritimus and I. uriae, for virus assays. New geographical localizations of O. (A.) maritimus were found along the coasts of Brittany. In addition we take sera from birds, small mammals and humans for serosurveys. Studies with this material are in progress.

## II. ISOLATION AND CHARACTERIZATION OF VIRUS STRAINS ISOLATED FROM ESSAOUIRA ISLAND, MOROCCO.

Three strains of viruses were isolated from 296 O. (A.) maritimus ticks collected in June 1979 on Essaouira island, off Essaouira (Mogador), Morocco (Fig. 2). The ticks parasited a colony of Larus argentatus michahellis, a mediterranean sub-species of the herring gull.

One of these strains is a HUG group virus, probably a Soldado-like virus, and the two others appear similar and are members of the Chenuda (CNU) complex of KEM group. These strains were sent to the Y.A.R.U. for further identification : A.J. Main Jr found one of the strain belonging to the CNU complex was closest to Chenuda and Baku viruses, by CF tests. Complete identification of the moroccan strains is in progress in the Y.A.R.U.

- 
- Dr C. Chastel and G. Roguès, Virus Laboratory, Faculty of Medicine, Brest, France.
  - H. Bailly-Choumara, Entomologist, "Office Scientifique", Rabat, Morocco.
  - J.Y. Monnat, Zoology, Faculty of Sciences, Brest, France.
  - Drs J.C. Beaucournu and C. Guigen, H. Launay, Medical Entomology, Faculty of Medicine, Rennes, France.

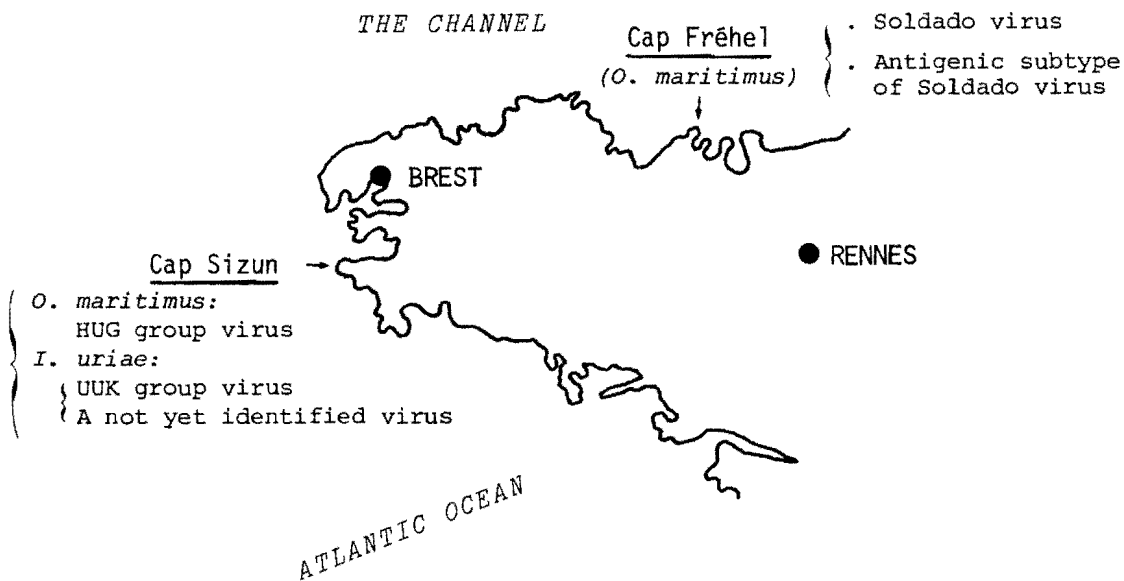


FIG. 1. MAP OF BRITTANY

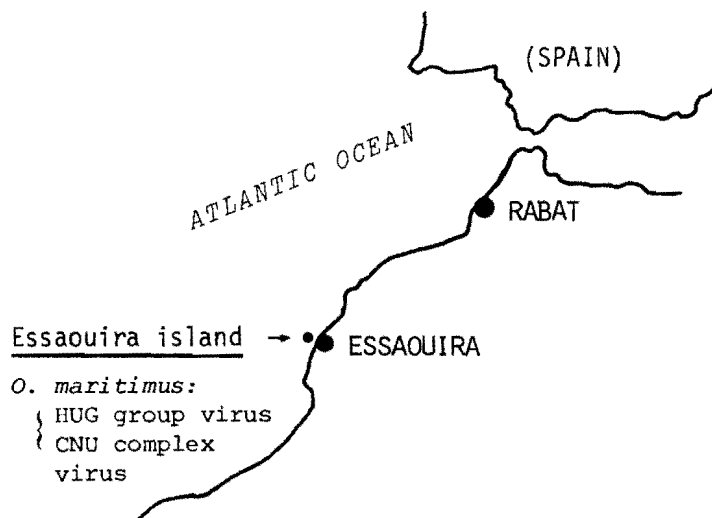


FIG. 2. MAP OF MORROCO

REPORT FROM THE UGANDA VIRUS RESEARCH INSTITUTE, ENTEBBE, UGANDA

1. Isolation of a Flavivirus from Human Blood in Uganda:

An isolate (SG 40284) of flavivirus was obtained from one of 160 human blood samples screened for virus isolation in 1979. It was acquired from a male patient aged 19 years. He came from Buvvi village and visited the Institute Clinic on the 12th February 1979 with complaints of fever, headache, muscle and joint pains. On examination, he was found to have an elevated temperature. A sample of his venous blood was therefore taken. It was inoculated into newborn mice for virus isolation.

On day 13 post-inoculation three of the inoculated mice were found sick, one of them with marked paralysis in the hind legs. Brain tissues from these mice were passaged into other groups of suckling mice. These became sick 7 days later. Brains from them were subsequently passaged into suckling and adult mice by IC and IP routes to study the agent's biological properties in mice. At the 3rd passage the agent had a good titre of  $\geq 8.5$  logs in baby mice inoculated by IC route. None of the inoculated adults mice displayed signs of disease. This virus was reisolated from the original blood sample.

This virus has been found to be sensitive to ether treatment. Other studies are continuing.

For identification, HI and CF tests have related this isolate to group B viruses. Lack of antigens of some flaviviruses have delayed identification of this isolate. Plans are underway to send it to Yale Arbovirus Research Unit for identification.

2. Transmission of "Kasokero" Virus by Ilobi Strain of *Aedes aegypti* Mosquitoes:

A virus tentatively called Kasokero virus was isolated from blood of a female fruit bat (*Rousettus aegypticus*) caught in 1977 from Kasokero cave in Masaka District, Uganda. Serological tests conducted in this Institute and at Yale Arbovirus Research Unit showed that this virus is not related to any of over 400 registered and unregistered arboviruses with the exception of a distant serological relationship with Yogue virus. A name Kasokero virus has been proposed for this virus.

Seventy-two newly hatched laboratory reared mosquitoes of the Ilobi strain of Aedes aegypti were starved for two days. They were then allowed to feed for  $3\frac{1}{2}$  hours on three viraemic mice which had been inoculated with Kasokero virus. These mice were later killed and their blood was pooled, titrated and then inoculated into suckling mice to determine the virus content used to infect mosquitoes.

Soon after feeding and then every two days for 18 days, three of the fed mosquitoes were each time triturated and suspensions were prepared. These were titrated in PBS containing 0.75% of bovine plasma albumin and then inoculated IC into newborn mice to find out whether Kasokero virus propagates in Ae. aegypti. Similarly, every two days the fed mosquitoes were allowed to feed on 10 one-day old mice to determine whether this virus could be transmitted to these mice by bite of Ae. aegypti. Bitten mice were examined for 21 days.

The results showed that virus titre of pooled mice blood was  $\geq 8.5$  log/0.02 ml after mosquito feeding indicating sufficient virus to infect mosquitoes. The virus concentration in 3 engorged mosquitoes was 7.1 log/0.02 ml soon after feeding. This amount was reduced to 1.5 log on day 2 and disappeared thereafter.

None of the 90 mice bitten by "infected" mosquitoes between day 2 and day 18 became infected with Kasokero virus.

The preliminary results from this experiment show that this virus did not propagate in Aedes aegypti mosquitoes and no virus was transmitted to the baby mice by the fed mosquitoes. This study continues with other species of mosquitoes.

(M. Kalunda, L.G. Mukwaya and M. Lule)

REPORT FROM THE ARBOVIRUS LABORATORY  
INSTITUT PASTEUR AND ORSTOM BANGUI  
CENTRAL AFRICAN REPUBLIC

Isolation of yellow fever virus from eggs and larvae of a tick *Amblyomma variegatum*.

From January 1977 to December 1978, 495 monospecific pools of eggs/ticks<sup>from</sup> collected from cattle at the slaughterhouses in Bangui were inoculated into suckling mice. One strain of yellow fever virus (WTB 1927) was isolated from eggs of a tick *Amblyomma variegatum*. The virus was also isolated from the larvae of the same laying and from the blood of one *Cercopithecus* on which they fed.

The same virus has been previously obtained from adults of the same species collected at the slaughterhouses in Bangui, in 1975 (Sureau and al).

An acarine appears for the first time as a sylvatic vector and reservoir (at least temporary) of yellow fever (1).

This WTB 1927 strain was compared with the strain of yellow fever virus previously isolated in Central African from mosquitoes, by serological tests (FC and NT) and by cytopathic effects (CPE) on different cell culture systems. No significant difference was observed (2).

J. F. SALUZZO, A. J. GEORGES (INSTITUT PASTEUR)

J. P. HERVE, J. P. GONZALEZ (O.R.S.T.O.M.)

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- (2) SALUZZO J. F., HERVE J. P., SALAUN J. J., GERMAIN M., CORNET J. P., CAMICAS J. P., HEME G. and ROBIN Y. - Caractéristiques des souches du virus de la fièvre jaune isolées de la ponte et des larves d'une tique *Amblyomma variegatum* récoltée à Bangui (Centrafrique). Ann. Virologie (Inst. Pasteur). In press.

Serological survey to Lassa, Ebola and Marburg viruses in Central African Republic.

A serological survey of the Central African human population for antibodies, to Lassa, Ebola and Marburg viruses was initiated in 1979. Serum samples were obtained from 996 individuals and were collected in two regions : in M'Bomou and Basse-Lobaye departements. Samples were tested using the indirect immunofluorescence technique. All samples were tested at a final 1/8 dilution on polyvalent slides ° Lassa, Ebola, Marburg. Samples positive at 1/8 were further tested on monospecific slides. All serum samples positive were checked in the Central for Disease Control in Atlanta.

The M'Bomou departement is located in the transition zone of the tropical rain forest to savannah in the south-east of the Central African Republic. Of 499 serum samples tested 17 were found positive for Ebola virus, 7 for Marburg virus and one for the two viruses. Two of the Marburg cases showed a high antibody titer of  $\gg 1/64$ . These antibodies were present at the same level in a second sample collected 10 months after.

The Basse-Lobaye region is included in the tropical rain forest in the northwestern Congo basin. Of 497 serum samples tested, 13 were found positive for Ebola virus, one for Marburg virus and 7 other were positive for Marburg and Ebola viruses.

The serological survey undertaken in Central African Republic confirm the findings of Heyman and al from the Tandala region of Zaire where they found 7 % of the inhabitants of this area have substantial I.F.A. titers to Ebola virus, beside epidemiological manifestations. These results indicate the potential risk of the manifestation of this virus in the Central African Republic. Also it is remarkable in two villages in the forest bloc, Loko and Gouga, showed a particularly high positive results respectively 17,2 % and 9,4 % for Ebola virus.

The finding of Marburg virus antibodies was one of the first detection of these antibodies, in Africa, beside epidemiological manifestations.

The presence of 8 specimens doubly positive for Ebola and Marburg viruses let one think that the preferal areas where these viruses circulate are the same.

J. F. SALUZZO, A. J. GEORGES (INSTITUT PASTEUR)

J. P. HERVE, J. P. GONZALEZ (O.R.S.T.O.M.)

° Slides were obtained through the courtesy of Dr. K. M. JOHNSON (C.D.C.).



REPORT FROM THE NATIONAL INSTITUTE FOR VIROLOGY, JOHANNESBURG

During the summer 1979/80 a larger number than usual of SIN virus infections in man in South Africa were diagnosed by laboratory procedures (Table). As South Africa seems to be the only country where regular human morbidity is recognized it seems an opportune time to review this infection in man in South Africa.

A systemic illness, accompanied by a rash, described as epidemic follicular keratosis or papular fever, of unknown aetiology, was recognized during the 1940s and 1950s in the temperate Highveld region (Cochrane and Loewenthal, 1955, 1957, Rudolf, 1955; Findlay and Whiting, 1968; Gear, pers. comm.). Retrospectively, this syndrome is now believed to have been caused largely by SIN virus. The chronology of events leading to this belief is as follows:-

- 1952 SIN virus isolated from mosquitoes and birds in Egypt; antibody in man and wild birds; virus transmitted experimentally by Cx. univittatus (Taylor et al. 1955).
- 1954 SIN virus isolated from Culex mosquitoes in Highveld, S. Africa; antibody in man (Weinbren et al. 1956).
- 1961 SIN virus isolated from blood of 5 human beings, with fever, headache, joint and body pains in Uganda (Ann. Rep. East African Virus Research Inst. 1962).
- 1963 SIN virus isolated from a vesicle in a patient with joint pain/rash syndrome in Johannesburg (Malherbe et al. 1963). Fourteen similar cases diagnosed serologically on Highveld. Cochrane recognizes the similarity of the illness to epidemic follicular keratosis and papular fever (McIntosh et al. 1964).
- 1967 Epidemic of SIN on Highveld described by Findlay and Whiting, 1968.

Since 1963 SIN infections, with accompanying illness, in man in South Africa, have been diagnosed by laboratory means in 11 of the 18 years of elapsed time (Table). Two were by isolation of virus, one from vesicle fluid, the other from blood. Thirty-eight were diagnosed by seroconversion and 48, presumptively, from high titre antibody in convalescent phase blood. Serological diagnosis is considered reliable as no closely-related virus is known to occur in South Africa. Sentinel bird studies have indicated that avian infection occurs regularly each summer on the plateau. The vector is Cx. univittatus and this mosquito is also the main cause of human infection. It is lowly anthropophilic which is probably the main factor limiting human infection on the plateau. The low isolation rate from human blood suggests that viraemia in man is transient and probably low. A man-mosquito-man cycle is improbable and human infection is, consequently, largely dependent upon avian infection to infect vectors.

It is difficult to assess the real incidence of human morbidity in South Africa. While 88 laboratory identified cases in 18 years is not an impressive figure, it should be remembered that blood specimens submitted for diagnosis were almost entirely unsolicited, and we would normally only

receive local specimens. Findlay and Whiting reported on 52 cases in 1967, of which 10 were admitted to hospital, and in only 11 were blood specimens submitted to us. They reported that other cases were recognized in widely separated localities on the plateau. Immune rates in human populations in South Africa have varied from about 1 to 40%, depending upon locality. Minor epidemics apparently occurred in 1940, 1945, 1954/56, 1963, 1967, 1976 and 1980. The nature of the infection is now well-known among medical practitioners. Consequently, the number of specimens received for diagnosis has increased latterly. The data suggest that SIN virus causes a significant amount of morbidity on the South African plateau.

Taylor et al. 1955. *Amer. J. Trop. Med. Hyg.* 4: 844; Weinbren et al. 1956. *S. Afr. Med. J.* 30: 631; Cochrane and Loewenthal, 1955. *Med. Proc.* 1: 19; Idem, 1957, *Ibid* 3: 520; Rudolf, 1955. *Ibid*: 1:71; Malherbe et al. 1963. *S. Afr. Med. J.* 37: 547; McIntosh et al. 1964. *Ibid* 38: 291; Findlay and Whiting 1968. *Br. J. Derm.* 80: 67.

(McIntosh, Jupp, Dos Santos)

TABLE. Number of laboratory diagnosed infections of SIN virus in man in South Africa.

Year	Virus isolation	Sero-conversion	Presumptive*
1963	1	14	
1966			2
1967			28
1968			1
1969		2	
1970		1	1
1974	1	1	1
1975		1	3
1976		5	2
1979			1
1980		14	9
	2	38	48

\*, high titre HI antibodies in convalescent phase blood

REPORT FROM THE SPECIAL PATHOGENS UNIT,  
NATIONAL INSTITUTE FOR VIROLOGY, SANDRINGHAM 2131,  
REPUBLIC OF SOUTH AFRICA.

Observations on Rift Valley fever in Zimbabwe, 1956 to 1979.

The history of Rift Valley fever (RVF) in Zimbabwe can conveniently be divided into five distinct periods:-

1. Prior to 1956 there was virtually no investigation on RVF in the country but interest was aroused by the diagnosis of the disease in neighbouring South Africa. RVF was suspected to be the cause of an outbreak of abortion in cattle in the Nyamandhlovu district near Bulawayo in 1955 and antibodies were subsequently demonstrated in sera from that area.
2. Routine screening of pathological specimens from livestock for the presence of RVF virus by the inoculation of mice was instituted during 1956 and the virus was first isolated in 1957 from aborted cattle fetuses and from a sheep foetus. Workers were not aware at the time of the occurrence of a major outbreak of the disease but from the fact that virus was isolated from 10 per cent (8/79) of the aborted cattle fetuses tested during 1957, it can be surmised that an epizootic situation existed. No further isolations were made and the practice of screening a proportion of livestock specimens submitted to the laboratory was discontinued in 1962, a total of 191 specimens being tested for virus from 1956 to 1962. A total of 474 cattle sera were screened for neutralising antibody to RVF virus during this same period but the exact locations from which the sera originated are not recorded.
3. From 1963 to 1968 no observations were made on RVF in Zimbabwe.
4. Early in 1969 there was an explosive outbreak of the disease, causing death and abortion in cattle and sheep. The epizootic started in February and was most intense during the latter part of the wet season in March, April, May and June, but cases of RVF continued to be diagnosed through the dry season until October. The epizootic flared up again with the rains in the early months of 1970 and finally subsided in May. Diagnosis was based on histopathological examination of specimens during the epizootic. Sera collected during the epizootic were tested retrospectively for haemagglutination-inhibition (HAI) antibodies to RVF virus. Taking into account the results of both histological and serological examinations, RVF was confirmed to have occurred in 193 locations during the 1969-1970 epizootic; the term location being used to indicate either a privately-owned farm, or a fixed point in a tribal area or in state-owned land.

5. Subsequent to the 1969-70 epizootic it became routine to subject all livestock specimens to histological and virological screening for evidence of RVF infection, while sera were routinely subjected to HAI tests. In addition, surveys were conducted on the sera of humans and wild animals. In the seven years from 1971 to 1977, the virus was isolated 10 times and viral antigen was demonstrated in specimens on a further three occasions by immunodiffusion (ID) test. Taking into account the evidence of both virological and serological tests, outbreaks of RVF in livestock were confirmed to have occurred in 131 locations during these years. There is little doubt that the diagnosis of RVF would almost invariably have been missed in the field during this inter-epizootic period had there not been routine screening of specimens in the laboratory. As it happened, it was found that a degree of RVF virus activity in livestock occurred in all years in what may be termed enzootic areas, with the virus being most active in 1974 and 1975.
6. A major epizootic occurred again with the particularly heavy rains which fell in 1978, but on this occasion there was little tendency for resurgence of the disease to occur in the succeeding year; 1979 being a very dry year. RVF infection was confirmed to have occurred in 234 locations during this two year period, taking into account both serological and virological evidence.

The observations made on specimens from cattle, sheep and goats over the 24 years from 1956 to 1979 are summarised in Table I. Not included in the table are 5440 sera plus other specimens from humans, rodents and wild animals, most of which were tested for survey rather than for diagnostic purposes. The specimens were derived overwhelmingly from cattle, there being comparatively few sheep in Zimbabwe and specimens from goats rarely being submitted to the laboratory. In testing specimens for the presence of virus it was usual to pool brain, lung, liver, spleen and kidney from dead animals or aborted fetuses and to inoculate suspensions of these pools into calf testis cell cultures and intracerebrally (ic) into infant mice. On occasion, virus was isolated from brain tissue submitted alone from adult cattle or fetuses, as well as from foetal stomach contents, placenta and blood. The sediments obtained from centrifuging tissue suspensions prior to inoculation of mice and cell cultures, were routinely tested for RVF antigen in micro-ID tests against immune sheep serum. In routine histopathological surveillance, liver and lung were examined from aborted fetuses and liver from other animals.

It was possible to trace and plot accurately over 95 per cent of the locations from which serologically or virologically positive RVF specimens were obtained over the past 24 years. Positive specimens originated with remarkable regularity from a discontinuous area lying along the northern half of the watershed plateau, which runs diagonally across the country from south-west to north-east (Figure 1). RVF must be considered to be enzootic in this area, a degree of virus activity being detected there in all years when tests were done. An adjacent area can be delineated in which RVF also appears during epizootics and a further small enzootic area can be identified near the eastern border.

Elsewhere RVF is diagnosed sporadically, but the delineation of the main enzootic area may, to some extent, be spurious in that cattle husbandry is most intensive in that area and a disproportionately large number of specimens are submitted from there to the laboratory. There are very few cattle in the Zambezi Valley in the north-west and far north of the country, an area still under reclamation from the tsetse fly and in which major concentrations of large wild ungulates occur. Large concentrations of game animal species also occur in the Limpopo Valley in the south and south-west, but there are many cattle in the southern half of the country. Yet, little evidence of RVF has been obtained in the south despite the fact that deliberate attempts were made on occasion to test large numbers of cattle sera from the area, as during the 1969 epizootic. Sera from wild animals and humans have also yielded little evidence of RVF in the Zambezi and Limpopo valleys.

There is little doubt that cattle and other livestock can serve as amplifiers of infection, particularly during epizootics, but the question of whether or not they can serve as sole reservoirs of the virus at other times, must remain open. Cattle are widely distributed in Zimbabwe and dense concentrations occur outside the enzootic areas. Vectors of RVF have been poorly studied in general and as far as is known, recognized mosquito vectors are also widely distributed in Zimbabwe, so that the distribution of neither cattle nor mosquitoes seems to determine which areas are enzootic. It has long been suspected that RVF virus may cycle cryptically in enzootic areas in an unknown wild vertebrate host or hosts with known or unknown vectors. Rodents have been suspected, but an investigation in Zimbabwe failed to implicate wild rats and mice in circulation of the virus.

Nevertheless, it was clearly established that RVF is enzootic in the same areas in which epizootics arise in livestock and that the generation of an epizootic, therefore, seems to involve intensification of virus activity in livestock areas where it is already present rather than lateral spread from cryptic enzootic foci. The distribution of outbreaks in South Africa and South West Africa over recent years, also suggests that the virus does not spread from enzootic foci on the coast, but is widely enzootic in the grasslands of the interior plateau.

Factors which precipitate epizootics are poorly understood, but exceptionally heavy rains which favour vectors appear to be involved. The total precipitation in Zimbabwe during 1969 was only average, but long dry spells were interspersed with heavy rains and floods. Virus activity increased in Zimbabwe with heavy rains in 1974 and 1975 and there is no doubt that the 1978 epizootic was associated with exceptionally heavy rains, this being the wettest year on record.

The climatic factors appear to act over broad areas and virus activity intensifies simultaneously in locations hundreds of kilometres apart during epizootics. The 1969 epizootic in Rhodesia was accompanied by a large outbreak of the disease to the east in Mocambique. The widespread epizootics in South Africa and South West Africa during 1974 and 1975, were echoed by increased virus activity in Zimbabwe and there is unpublished evidence that a major epizootic may have occurred in Zambia at the same time. It should be stressed that total rainfall in an area does not appear to be as important as whether rainfall exceeds what is normal for the area. Rainfall in the relatively dry south-western Bulawayo-Nyamandhlovu RVF area never approaches that of the Salisbury area.

A large proportion of livestock must be immune following epizootics and the need for the population to be replenished by susceptible generations probably contributes to the spacing out of epizootics.

It is difficult to arrive at an estimate of the losses caused by RVF. Losses of livestock were often dramatic in individual herds and flocks during the 1969-1970 and 1978 epizootics and in one herd, for instance, 28 out of 1000 cows died and 85 per cent of the remainder aborted in 1978. Available information suggests that an average of 15 per cent of cows aborted in the affected areas in 1978 and average mortalities probably did not approach 5 per cent. Relating these figures to cattle census figures for the affected areas suggested that abortions totalled 60 000 and less than 20 000 cattle died in 1978. Losses in 1969-1970 were probably similar.

R. SWANEPOEL.

TABLE I. Observations on Rift Valley fever of livestock in Zimbabwe, 1956-1979.

Period	Sera tested for antibodies	Specimens for virology	Locations tested	Locations positive	"Virus isolations"*
Before 1956	—————	No RVF investigations	—————	—————	—————
1956 - 1962	474**	191	95	7	9
1963 - 1968	—————	No RVF investigations	—————	—————	—————
1969 - 1970	2 264	1 438	798	193	171
1971 - 1977	10 955	1 491	920	131	13
1978 - 1979	3 199	882	541	234	152

\* Includes virus isolations, histopathological diagnoses and positive immunodiffusion tests - see text.

\*\* Exact locations of origin not recorded.

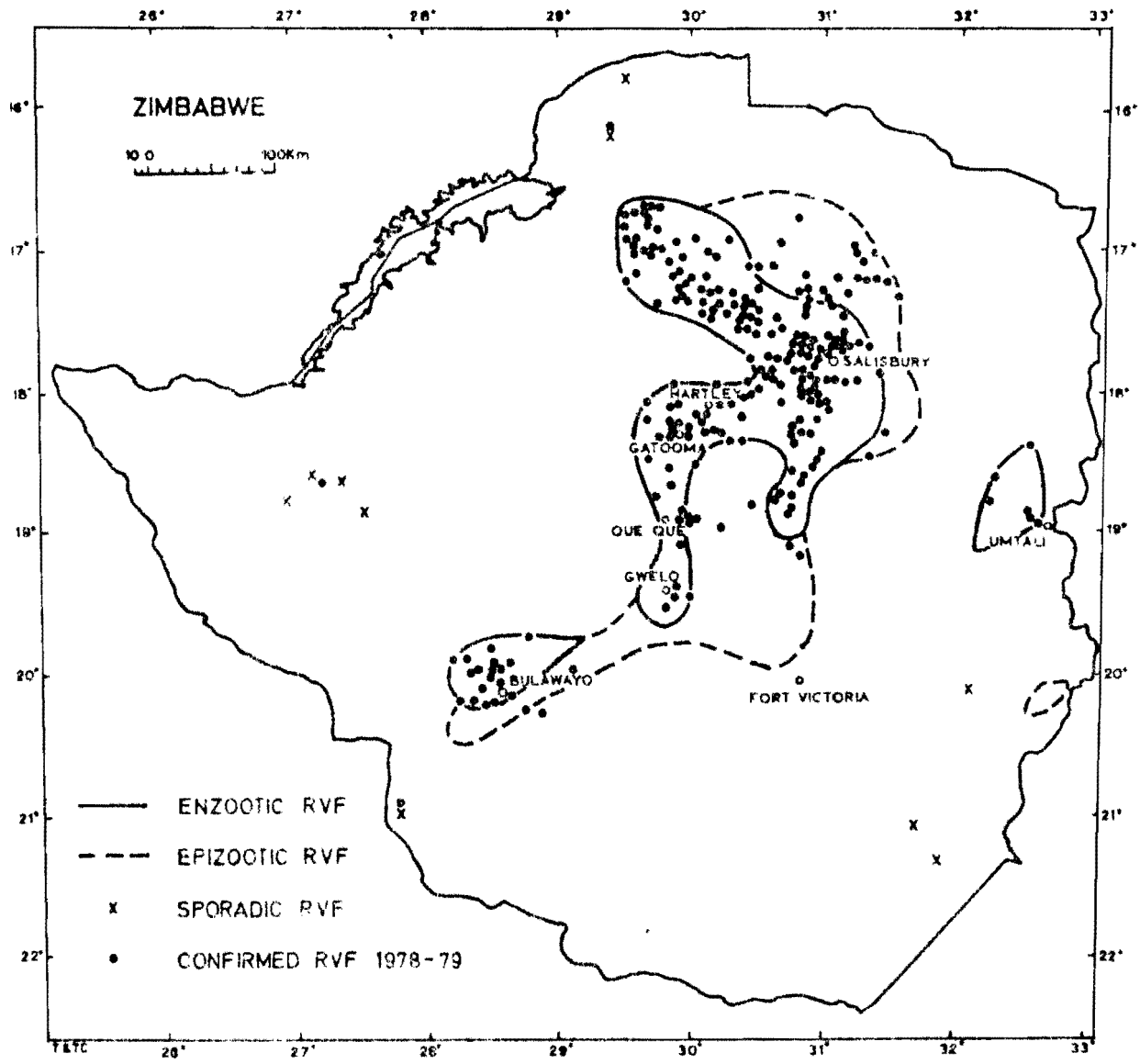


Figure 1. Areas of enzootic, epizootic and sporadic Rift Valley fever in Zimbabwe 1956-1979, showing locations where the disease was confirmed in 1978 and 1979.



REPORT FROM THE LABORATORY OF ENZIMOLOGY, DEPARTMENT OF MOLECULAR BIOPHYSICS, INSTITUT OF BIOPHYSICS, FEDERAL UNIVERSITY OF RIO DE JANEIRO, R.J., BRAZIL.

Inhibition of RNA and protein synthesis in mouse L cells infected with Marituba virus

Infection of mouse (L-A9) fibroblasts cells with Marituba (MTB) virus resulted in rapid and marked reduction in cellular RNA and protein synthesis considerably before cell viability was compromised. The incorporation of  $^3\text{H}$  (Uridine and Leucine) into acid insoluble material during 30 minutes pulses, declined exponentially in infected cells and this decrease and the time lag before its initiation was a function of the multiplicity of infection. In cells infected with m.o.i. higher than 0.1 we found the same value after an initial lag that depends on the m.o.i. Biphasic curves can be observed in cells infected with 1, 5 and 10 pfu/cell and this may be interpreted as precursor incorporation into viral-directed RNA and protein synthesis. No significant difference in inhibition of RNA and protein synthesis can be detected in cells infected at multiplicities of 5 or 10 but a delay of approximately 6 hours is observed in cells infected with 1 pfu/cell. The protein synthesis in L cells infected with MTB virus was also analysed using polyacrilamide gel electrophoresis. L-A9 cells infected at an input multiplicity of 5 pfu/cell were labelled for 2 hours with  $^{35}\text{S}$ -methionine (5  $\mu\text{Ci/ml}$ ) at various times after infection and the cells processed for electrophoresis and autoradiography. It is apparent that synthesis of cellular proteins is declining by 4-6 hours after infection. Labelling of cellular proteins is dramatically reduced by 8-10 hours after infection and completely inhibited thereafter when viral structural proteins first appear.

(M.A. Rebello, N. Volkmer, I.C. Frugulhetti and M.C.M. Soares).

REPORT FROM ARBOVIRUS LABORATORY  
INSTITUT PASTEUR  
BP 304  
97305 - CAYENNE CEDEX - FRENCH GUIANA

RAPID DENGUE DIAGNOSIS  
COMPARISON OF THREE METHODS

Following the workshop on dengue laboratory diagnosis held in the Center for Disease Control Laboratory, San Juan, Puerto-Rico, 5-9 february 1979, we decided to compare the different techniques used for the isolation and identification of dengue viruses. Using aliquotes of serum samples, we have compared the following methods : suckling mouse inoculation, fluorescent focus method, Toxorhynchites mosquito inoculation, plaque method in LLC-MK2 cells and Aedes pseudoscutellaris mosquito cells (AP 61) inoculation.

MATERIALS AND METHODS

1. SERA

23 serum samples found positive for dengue virus in 1977 and 1978 and kept since that time at  $-70^{\circ}$  C were studied. The minimum necessary volume of serum was 1.0 ml and most of the samples have to be diluted before use (see table 1).

2. SUCKLING MOUSE INOCULATION.

Each of 6 suckling mice one day old were inoculated intracerebrally with 0.02 ml of serum samples. They were observed daily for 21 days. Blind passages were not used.

3. PLAQUING METHOD IN LLC-MK2 CELLS.

Serum samples were inoculated onto monolayer cultures of LLC MK2 cells - After adding the agar overlay, cultures were inverted and incubated at  $34^{\circ}$ C for 7 days - Neutralizing tests were performed on the 7th day and read on the 14th day.

4. FLUORESCENT FOCUS METHOD.

Serum samples were inoculated onto LLC-MK2 monolayers in Lab Tek slides (8 chambers) and into Toxynrhynchites amboinensis mosquitoes. After 3 days Lab Tek slides were FA stained using a broad reacting dengue 2 immune fluid. Mosquitoes were ground up on the 4th day and the suspension was inoculated onto Lab Tek slides either directly or after neutralization by specific dengue 1, 2 and 3 immune fluids according to the results of FA staining on the 3 rd day.

#### 5. TOXORHYNCHITES AMBOINENSIS.

Mosquitoes were immobilized by immersing the tubes in wet ice for 5 to 10 minutes and 0.75µl of serum was inoculated in the mesothoracic spiracular chamber. Each serum sample was inoculated to 15 mosquitoes. Mosquitoes to be used for CF antigen preparation were kept for 7 to 10 days. After sonication in borate buffer, pH 9.0, and centrifugation, the supernatant was used as CF antigen.

#### 6. Aedes pseudoscutellaris CELLS (AP 61)

This mosquito cell line was kindly provided by Mrs Barbara Hull from CAREC - 25 cm<sup>2</sup> disposable flasks were inoculated directly so as to obtain a 1 : 40 final serum dilution in the culture medium - After the first 24 hours incubation, cultures were examined every day up to 14 days.

#### RESULTS

Results are summarized in tables 1 and 2. As the aim of our work was to compare the rapidity of diagnosis, only one test was performed for each method and several isolates were not identified in time.

Only one strain was isolated in suckling mice.

Using plaque assay 5 strains were isolated but no one was identified within the 14 days period.

Using mosquito (Toxorhynchites) inoculation method and mosquito suspension as CF antigen, 13 strains were isolated and 3 identified within 10 days.

By the fluorescent focus method, 13 strains were isolated and 9 identified within 10 days.

We did not get any reproducible results with the AP 61 cell line and we have discarded it from the tables.

As we can see in table 2, using fluorescent focus method and Toxorhynchites mosquito inoculation we were able to isolate 14 viruses from 23 samples (61 %) and to identify 11 dengue viruses on 14 isolates (78.6 %).

From 6 serum samples collected in 1977, 5 viruses were recovered (83.3 %) and only 9 from 17 specimens in 1978 (53 %).

(M. L'HUILLIER, G. GIRAULT, Y. ROBIN)

TABLE 1 - Results of comparative studies on dengue virus diagnosis

SERUM No	Serum ml	Diluent ml	SM	PLAQUES		F.F.		TOXOR		RESULTS
				J 7	J 14	J 3	J 10	J 7	J 10	
77-1486	0.6	0.4		+	0	0	+ ?	+	+	Dengue ?
77-1772	0.3	0.7				+	+ D2	+	+	Dengue 2
77-1893	0.2	0.8				+(14c)	0			Neg.
77-2210	0.2	0.8		+	+	+	+ ?	+	0	Dengue ?
77-2335	0.2	0.8		0	+	+	+D2	+D2	NT	Dengue 2
77-2357	0.2	0.8		+	+	+	+D2			Dengue 2
78-851	1.0	0								Neg.
78-1117	1.0	0								Neg.
78-1209	1.0	0				+	+D1	+	NT	Dengue 1
78-1327	0.5	0.5	+	0	+	+	+ ?	+	+D1	Dengue 1
78-1356	1.0	0				Cont	+D1	+	NT	Dengue 1
78-1370	1.0	0				+	+D1	+	NT	Dengue 1
78-1480	0.6	0.4								Neg.
78-1482	1.0	0								Neg.
78-1570	0.1	0.9						NT	+D1	Dengue 1
78-1646	0.1	0.9								Neg.
78-1680	0.3	0.7				+(2c)	0			Neg.
78-1694	0.5	0.5								Neg.
78-1696	0.1	0.9				+	+D1	+	+	Dengue 1
78-1726	0.1	0.9								Neg.
78-1761	0.3	0.7				+	+D1	+	NT	Dengue 1
78-2017	0.1	0.9				+	+ ?	+	NT	Dengue ?
78-2341	0.3	0.7				Cont	+D1	+	+	Dengue 1
23			1	3	4	12	13	12	6	14
				5		15 (9)		13 (3)		

TABLE 2 - Results of comparative studies on rapid dengue virus diagnosis - Summary.

Viruses Isolated	Method of Isolation			
	S.M.	PLAQUES	F.F.	TOXOR.
1	+	+	+	+
3		+	+	+
1		+	+	
8			+	+
1				+
14	1	5	13	13

REPORT FROM THE INSTITUTOS NACIONALES DE SALUD

Apartado No. 451 - LIMA, PERU

Coendu Prehensilis as a Possible Reservoir in the

Wild Cycle of Some Arboviruses

María R. Méndez,<sup>(1)</sup> Guillermo Calderon,<sup>(2)</sup> and Sara M. Sanchez<sup>(1)</sup>

Because of the recurrent appearance of human yellow fever cases in the forest of the upper Amazon river basin of Peru, some animals were captured with the aim of getting an idea if other vertebrates as the monkeys could be involved in the wild cycle of the virus. The initial collection was made in June 1978 in Bellavista, Departamen to San Martin and consisted of 21 Didelphis (species undetermined); 14 wild rodents and one porcupine (Coendu Prehensilis).

HI testing of the sera from all these animals against antigens of the following viruses were performed: yellow fever, (YE) St Louis, (SLE) Ilheus, (ILH) Dengue 2, (DEN2) Venezuelan Equine Encephalitis, (VEE) Eastern, (EEE) and Western (WEE) were negative; except the porcupine specimen which gave the following results:

YF	SLE	ILH	DEN2	VEE	EEE	WEE
320	80	80	80	20	320	40

After this finding, another capture, specifically guided for secure porcupines in the same area was made last March, 1980. It was possible to get three Coendu prehensilis and two other porcupine, Coendu sp. The former species usually lives between 300 and 400 mts. above sea level, while the unidentified species was found

at higher altitudes (400-1200 mts).

The HI tests done with the above mentioned antigens were all negative with the two sera from the undetermined species of porcupine while all the three Coendu prehensilis sera did react with all the antigens. The following table shows the HI results with these three sera.

ANIMALS N°	YF	SLE	ILH	DEN2	VEE	EEE	WEE
1	320	160	ND	80	20	160	20
2	80	80	40	80	80	640	160
3	40	40	40	80	40	320	40

The fact that all four Coendu Prehensilis sera did react with all the seven antigens included in the test, indicate that they should be repeated with acetone - extracted sera and further confirmed, if necessary, by neutralization and complement fixation tests. The results with the flavivirus antigens could represent exposure to yellow fever and/or other viruses within the group. On the other hand the finding with the group A antigens would suggest EEE as the most likely infecting agent. These results would also indicate that Coendu prehensilis are perhaps exposed to the bites of mosquitoes of both diurnal and nocturnal activity.

The main conclusion is, of course, that the possible role of this vertebrate in the epidemiology of arboviruses should be defined and that experimental laboratory infections should be carried out.

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REPORT FROM THE INSTITUTO NACIONAL  
DE SALUD, BOGOTA, COLOMBIA

OUTBREAK OF DENGUE 1

Aedes aegypti was eradicated from Colombia, with the exception of the city of Cúcuta, during the fifties. However, the country became reinfested with the mosquito in 1968. Thereafter three serious dengue outbreaks were observed. The first one, caused by dengue 2 was observed in northern Colombia during 1971 and 1972, produced no less than half a million cases. The second one, caused by dengue 3, was observed particularly in Central Colombia during 1976 and 1977. It caused at least 200.000 cases, and possibly twice as many. The third one, caused by dengue 1, swept over the country during 1978 and 1979 and produced about one million cases. The last epidemic apparently subsided in 1979, but more recently, in mid-1980, has flared up again. Dengue - 1 cases have been observed in July 1980 in places in Central Colombia ( Utica, for example ) where the dengue - 1 outbreak reached its peak eighteen months ago. At the same time, dengue 1 has been observed in places invaded by A aegypti in 1978, where the mosquito had never been found before and where there were no records of dengue epidemics in years past. Such is the case of Villavicencio, in Eastern Colombia, not far from the sylvatic yellow fever area, where numerous cases of dengue 1 infection have been observed during June, July and August, 1980. A limited serologic survey ( HI tests ) conducted during August 1980 on 149 individuals indicated that 39 ( 26% ) exhibited evidence of past dengue 1 infection, 16 ( 11% ) showed secondary broad responses to flaviviruses at titers 1:320 or higher and 30 showed broad responses at lower titers. The HI picture was obscured undoubtedly by the repeated yellow fever vaccinations which have been carried out in Villavicencio.

( Hernando Groot, Jorge Boshell - Samper, Gladys Marquez and Hernando Vidales )



Report from the  
CARIBBEAN EPIDEMIOLOGY CENTRE, PAHO/WHO  
Port of Spain, Trinidad

The 1978/79 Yellow Fever Epidemic  
in Trinidad

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The yellow fever epidemic in Trinidad began with an epizootic in Alouatta monkeys in November 1978. First reports centered around the Guayaguayare forests of South Trinidad and the epizootic spread within one month to the Moruga area. With the co-operation of the Trinidad Ministry of Health and Ministry of Agriculture 9 dead or ill Alouatta monkeys were submitted to CAREC and yellow fever virus was isolated from 6 in the AP-61 cell line and from 3 in suckling mice (Table 1). Haemagogus mosquitoes were collected from the same areas and 7 of 26 mosquito pools were positive in the AP-61 system and 6 of 26 in suckling mice.

Surveillance of human febrile cases was intensified, and specimens were received from hospitals and health clinics. These were inoculated into AP-61 cells and suckling mice, isolates were identified by complement fixation and mouse neutralization and antibody development in recovered cases was demonstrated by haemagglutination inhibition and mouse neutralization tests. Data on the first 8 cases identified are shown in Table 2. There were 6 virus isolates in AP-61 and 5 in suckling mice. Liver samples were referred to the Armed Forces Institute of Pathology at Walter Reed and one diagnosis was made histologically in the absence of virus isolation.

This phase of the epidemic ended with the last positive mosquito and monkey specimens in February and the last human case on 9th March, 1979. No further activity was reported until August 1979, when dead monkeys were again sighted in forests north of the original areas (see map).

Alouatta monkeys and Haemagogus mosquitoes were again referred to the laboratory and virus was isolated from 5 of 8 animals and from 8 of 87 Haemagogus pools.

There were a further 10 human cases whose data are shown on Table 2. Virus was isolated from 7 of these in AP-61 and from 5 in mice. Three diagnoses were made on the basis of liver pathology only. The 18 cases confirmed in this epidemic were unvaccinated males with the exception of one man who had been vaccinated in 1973, and with one exception there was exposure in yellow fever affected forested areas. There were 7 deaths.

(P.J.S. Hamilton)

TABLE 1. YELLOW FEVER ISOLATIONS MADE DURING THE OUTBREAK IN TRINIDAD -  
November 1978 - November 1979.

PHASE OF OUTBREAK	SOURCE	RESULTS IN AP-61 TISSUE CULTURE	RESULTS IN SUCKLING MICE
I	<u>Alouatta</u> monkeys	6/ 9*	3/ 9
November 1978	<u>Haemagogus</u> mosquitoes	7/ 26*	6/ 26
to			
March 1979	Man	6/287*	5/287
II	<u>Alouatta</u> monkeys	5/ 8	5/ 8
August 1978	<u>Haemagogus</u> mosquitoes	8/ 87	5/ 86
to			
November 1979	Man	7/129	5/129

\* Number of animals, mosquito pools or humans positive/  
Number tested.

TABLE 2.

DATA ON YELLOW FEVER CASES - TRINIDAD  
JANUARY - MARCH 1979

Case No.	Collection Dates	Onset Dates	Result of Illness	Isolation		Identification		Serology		Histology
				Mice	Ap-61	CFT	NEUT	HI	NEUT	
1 FS	5.1.79 8.1.79	27.12.78	Died 7.1.79	-	-	-	-	-	-	Positive Trinidad & AFIP
2 BG	23.1.79 24.1.79  25.1.79 26.1.79	15.1.79	Died 25.1.79	- -	+ (Serum) + (Urine)	+ +	+ +	- -	- -	
3 SM	29.1.79 8.2.79	26.1.79	Recovered	+	-	+	+	1/10 1/1280	Neg. 4 logs	
4 FR	8.2.79	2.2.79	Recovered	+	+	+	+	Neg. 1/1280	1 log 3 logs	
5 LH	16.2.79 2.3.79	12.2.79	Recovered	+	+	+	+	Neg. 1/80	0.7 log 3 logs	
6 AS	18.2.79 23.3.79	17.2.79	Recovered	-	+	+	+	Neg. 1/160	1 log 3.5 log	
7 MK	21.2.79 6.3.79	20.2.79	Recovered	+	+	+	+	Neg. 1/640	Neg. 3 logs	
8 LC	9.3.79	6.3.79	Recovered	+	+	+	+	-	-	

TABLE 3.

## DATA ON YELLOW FEVER CASES - TRINIDAD

AUGUST 1979

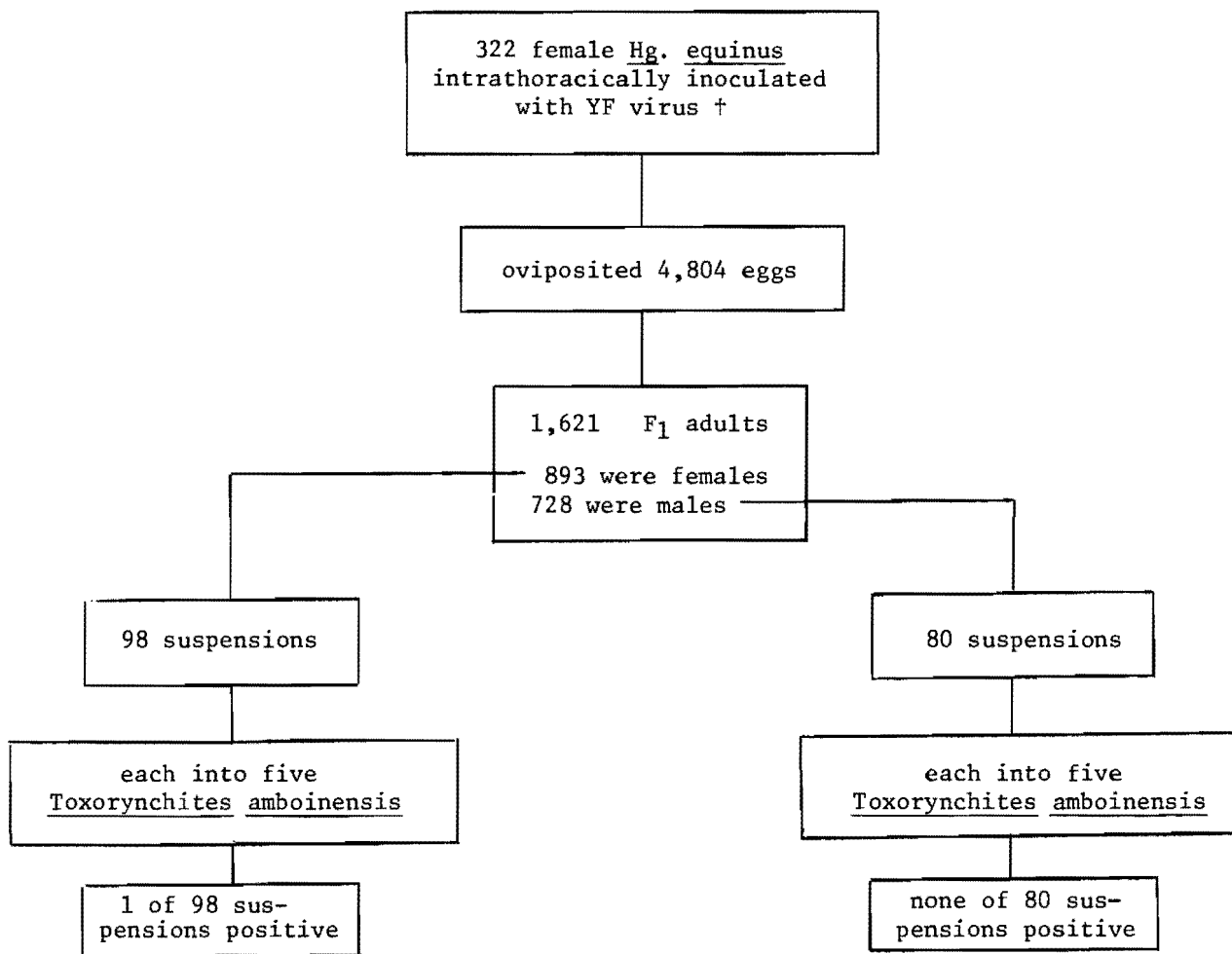
Case No.	Collection Dates	Onset Dates	Result of Illness	Isolation		Identification		Serology		Histology
				Mice	AP-61	CFT	NEUT	HI	NEUT	
RM 9	21.8.79	4.8.79	Died	-	-					Trinidad + AFIP +
KD 10	10.8.79	8.8.79	Recovered	+	+	+	+	NT	Neg > 4 logs	
KB 11	15.8.79	10.8.79	Recovered	+	+	+	+	Neg. 1/320	0.5 log > 4 logs	
BS 12	16.8.79	13.8.79	Died	+	+	+	+			Trinidad + AFIP +
ES 13	28.8.79	19.8.79	Died	-	-					Trinidad AFIP +
JC 14	28.8.79	24.8.79	Recovered	+	+	+	+	Neg. 1/10	Neg. 3 logs	
SS 15	29.8.79	28.8.79	Recovered	+	+	+	+	Neg. 1/80	Neg. 4 logs	
SR 16	1.9.79	28.8.79	Died	-	-					Trinidad + AFIP +
AS 17	29.8.79	17.8.79	Recovered	-	+	+	+	Neg. 1/80	Neg. 4 logs	
AR 18	7.9.79	5.9.79	Died	-	+	+	+			Trinidad +

REPORT FROM THE GORGAS MEMORIAL LABORATORY, PANAMA\*

I. Transovarial Transmission of Yellow Fever Virus by its Sylvatic Vector, Haemagogus equinus.

We have designed experiments to determine if transovarial transmission of yellow fever (YF) virus occurs in Haemagogus equinus, a known sylvatic vector of YF in tropical America. In this communication we report the first evidence of transovarial transmission of YF virus by this sylvatic vector.

The following diagram illustrates the method followed:



† Local YF isolate from a fatal human infection, passed once in an Aotus monkey and once in Vero cell culture.

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- or -

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This is the first time transovarial transmission of YF virus has been demonstrated in a New World sylvatic vector. We concentrated on Hg. equinus initially because YF virus has been isolated many times from Haemagogus mosquitoes in tropical America and because Hg. equinus was the only sylvatic YF vector abundantly present during the 1954 Guatemala-Honduras jungle YF epidemic in which the virus persisted for almost a year and a half in the absence of evidence of vertebrate mediated transmission.

Demonstration of transovarial transmission of YF virus by parenterally infected Hg. equinus suggests that this mechanism may contribute to virus maintenance during adverse environmental conditions. Additional studies are now in progress to test for vertical transmission following oral rather than parenteral infection, to further quantify the rate at which transovarial transmission occurs, and to test for virus transmission following feeding by vertically infected F<sub>1</sub> progeny.

(B. Dutary and J.W. LeDuc)

## II. Serological Survey in Bocas del Toro Province, Panama.

Between January and April, 1980, a serological survey was made of residents of the Teribe, Changuinola and Oeste River systems, Bocas del Toro Province, Panama. The objective of this survey was to determine the prevalence of antibody to selected arboviruses known or thought to occur in the region. This information was included in an environmental impact study conducted by GML on behalf of the Panamanian national Institute of Hydraulic Resources and Electrification (IRHE) and prepared prior to initiating construction of hydroelectric projects there.

Human sera were collected from residents by field medical teams which visited the region several times during the study. A total of 1300 sera were collected and tested from residents. This sample represents roughly 50% of the estimated total population of the area. Sera from an additional 97 non-resident employees of the project who had begun establishing field camps in the Changuinola River valley within the previous 6 months were also included.

Sera were tested at a 1:8 dilution by plaque reduction neutralization tests on Vero cell cultures grown in 96 well panels against 20 to 100 plaque forming units of virus with overnight incubation at 4°C. All sera collected were tested against Venezuelan equine encephalitis (VEE), Ilheus (ILH), Punta Toro (PT), Chagres CHG) and vesicular stomatitis-Indiana serotype (VSI) viruses. In addition, a representative sample of 450 sera was selected and tested against Mayaro (MAY), St. Louis encephalitis (SLE), Bussuquara (BSQ), Madrid (MAD), Ossa (OSSA), Wyeomyia (WYO), Guaroa (GRO) and Guama (GMA) viruses. This sample was stratified random sample designed to include representatives of all age groups and both sexes of residents of the Teribe and Changuinola River valleys and the workers, but did not include residents of the Oeste River valley.

A preliminary summary of antibody prevalence rates by area of residency is presented in Table 1. Prevalence rates ranged from a low of less than 1% (3/1395) for CHG virus to 65% (868/1328) for VSI virus. Seropositive individuals were identified in nearly all areas sampled for all viruses tested with the exception of CHG virus, which was found only among non-resident workers and thus is apparently absent from the region. A more detailed analysis of results of this serological survey is in preparation.

(J.W. LeDuc, G. Justines, P.H. Peralta and A.J. Adames)

### III. Differences in Plaque Sizes in Guaroa Virus Strains from Colombia, Brazil and Panama.

Guaroa (GRO) virus was first isolated from blood of apparently healthy farmers in the settlement of Guaroa, Colombia (Groot *et. al.*, 1959). Analysis of plaque composition of this prototype GRO virus found 2 distinct plaque sizes, one large (LP, 2.5-3.0 mm diameter) and one small (SP, 1.0-1.5 mm), in a ratio of 10 LP : 1 SP (Tauraso 1969).

Guaroa virus was subsequently recovered from the blood of sick forest workers in Brazil (Causey *et. al.*, 1962) and we recently isolated it from the blood of a black spider monkey (*Ateles fusciceps*) collected in Darien Province, Panama in 1979 during YF surveillance. Guaroa virus had previously been isolated in Panama from *Anopheles neivai* mosquitoes collected in Almirante, Bocas del Toro Province in 1960 (Galindo *et. al.*, 1961). A laboratory technician, accidentally infected with the GRO *Ateles* isolate, suffered a febrile illness of about 3 days duration, confirming that this Panamanian strain of GRO virus, like the Brazilian strain, causes human illness.

Analysis of plaque composition of both the Panamanian and Brazilian isolates also found both LP and SP, but in a ratio of 1 LP : 80-100 SP. Additional studies are in progress to determine if the LP:SP ratio correlates with the virulence of GRO virus for man as suggested by the differences in plaque ratios noted here between the apparently avirulent Colombian strain (10 LP : 1 SP) and the apparently virulent Panamanian and Brazilian strains (1 LP: 80-100 SP).

(G. Justines)

### IV. Outbreak of Equine Encephalitis in Herrera Province, Panama.

An outbreak of equine encephalitis is currently in progress on the Azuero Peninsula in Panama. Initial reports by local veterinarians stated that the presumed index case was seen during the first week of August and that approximately 30 horses had already died. On 19 August 1980 field investigations were begun in the areas of Parita, La Arena and Monagrillo, near Chitre in Herrera Province by GML and the Ministerio de Desarrollo Agropecuario. The

field team collected serum samples from horses with symptoms of acute encephalitis and from convalescent horses. Hemagglutination inhibition tests on sera from convalescent horses showed high titered antibody to eastern equine encephalitis virus. When treated with 2 mercaptoethanol, 7 of 10 showed a significant drop in titer, indicative of recent infections, as shown in Table 2. Field and laboratory investigations of this outbreak are continuing.

(G. Justines and Ministerio de Desarrollo Agropecuario, R. de P.)

Table 2. Hemagglutination inhibiting (HI) antibody titers to eastern equine encephalitis (EEE) virus in sera treated and untreated with 2-mercaptoethanol collected from convalescent horses, Herrera Province, Panama, 19 August 1980.

Horse #	Days after onset of illness*	HI antibody titer to EEE virus			
		2 hr. incubation		Overnight incubation	
		Untreated	Treated**	Untreated	Treated**
1	2-3	<20	<20	80	<20
2	2-3	<20	<20	80	<20
3	4-6	80	<20	640	160
4	6	80	<20	320	160
5	approx. 14	40	20	640	160
6	approx. 14	40	20	80	20
7	approx. 14	160	80	320	320
8	unknown	<20	<20	<20	<20
9	unknown	80	40	320	320
10	unknown	80	40	640	160

\* Approximate days after onset of encephalitis-like illness according to local veterinarians.

\*\* Equal vols. of 0.1 M 2-mercaptoethanol and serum incubated 1 hr. at room temperature prior to dilution.



Table 1. Neutralizing antibody prevalence rates to 13 arboviruses by area of residency of people surveyed; Teribe-Changuinola and Oeste River systems, Bocas del Toro Province, Panama, 1980.

	<u>VEE</u>	<u>MAY</u>	<u>ILH</u>	<u>SLE</u>	<u>BSQ</u>	<u>MAD</u>	<u>OSSA</u>	<u>PT</u>	<u>CHG</u>	<u>WYO</u>	<u>GRO</u>	<u>GMA</u>	<u>VSI</u>
<b>Rio Teribe</b>	12% 60/496	3% 3/120	15% 76/496	26% 31/121	10% 12/121	2% 3/121	2% 2/117	9% 46/508	0% 0/508	5% 6/120	12% 14/120	0% 0/122	78% 346/445
<b>Rio Changuinola</b>													
Below impoundment	21% 80/378	2% 3/114	20% 76/373	17% 19/111	13% 15/113	4% 4/113	9% 10/110	4% 14/378	0% 0/380	10% 11/113	5% 5/111	10% 11/108	60% 224/375
Above impoundment	19% 65/340	11% 20/179	11% 38/343	10% 17/178	21% 39/180	5% 9/180	15% 27/178	16% 34/341	0% 0/343	5% 8/178	7% 12/179	10% 17/175	60% 205/345
<b>Rio Oeste</b>	37% 25/67	NT	13% 9/67	NT	NT	NT	NT	4% 3/67	0% 0/67	NT	NT	NT	72% 48/67
<b>Workers</b>	20% 20/97	6% 2/34	16% 16/97	23% 8/34	14% 5/34	14% 5/34	6% 2/36	16% 16/97	3% 3/97	32% 10/31	15% 5/33	3% 1/34	47% 45/96
<b>Total</b>	18% 250/1378	6% 28/447	16% 215/1376	17% 75/444	16% 71/448	5% 21/448	9% 41/441	8% 113/1391	< 1% 3/1395	8% 35/442	8% 36/443	7% 29/439	65% 868/1328

REPORT FROM THE SAN JUAN LABORATORIES  
CENTER FOR DISEASE CONTROL  
SAN JUAN, PUERTO RICO

Dengue in Puerto Rico, 1980

Through August 27, 1980 205 cases of dengue-like illness were reported to the San Juan Laboratories, compared with 684 during the same period in 1979. Serological confirmation was obtained for 40% of the 96 patients from whom paired blood specimens were submitted. Virus was isolated from the blood of 14 patients with onset of illness during 1980, the most recent having onset in August. All isolates have been dengue type 1, so that this type has now been circulating for 24 months in Puerto Rico. Confirmed cases came from 5 municipalities in the Metropolitan San Juan area, from the municipality of Ponce, and from 5 rural municipalities.

Ades aegypti population indices in Puerto Rico

One hundred flower urns in 10 cemeteries island-wide were inspected weekly for *A. aegypti* larvae. Positivity averaged from 2% - 9% during the first 35 weeks of 1980, with one cemetery consistently near zero and another usually between 10% and 25%. Sand or larvicide was placed in the urns in some cemeteries as a control measure. Every time a suspect dengue case is reported, ULV spraying with malathion is carried out around the infected premises in 2 cycles during one week; 12/17 truck-mounted spraying machines are currently in operating order at the Puerto Rico Health Department.

Eight modified New Jersey traps have been operated fairly continuously since the beginning of 1980 at sites in 5 cities around the island. These traps have been painted black and the lights have been removed, so that they act as attractively-colored (to mosquitoes) air samplers. The catch is collected at approximately weekly intervals. Average catches (both sexes combined) of *A. aegypti* for the first 5 months of 1980 ranged from zero in Arecibo in January to 44 in San Juan in March. Catches tended to increase through the year.

Dengue in the U.S. Virgin Islands

No confirmed cases of dengue have been reported from these islands during 1980.

Dengue in the Continental United States

A number of specimens have been received in 1980 from suspect cases of dengue imported into the United States. Only 3 have been serologically confirmed: a case from California, exposed in the Phillipines, a case from New York, exposed in Haiti, and a case from Oregon, whose travel history has not yet been provided.

A surveillance system set up along the Texas-Mexico border by Dr. R. Fontaine has sent some specimens from cases of febrile illness. One 28-year-old patient from Laredo, Texas with onset of illness in June had dengue HI antibody, not necessarily from a recent infection.

#### Dengue in Hispaniola, 1980

Local epidemiologists in Haiti and the Dominican Republic have been contracted to obtain a minimum of 25 acute blood specimens monthly from cases of illness compatible with dengue, and to get as many paired convalescent specimens as possible from these patients. The number of acute specimens received from Haiti by month of onset January through July 1980 varied from 28 to 88. Paired samples were obtained in 24% of all cases. The serological confirmation rate declined from  $\geq 50\%$  in January and February to zero in July, whereas the rate for dengue antibody in acute specimens (indicating the prevalence of infection) remained level throughout the period, with an average of 74%. The results confirm a high level of endemic dengue in Haiti, with possibly a seasonal drop in transmission during the middle of the year.

The Dominican Republic reported 203 cases of dengue through their communicable disease reports during 1979, but sent only 31 acute and 19 paired convalescent samples in 1980, from cases with onset from February to May, plus 194 survey specimens from 2 schools. Testing of the school survey will be done later in the year. Of 16 paired samples tested, one case was confirmed as current dengue, with onset in April, one had an inconclusive result, and 6 (38%) had dengue antibodies in the acute serum, indicating a lower prevalence than in Haiti.

No virus was isolated from specimens from either country.

A monthly inspection of 200 premises in 10 sectors of Santo Domingo for Aedes aegypti larvae revealed house indices ranging from zero to 50%.

#### Dengue in Cuba

Serum samples taken from Cuban refugees from the 1980 exodus for routine VDRL tests were also tested for dengue antibodies. The majority of specimens were selected from males aged 18-30 years, and 267 of 569 (47%) had HI antibody. This must have been the results of the 1977 epidemic, and of an unreported resurgence of the disease in 1979 referred to by the refugees. None of the titers indicated very recent infection.

#### Dengue in Honduras

DEN-1 virus was isolated from sera sent to us from 4 patients from San Lorenzo, on the Pacific Coast, who had onset of illness in January 1980. Dengue is apparently still active in Honduras more than a year after the epidemic peak.

### Dengue in El Salvador

Plaque neutralization test studies of selected sera from patients bled during the 1978 epidemic have revealed one patient with monotypic DEN-2 antibodies. This finding lends additional support to the discovery of DEN-2 virus and antibody in Guatemala during the 1978 DEN-1 epidemic by the Gorgas Memorial Laboratory.

### Dengue in Mexico

Results of the household surveys carried out in February in Mérida, Yucatán State, and Tampico, Tamaulipas State, at the invitation of the Mexican government, are as follows. In Mérida, the A. aegypti house index was 18% and 19% in the two areas surveyed, while the antibody prevalence was 13% and 45%, respectively. In Tampico one area had a house index of 2% and a prevalence of 12%, the other had 14% and 27% respectively. That is, prevalence of infection correlated with the vector index in Tampico but not in Mérida.

The epidemic is expected to move north towards the Texas border since the abnormal drought conditions of early summer in the area were broken by Hurricane Allen.

### Dengue 2 Candidate Vaccine Tests

The San Juan Labs are assisting the Dengue Laboratory of the University of Puerto Rico School of Medicine, under Dr. E. Kraiselburd, in evaluating the Walter Reed Army Institute of Research dengue vaccine in rhesus monkeys at the Caribbean Primate Research Center in Puerto Rico. Four groups of 6 seronegative juvenile male rhesus monkeys were vaccinated as follows: Group A with undiluted vaccine, Group B with undiluted vaccine plus human serum containing dengue antibody, Group C with a 10-fold dilution of the vaccine, and Group D with the diluted vaccine plus human antibody. The objective was to determine whether dilute antibody would enhance the infectivity of vaccine when administered subcutaneously after a short incubation period at room temperature. Control monkeys received diluent and antibody. Monkeys were bled for viremia testing in tissue culture and Toxorhynchites mosquitoes for 12 consecutive days, and no viremia was detected. By the HI test 9 monkeys had sero-converted by post-inoculation day 33, and 14 by day 89. By the plaque reduction neutralization test, 21 had seroconverted by day 58, but only 8 retained detectable antibody by day 89. There was no significant relationship to either dose or antibody added in the proportion of monkeys converting by PRNT.

(J.P. Woodall, C.G. Moore, G.E. Sather, G. Kuno, R. Craven, D. Eliason, M. Moore, Center for Disease Control, San Juan, Puerto Rico and Atlanta, Georgia.)

REPORT FROM THE TEXAS DEPARTMENT OF HEALTH, AUSTIN, TEXAS 78756

Report for the period January 1-June 30, 1980

For the period indicated, 13,811 mosquitoes were divided into 1274 pools and inoculated into suckling mice. The following isolates were obtained:

<u>Locality</u>	<u>Collection Date</u>	<u>Pooled Species</u>	<u>Number of Isolations</u>	<u>Virus</u>
Dallas	March 18-19	C. tarsalis	1	Hart Park
Beaumont	May 21	C. quinquefasciatus	1	Hart Park
		C. salinarius		
		C. salinarius	1	Hart Park
		C. quinquefasciatus		
Dallas	May 22-27	C. quinquefasciatus	1	Hart Park
		C. restuans		
		C. tarsalis		
Dallas	May 27-30	A. quadrimaculatus	1	Hart Park
		C. quinquefasciatus		
		C. tarsalis		
Beaumont	May 28	C. (Melanoconion) sp.	1	Hart Park
		C. salinarius		
		C. quinquefasciatus		
Dallas	June 2	C. tarsalis	1	Hart Park
		C. territans		
		C. quinquefasciatus		
		C. restauns	1	Hart Park
		C. salinarius		
Alvin	June 3	C. quinquefasciatus	1	Hart Park
Dallas	June 9-10	C. quinquefasciatus	1	Hart Park
		C. quinquefasciatus		

Dallas and Lubbock submitted a total of 803 sentinel bird blood. No serological conversions were detected in any by the hemagglutination inhibition test.

Of 25 wild bird bloods submitted from Dallas and San Antonio for virus isolation studies, all were negative by inoculation of suckling mice.

ST LOUIS ENCEPHALITIS 1980

The Bureau of Communicable Disease Services and Houston City Health Department reported their first case of St. Louis Encephalitis (SLE) during week 17 (ending April 26, 1980). The patient, a 39 year-old female resident of Houston, became ill on March 7, 1980 with fever, headache, malaise, nausea, vomiting, and rash. She was hospitalized seven days following the onset of illness and was later discharged after recovering completely.

Serologic tests performed at the Houston City Health Department Laboratories, Texas Department of Health and the Center for Disease Control all confirmed the diagnosis of SLE. Texas Department of Health results were 1:20 (Serum 1) by IHA and 1:320 (Serum 2) by IHA. Serum 1 was collected 3/5/80; Serum 2 was collected 3/27/80.

(Charles E. Sweet)

REPORT FROM THE OFFICE OF LABORATORY SERVICES AND ENTOMOLOGY  
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES  
JACKSONVILLE, FLORIDA

Arbovirus surveillance in Florida was continued year round in several counties because of the six laboratory confirmed cases of St. Louis Encephalitis (SLE) which occurred in 1979. Year round sentinel chicken flocks, in addition to the flocks established in May and June, 1980, yielded eleven SLE reactors out of 894 chicken sera tested. All occurred in the early part of the year, most probably representing exposure during the end of 1979 season when virus circulation was known to be present. There were five EEE reactors.

From January through June, 1980, a mammal study was continued in the Tampa Bay area. Of the 130 wild caught raccoons, 12 were SLE reactors and three were EEE reactors. The 86 wild caught opossums yielded six SLE reactors and three EEE reactors. Twenty-three other mammals and amphibians yielded 14 with titers to SLE.

A total of 936 sera from patients with CNS symptoms were tested by HI against SLE, EEE, VEE, Dengue and CAL antigens. Twenty patients had constant titers to Group B antigens or EEE, indicating previous infection at some undetermined time. At this writing (August 29, 1980), there have been three confirmed and two presumptive cases of EEE, with two deaths. There have been two confirmed and one presumptive cases of SLE. All these cases of SLE live in the same general area in the panhandle of Florida.

The population of the Culex nigripalpus has been unusually low for the past 6 months due to the shortage of rainfall. No virus has been isolated from the mosquito pools tested.

(N.J. Schneider, F.M. Wellings, E.E. Buff and J.A. Mulrennan, Jr.)

REPORT FROM THE VIRAL AND RICKETTSIAL PRODUCTS BRANCH,  
BIOLOGICAL PRODUCTS DIVISION, BUREAU OF LABORATORIES,  
CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA 30333

Modification of a Production Procedure for Arbovirus Antigens

In 1958, Clarke and Casals described the sucrose-acetone extraction procedure for producing arbovirus complement-fixing (CF) antigen and hemagglutinating antigen (HA) from infected mouse brains (Am. J. Trop. Med. & Hyg. 7:561-573, 1958). This procedure has been used successfully by many investigators to produce a great variety of arbovirus antigens. We find it to be quite satisfactory for producing most of our arbovirus antigens; however, we are concerned about the potential biohazards associated with processing large volumes of infectious brain suspension.

Since most arboviruses are not inactivated by acetone, antigens extracted by the Clarke and Casals procedure are infectious. It is possible, however, to inactivate extracted antigens by gamma irradiation or by treatment with certain chemicals. This renders the finished product safe for shipping and for use in the serology lab; however, it does not remove the hazards associated with the extraction procedures.

We have modified the Clarke and Casals procedure by inactivating the brain suspension before extraction with acetone. After aspirating brains from suckling mice, we make a 40% brain suspension in borate saline (pH 9.0) by homogenizing in an ice bath for 2 minutes. We then add enough 1.0 M Tris buffer (prepared in borate saline at pH 9.0) to the brain suspension to equal one-tenth of the volume of brain suspension. Betapropiolactone (BPL), prepared as a 10% solution in physiological saline, is added to give a final concentration ranging from 0.05 to 0.4%, depending upon the arbovirus being inactivated. The suspension is stirred gently at 40°C for 18 to 24 hours. After inactivation, the brain suspension is diluted with an equal volume of aqueous 17% sucrose solution. The noninfectious brain suspension is then extracted with acetone as originally described by Clarke and Casals.

We have found this modified extraction procedure to be satisfactory for producing the following CF and HA antigens:

Eastern equine encephalomyelitis  
Western equine encephalomyelitis  
Venezuelan equine encephalomyelitis  
St. Louis encephalitis  
Yellow fever  
Powassan  
Rocio  
Colorado tick fever  
La Crosse (CF antigen only)



The modified extraction procedure is not consistently reproducible for all four types of dengue viruses; therefore, we still use Clarke and Casals' procedure to produce some of the dengue antigens.

(W. Adrian Chappell, Lendell A. White, Dane W. Sanderlin, and Baldev K. Nottay)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, THE UNIVERSITY OF  
MARYLAND MEDICAL SCHOOL, BALTIMORE, MARYLAND 21201

Immunoprecipitation Analysis of Proteins Specified by  
Phlebotomus Fever Serogroup Viruses.

There are currently 31 serologically distinct viruses which have been placed in the phlebotomus fever serogroup on the basis of reciprocal HI reactions. The majority of these viruses have not been examined by morphological or biochemical means; however, where such data do exist these viruses appear to satisfy the criteria for classification as bunyaviruses and are currently forming the genus, phlebovirus, among the Bunyaviridae family. Analysis of the virus-specified proteins of these agents has been complicated by several observations, specifically 1) virus particles tend to be unstable during purification on density gradients, 2) the inhibition of host cell-specific protein synthesis following infection by these viruses is both delayed and incomplete, and 3) DNA-specific transcription inhibitors (actinomycin D, DRB) in concentrations required to depress host cell synthesis also partially inhibit the synthesis of viral proteins.

We have found that immune selection of isotopically labeled viral proteins, either by direct or indirect precipitation or by immuno-affinity chromatography (1), followed by polyacrylamide gel electrophoresis and fluorography to be highly sensitive and specific procedures for the identification and analysis of the virus-specific proteins of this group of viruses. The specificity of precipitation is easily controlled in parallel experiments using mock-infected cells or normal ascitic fluid and by the demonstration of the same polypeptides in infected, but unrelated, host systems (vero and BHK cell cultures). These procedures have proved useful in the analysis of viral proteins in partially purified virions, in lysates of infected cells prepared with non-ionic detergents (1), and in *in vitro* translation systems. In addition, immunoprecipitation methodology has allowed us to determine the specificity of monoclonal antibodies and in tracing the cross-reacting determinants of several phlebotomus virus agents to specific viral proteins. We report here the characterization of the major proteins coded by 12 different phlebotomus agents.

Bunyaviruses (see references 2 and 3 for review) have generally been reported to contain three major structural proteins, two glycoprotein species and an unglycosylated, internal nucleocapsid protein complexed with the viral RNA segments. In addition, the negative-stranded nature of the genomes of these viruses also presupposes a virion-associated polymerase. Immunoprecipitation and polyacrylamide gel analysis of the 12 phlebotomus viruses listed below (Table 1) has shown that although there are clearly broad similarities among the major virus-coded proteins, each produces a characteristic polypeptide profile when run in parallel on discontinuous slab gels. Although it might be expected that different antisera preparations would give varying results, this has not been our experience insofar as mouse ascitic fluids or antisera prepared here, or obtained from Dr. W. Brandt and Dr. J. Dalrymple (Walter Reed Army Institute of Research), or from Dr. C. J. Peters (US Army Medical Research Institute of Infectious Diseases) have all produced identical gel profiles.

These polypeptides listed as structural polypeptides were identified either by their presence in peak fractions of density gradients (assayed for infectivity or by electron microscopy) or in direct immunoprecipitates of intact viral particles from clarified and concentrated media preparations. Glycoproteins were identified by monitoring the incorporation of D(2-<sup>3</sup>H-mannose) rather than L-(4, 5-<sup>3</sup>H-leucine). Although

heterogeneity was observed in the glycoprotein species of all of the viruses examined, only those from Punta Toro, Arumowot, Naples and Saint-Floris were sufficiently resolved to exclude the possibility that multiple banding was due to glycosylation microheterogeneity. However, when Karimabad viral proteins were immunoprecipitated from cells treated with Tunicamycin, or from *in vitro* translation lysates, two unglycosylated polypeptides of slightly increased mobility were regularly observed. It is therefore considered probable that each of these viruses codes for two glycoproteins of distinct amino acid sequence but of similar molecular weight. This is in marked contrast to results reported for several other bunyaviruses groups in which the two glycoproteins exhibit widely divergent molecular weights (2, 3). A fourth structural polypeptide has been detected in immunoprecipitates from Karimabad and Punta Toro virions, or cells infected with these viruses, which possesses a molecular weight in excess of 140,000 and conceivably could represent the required particle-associated polymerase.

Using homologous hyperimmune ascitic fluids prepared by the immunization of adult mice with infected suckling mouse brain homogenates, all of these structural proteins were detected in <sup>3</sup>H-leucine-labeled lysates of infected cell cultures. However, other polypeptides were also detected with these antibody preparations which are apparently virus-coded insofar as they are not precipitated by normal ascitic fluids or from uninfected cells, but are detected in both infected BHK and vero cells. These are tentatively considered to be non-structural proteins and are currently under study using peptide analysis and monoclonal antibodies to determine if they contain amino acid sequences homologous to the known structural proteins. It should be noted that the estimated molecular weight of several phlebotomus virus genomes (4) is in excess of that required to code for the known structural proteins, and at least theoretically could code for additional proteins with unique functions. Pulse-chase procedures, in the presence or absence of amino acid analogues, have failed to demonstrate precursor-product relationships among any of the proteins precipitated from Karimabad or Punta Toro virus-infected cells. Such experiments, however, cannot eliminate the possibility of rapid precursor processing, and preliminary tryptic peptide analysis has suggested that the Karimabad virus 25K and the 30K polypeptides appear to share approximately 20% of their leucine-containing peptides. Assuming that all viral RNA sequences are transcribed into single unambiguous messenger RNA molecules, the reason for this apparent homology remains unclear.

Another useful application of immunoprecipitation lies in reacting lysates of infected cells with heterologous antisera. Electrophoretic analysis of the precipitated polypeptides will then indicate which polypeptides contain cross-reacting determinants which should include, but not be limited to, those determinants monitored by reciprocal HI or neutralization tests. We have analyzed, for example, the reactivity of several heterologous antisera to lysates of Punta Toro virus-infected cells and have shown that 1) antisera raised against Rift Valley Fever virus or Karimabad virus precipitates the Punta Toro virus nucleocapsid protein (27K) and the 56K glycoprotein, 2) antisera prepared against Saint-Floris reacts with the 66K Punta Toro virus glycoprotein, 3) antisera prepared against Gordil virus precipitates only the Punta Toro virus nucleocapsid protein, and 4) antisera prepared against Sicilian, Rio Grande, or Arumowot are non-reactive in this system. When expanded, such data may be useful in determining whether genome segment reassortment has occurred in nature and also, by comparison to HI or neutralization tests, which polypeptides contain the determinants responsible for these serological reactions. It would seem, however, that the phlebotomus group viruses are rather distantly related. By contrast, antisera prepared against LaCrosse, Snowshoe Hare, Trivattatus, or CEV, when reacted against lysates of LaCrosse virus-infected cells, all generate identical polypeptide patterns - indicating that conserved antigenic determinants exist on all major polypeptides.

Table 1

APPARENT MOLECULAR WEIGHTS ( $\times 10^{-3}$ ) OF POLYPEPTIDES  
SPECIFIED BY SELECTED PHLEBOTOMUS SEROGROUP VIRUSES

	<u>NUCLEOCAPSID</u>	<u>STRUCTURAL GLYCOPROTEIN</u>	<u>OTHER</u>	<u>PUTATIVE NONSTRUCTURAL</u>
Punta Toro	27	66, 56	>140	(46?), 31, 25, 22
Karimabad	24	59	>140	74, 51, 31, 22*
Arumowot	23	59, 54		
Naples	25	59, 50		74
Sicilian	26	59		74, 31
Rio Grande	32	66		
Saint-Floris	26	66, 52		74
Itaporanga	24	66		26, 35
Chagres	24	59		
Candiru	26	63		
Gabek Forest	24	57		32
I-47	27	59		

\* detected only in in vitro translation of viral mRNA.

(J. Smith, D. Prather, D. Pifat)

1. Smith, J. F. and D. T. Brown (1977). Envelopment of Sindbis Virus: Synthesis and Organization of Proteins in Cells Infected with Wild Type and Maturation-Defective Mutants. *J. Virol.* 22:662-678.
2. Bishop, D. H. L. and R. E. Shope (1979). Bunyaviridae. In *Comprehensive Virology*, Volume 14, Plenum Press, New York, pp 1-156.
3. O'Bijeski, J. F. and F. A. Murphy (1977). Bunyaviridae: Recent Biochemical Developments. *J. Gen. Virol.* 37:1-14.
4. Robeson, G. and L. H. EL Said, W. Brandt, J. Dalrymple, and D. H. L. Bishop (1979). Biochemical Studies on the Phlebotomus Fever Group Viruses (Bunyaviridae Family). *J. Virol.* 30:339-350.

REPORT FROM THE DIVISION OF VIROLOGY AND IMMUNOLOGY  
BUREAU OF LABORATORIES  
PENNSYLVANIA DEPARTMENT OF HEALTH  
LIONVILLE, PENNSYLVANIA

Arbovirus Surveillance in Pennsylvania, 1980

The Pennsylvania Departments of Health and Environmental Resources reinstated, on a somewhat larger scale, an arbovirus surveillance program similar to ones conducted during the summers of 1978 and 1979.

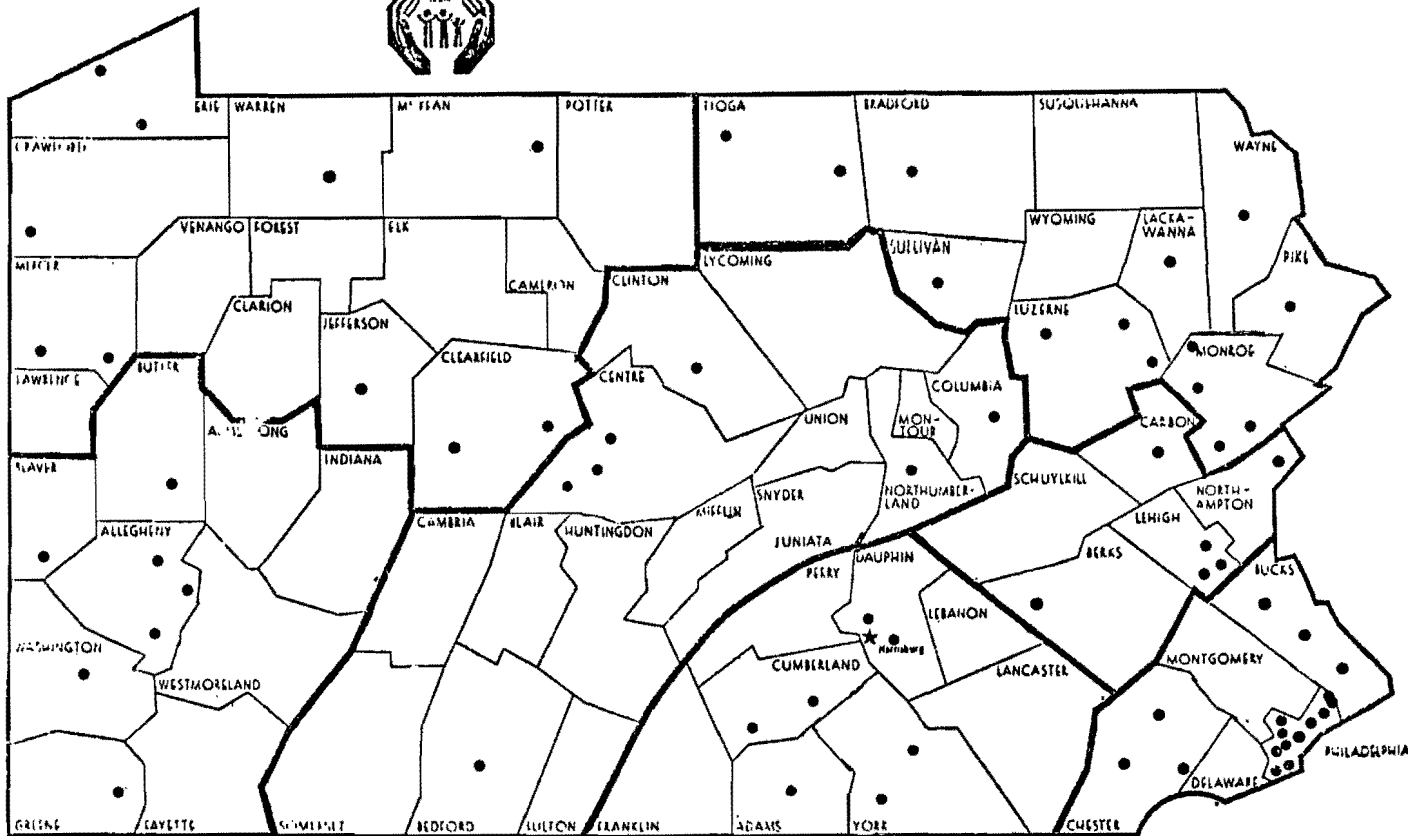
Sentinel flocks of four (4) cockerels were placed at 68 sites throughout the state at the locations shown in the Figure. This compares with 50 sentinel sites in 1979. This year, there was at least 1 sentinel flock in 36 of the 67 counties as compared with coverage in 28 counties in 1979. The cockerels were bled weekly and the sera tested, after protamine sulfate-acetone extraction, for hemagglutination-inhibition (HI) antibodies against St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), Eastern Equine Encephalitis (EEE), and California Encephalitis (CE) viruses. There were no seroconversions in 5376 HI tests performed through August 20, 1980.

Through August 20, 1980, twenty-three (23) patients with a clinical diagnosis of central nervous system disease were tested for serologic evidence of infection with SLE, WEE, EEE, and CE viruses. Serum samples collected from a 7-year-old boy 2 and 9 days after onset of encephalitis had stationary titers of 1:10 against SLE. The patient died on the day of the second serum collection and there were no autopsy specimens. There was no evidence of other cases.

The surveillance program will be continued through September 1980.

(Bruce Kleger, Philip Nash, and Vern Pidcoe)

SENTINEL POULTRY FLOCK LOCATIONS  
 Encephalitis Surveillance Program, Pennsylvania, 1980



REPORT FROM THE STATE OF NEW JERSEY DEPARTMENT OF HEALTH

Trenton, NJ 08625

The following is a report of arbovirus activity in New Jersey for the third quarter, 1980.

Table 1  
Isolations from Arthropods in New Jersey\*  
3rd Quarter 1980

<u>Group</u>	<u>EE</u>	<u>WE</u>	<u>Isolated from</u>	<u>Collected in</u>	<u>Month</u>
A		1	C. melanura	Bass River	July
A	1		C. melanura	Bass River	August
A	1	1	C. melanura	Burlington Co.	August
A		8	C. melanura	Dennisville	July
A	5	5	C. melanura	Dennisville	August
A	3		C. melanura	New Gretna	August
A	1	9	C. melanura	Woodbine	July
A		2	C. salinarius	Woodbine	July
A	1	2	C. melanura	Woodbine	August
A		1	C. salinarius	Woodbine	August
Totals: 12		29			

\*These include collections from the New Jersey Agricultural Experiment Station, Rutgers University.

Table 2  
Isolations from Avian Bloods in New Jersey\*

<u>Species</u>	<u>Isolate</u>	<u>Date Collected</u>	<u>Location</u>
Blue Jay	EE	8-5-80	Dennisville
Wood Thrush	EE	8-6-80	"
Oven Bird	WE	8-6-80	"
Oven Bird	EE	8-7-80	"
Black Capped Chickadee	WE	8-7-80	"
Purple Crackle	WE	8-13-80	"

\*Collected by the New Jersey Agricultural Experiment Station, Rutgers University.

(W. Pizzuti)

REPORT FROM THE STATE OF NEW YORK DEPARTMENT OF HEALTH  
DIVISION OF LABORATORIES AND RESEARCH, ALBANY, NEW YORK

Arbovirus Surveillance, 1980

During a two-month period ending August 15, 1980 a total of 1,858 pools of wild-caught mosquitoes (approximately 50-100 per pool) were received and tested. Aedes mosquitoes accounted for 1,298 of these pools. Isolates of California encephalitis complex virus were obtained only from 14 of 885 pools of Aedes mosquitoes collected in the Northeastern region of the state.

Blood samples from 97 patients with signs of acute infections of the central nervous system were received from May to August and tested by HI with California encephalitis, Powassan, St. Louis encephalitis, and Eastern and Western equine encephalitis antigens. In none of the patients was there serologic evidence that these arboviruses were involved in the etiology of their disease.

(Sunthorn Srihongse, Margaret A. Grayson and Rudolf Deibel)



Report from **Yale Arbovirus Research Unit**  
Yale University School of Medicine  
Department of Epidemiology and Public Health  
60 College Street  
New Haven, CT 06510

Colorado tick fever virus is a member of the family, Reoviridae, and the genus, Orbivirus. The genomes of the five genera of Reoviridae are segmented, double-stranded(ds) RNA, and the number of segments is ten to twelve depending on the genus. In general, orbiviruses exhibit ten segments of dsRNA. Colorado tick fever virus is a novel member of the genus because its genome is comprised of twelve segments of dsRNA.

Colorado tick fever was grown in BHK-21 clone 13 cells and purified following the reovirus protocols. The dsRNA was purified from the virus sample following a SDS, chloroform-isoamyl alcohol extraction procedure. The dsRNA segments were end labeled with (5'-<sup>32</sup>P)pCp and separated by electrophoresis through a 10% acrylamide gel. Examination of an autoradiograph of the gel revealed that CTF virus has twelve equimolar segments of dsRNA. The molecular weight of the dsRNA segments were calculated using the reported values of reovirus 3D, and the data suggest that CTF virus exhibits an apparent total molecular weight of  $18 \times 10^6$  daltons which is significantly larger than those of the other Reoviridae.

Three additional isolates of CTF were similarly analyzed, and they also exhibited twelve bands. The isolate used in this study was CTF-Florio strain. The dsRNA profile of 24 clones of this isolate which were randomly picked from plaques also yielded twelve segments, and one clone was plaque-purified three times in BHK-21 cells. A tissue culture virus stock was prepared of the multiply cloned virus, and a suckling mouse brain virus stock was also prepared. Identical dsRNA profiles were observed using both virus stocks as inoculum. Complement-fixation test were also conducted using this cloned virus and NIH reference reagents. The results of these tests confirm that CTF-Florio strain virus belongs to CTF serogroup.

Since CTF virus is a novel member of the genus Orbivirus, additional characterization of the viral genome is needed. Then, a re-evaluation of its taxonomic status may be appropriate.

D. L. Knudson

REPORT FROM THE STATE LABORATORY INSTITUTE, MASSACHUSETTS DEPARTMENT PUBLIC HEALTH

305 South Street, Boston, Massachusetts 02130

Eastern Equine Encephalitis Surveillance in Massachusetts, 1976-1979

Horses described as dying of "brain" disease during the "wet" summer of 1831 appears to be the earliest recorded observation of equine encephalitis of arboviral origin in Massachusetts. Eastern equine encephalitis (EEE) was first recognized as the cause of human disease in Massachusetts in 1938 during the largest outbreak documented in the state. Several hundred horse deaths occurred and thirty-four human infections were identified. In 1955 and 1956 sixteen human cases of EEE were identified; ten were fatal.

Epidemiologic surveillance of human and horse disease and mosquito infections for EEE and western equine encephalitis (WEE) or Highland J, as the eastern variant of WEE has been recently designated, was instituted in 1956. Studies were also undertaken at that time in a search for mammal, reptile and amphibian reservoirs; for amplification of virus in bird populations; climatologic factors; and for human mosquito vector(s).

In 1973 through 1975 another cycle of intense EEE activity occurred, resulting in six fatal human EEE infections and twenty-six horse deaths. One hundred seventy-eight (178) isolates of EEE were obtained from nine thousand two hundred and two (9202) mosquito pools. During 1973, EEE was isolated from thirty-three birds.

Budget restraints forced curtailment of bird surveillance in 1974. Vandals and monetary constraints have not permitted the use of sentinel animals or birds for systematic studies on transmission rates. In addition, active and effective immunization of horses against both EEE and WEE in 1974 has essentially eliminated the horse as a sentinel. With elimination of the horse and the inability to sustain long-term bird studies, intensified mosquito surveillance along with climatologic analysis have become the main mechanisms for predicting and assessing epidemiologic risk of human infection.

An extensive description of human disease, attack rates in horses and mosquito analyses in Massachusetts from 1957 through 1976 has been presented in a previous publication.<sup>1</sup>

The present report summarized results of mosquito, horse and human EEEV surveillance from 1976 through 1980 (Table 1).

<sup>1</sup> Grady, GF, Maxfield HK, Hildreth SW, Timperi RJ, Gilfillan RF, Rosenau BJ, Franczy BD, Calisher CH, Marcus LC, Madoff MA: Eastern equine encephalitis in Massachusetts, 1957-75: a prospective study entered upon analysis of mosquitos. *Am J Epidemiology*, 107: 170-178, 1978.

Table 1

EASTERN EQUINE ENCEPHALITIS (EEE) VIRUS INFECTIONS IN MOSQUITOS, HORSES  
AND HUMANS IN MASSACHUSETTS, 1976-1980

Year	Mosquitos			Horses	Human
	Mosquito pools tested	EEEV/total mosquitos tested	Minimum infection rate per 1000	confirmed/suspected	identified/tested
1976	1957	3/27,005	0.11	0/0	0/56
1977	2547	34/65,174	0.52	1/3	0/139*
1978	3607	37/64,167	0.58	5/7	0/95
1979	2583	56/44,358	1.26	2/4	0/92
1980**	1864	2/31,860	0.10	1/1	0/19

\* During serosurveillance in September 1977 a high level of plaque reducing neutralizing (PRN) antibody to EEEV was detected in an infant suffering from meningoencephalitis. Antibody to EEEV was subsequently detected in the infant's asymptomatic mother. Multiple specimens from the infant and mother were obtained and Cocksackie B1 was isolated from the infant's stool. Sucrose fractionation of mother's and infant's serum specimens showed PRN in the IgG but not IgM fractions suggesting infection at undetermined time in past in the mother with passively transferred antibody in the baby. The mother who lived near the eastern New Hampshire border suffered an undiagnosed meningoencephalitis in September 1973.

\*\* Data through September 2.

(Robert Gilfillan, Barbara Rosenau, Herbert Maxfield, Wayne Andrews and Leonard Marcus)

REPORT FROM THE VIROLOGY RESEARCH CENTRE, INSTITUT ARMAND-FRAPPIER (I.A.F.) CITY OF LAVAL, AND THE BITING INSECT RESEARCH GROUP, TROIS-RIVIERES, QUEBEC, CANADA

Follow-up studies on California group virus activity in Entrelacs (Province of Quebec) during 1979

Field work was undertaken during spring and summer of 1979 in Entrelacs, a region where human cases of California encephalitis (CE) had been serologically diagnosed, a year before, in order to detect CE group virus (es) as well as to determine the persistence of these viruses within the area with their vectors. A previous report (Arthropod-Borne Virus Information Exchange 37: 145, 1979) had shown that seroconversions to CE antigens were detected in 6 indicator rabbits and that 2 virus strains were isolated from the viremic phase serum of the rabbits. The viruses were identified as members of the CE virus group.

Since then, one additional isolate obtained from the viremic blood of a rabbit was also identified as a CE group member. Neutralization tests were carried out with Snowshoe hare, La Crosse and Jamestown Canyon viruses on serial serum samples from two of the sentinel rabbits : results showed highest antibody titers to Snowshoe hare virus.

From 17116 mosquitoes tested, 21 virus strains were isolated and identified as members of the California group. Of the mosquitoes tested 52% were identified as Aedes punctator, 27% as Ae. communis, 5% as Ae. excrucians and these showed a minimal field infection rate of 1:1203, 1:431 and 1:466 respectively.

One additional strain was isolated from Culex pipiens and was identified as Flanders virus by H. Artsob (National Arbovirus Reference Service).

One virus strain of the California group was isolated from a pool of Ae. communis adults reared in the laboratory from field collected larvae.

The high number of isolates obtained from mosquitoes and the seroconversions observed in indicator rabbits suggest important CE group virus activity in the region of Entrelacs (Laurentians, Province of Quebec).

The isolation of a virus in adult mosquitoes reared from field collected larvae adds an element of comprehension of the mode of persistence of these viruses in Entrelacs, via transovarian transmission.

The vectors Ae. communis, Ae. punctor and Ae. excrucians accounted for the predominant species and their respective minimum field infection rates have shown a strong prevalence of California group virus (es) in this region.

Results of neutralization tests with convalescent phase rabbit sera incriminate Snowshoe hare as the California group virus present in Entrelacs.

L. Poulin, S. Belloncik and M. Fauvel, Virology Research Centre,  
Institut Armand-Frappier, City of Laval, Quebec, Canada.

A. Maire and A. Aubin, Biting insect research group, Trois-  
Rivières, Quebec, Canada.

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE,  
DEPARTMENT OF MEDICAL MICROBIOLOGY,  
UNIVERSITY OF TORONTO,  
TORONTO, ONTARIO, CANADA.

Isolation of an Agent from Dermacentor Variabilis in Ontario, 1979.

In 1979 a total of 877 ticks was collected from parts of Ontario to monitor for arboviruses. This included 367 Ixodes cookei, 3 I. angustus and 507 Dermacentor variabilis. More complete information concerning the ticks collected is presented in Table 1.

The ticks were separated into 53 pools, ground up, and injected into 3-5 day old Swiss white mice by intracerebral (i.c.) inoculation. A total of 5 isolates was obtained. All isolations were achieved from D. variabilis pools collected at the Six Nations Reserve near Brantford. Four of the pools from which an isolate was obtained were collected on June 19 while the fifth pool was collected on July 16.

The isolates obtained, all of which appear to be identical, are currently unidentified. They killed suckling Swiss white mice 5 days post i.c. inoculation and adult mice after intraperitoneal inoculation but did not propagate in several tissue culture systems including BHK-21, Vero, LLC-MK2 and Madin-Darby canine Kidney. The isolates were ether sensitive.

No haemagglutinin was demonstrated from sucrose-acetone extracted mouse brain material of one isolate. Furthermore no reaction was observed between this isolate and ascitic fluids prepared to arboviruses isolated previously in Canada nor with 26 grouping ascitic fluids supplied by NIH.

These isolates appear not to be adventitious mouse agents since re-isolation was achieved from original material. In addition, one of eleven dogs from the area in which the ticks were collected showed complement fixing antibodies to these isolates.

Attempts to further characterize these isolates are in progress.

H. Artsob, L. Spence and C.Th'ng, National Arbovirus Reference Service, Toronto, in collaboration with G.A. Surgeoner and J. McCreadie, Department of Environmental Biology, University of Guelph.

Table 1  
 Ticks collected in Ontario in 1979 for arbovirus screening

TICK SPECIES	AREA OF COLLECTION	TOTAL TICKS	TOTAL POOLS
<i>Ixodes cookei</i>	Buckhorn	1	1
<i>Ixodes cookei</i>	Erin	15	1
<i>Ixodes cookei</i>	Guelph	173	13
<i>Ixodes cookei</i>	Orangeville	71	6
<i>Ixodes cookei</i>	Stratford	1	1
<i>Ixodes cookei</i>	Warkworth	106	11
<i>Ixodes angustus</i>	Buckhorn	3	1
<i>Dermacentor variabilis</i>	Bradford	35	2
<i>Dermacentor variabilis</i>	Guelph	1	1
<i>Dermacentor variabilis</i>	Six Nations Reserve	471	16

REPORT FROM THE LABORATORY FOR ARBOVIRUS RESEARCH AND SURVEILLANCE,  
DEPARTMENT OF BIOLOGY, UNIVERSITY OF NOTRE DAME, NOTRE DAME, INDIANA

This summer we again participated in an arboviral encephalitis surveillance program with the Indiana State Board of Health; this cooperative program has continued since early 1977.

We saw a sudden rise in St. Louis encephalitis virus antibody prevalence rates late last summer in juvenile house sparrows from the southwestern area of Indiana coincident with the onset of SLE in the first of 4 persons in the state. One juvenile sparrow was collected in Evansville on 8/27; the HI titer was  $\geq 1:80$ . Onset of the first human illness in Evansville was 9/10 and the second on 10/17. The other 2 cases occurred in areas of the state where avian surveillance was not conducted. However, onset in these two cases was within the 9/10-10/17 period.

Culex spp. populations were higher than usual in numerous areas of the state late in the summer and early fall of 1979 and it was assumed that a large overwintering population entered hibernacula. The very mild winter that extended into early February raised concern that the summer of 1980 might see increased SLE virus amplification. A period of cold weather in February and March followed by a cool dry spring apparently contributed to reduced Culex spp. populations this past spring. Areas of Indiana where SLE virus has been enzootic in past years were approaching drought conditions by late June. We saw no early-season SLE virus activity as we had seen every previous spring; only one sparrow was found by late June with antibody titers (HI and SDN) suggestive of a recent infection. This bird was captured in Bartholomew County in mid-June. Bartholomew County had the second highest case rate (71.9/100,000) nationally in the 1975 epidemic. We have found seropositive birds with titers suggestive of recent infection in that county every year of our survey (1977-1980).

The rain pattern has been quite varied over the state while all of Indiana has experienced higher than normal temperatures. To date (8/26) there have been only a few additional seropositive birds, none with elevated titers suggestive of recent infection with the exception of one juvenile that was HI negative but had a SDN titer of 1:4. It does not appear at this time that SLE virus amplification has been sufficient to produce many human cases. A few might still be expected, however; the low level endemicity of SLE virus has been apparent in the state since 1975. A total of 5,300 avian sera (primarily house sparrows) has been tested by HI for antibodies to SLE, EEE, and WEE viruses. No birds have been seropositive for either of the latter two viruses. This could rapidly change as there currently is an epizootic of EEE in horses just across the state line in Michigan (just north of Indiana's La Porte and St. Joseph Counties) which CDC/Ft. Collins is investigating.

No cases of La Crosse encephalitis have been reported in Indiana this year. Four were confirmed last year, however. We feel that



case recognition remains the greatest problem in detection of this disease in the state.

Our statewide serological survey of 10,200 Hoosiers for antibodies to 6 arboviruses revealed a 1.7% antibody prevalence rate for LAC virus in Marion (Indianapolis) County. This summer a network of 300 ovitraps were set out in that county; the target is transovarially infected Aedes triseriatus eggs. Oviposition substrate strips are being collected weekly, eggs hatched and larve (4th instar) pooled. We expect to complete virus isolation trials by next spring.

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We are continuing our studies on the genetics of susceptibility of Ae. triseriatus to LAC virus by selecting for resistant and susceptible lines of this mosquito. We are additionally selecting for genetic marker stocks of this species and the sibling species, Aedes hendersoni, for use in formal genetic studies and analysis of viral susceptibility. We have found that Toxorhynchites brevipalpus intrathorasic inoculation of LAC virus followed by immunofluorescent detection is a sensitive assay for this agent; this is consistent with other reports of the use of this technique for dengue viruses.

P. R. Grimstad

REPORT FROM THE ARBOVIRUS SURVEILLANCE PROGRAM

Division of Laboratories  
Illinois Department of Public Health  
Chicago, Illinois

Surveillance activities in Illinois this year have emphasized collection and testing of avian sera for hemagglutination-inhibition (HI) antibodies to St. Louis encephalitis (SLE) virus. Through August 26, 33 of 3,333 (1%) birds tested had HI antibodies to SLE virus (Table 1). This group consisted of 20 juvenile house sparrows (Passer domesticus), 12 adult house sparrows, and one common grackle (Quiscalus quiscula). This is almost three times as many positives as the 13 of 3,788 (0.3%) we found with SLE antibodies during the entire 1979 season. Sampling patterns were basically the same both years. The principal area of virus activity this year from the areas we sampled was near East St. Louis, adjacent to St. Louis, Missouri. When a direct comparison of the seven frequently sampled central and southern Illinois counties is made, the percentage of juvenile birds with SLE antibodies is 1.8 for June, 1.2 for July, and 1.5 for August.

Even with the increase in activity, relative to last year, no human SLE infections have been detected. Typically, we have had two confirmed cases of California Encephalitis (due to LaCrosse virus) and both were boys from Peoria County. They were 8 and 9 years of age and had onsets of June 20 and 28, respectively. Four additional clinical cases with single high titers are under investigation.

Testing was recently completed on 44,766 Aedes triseriatus mosquitoes that originated from 15 sites in Peoria County and five forest preserves in suburban Cook County (Table 2). These mosquitoes were collected as eggs in ovitraps. The ovitrap consisted of 12 ounce beverage cans with top removed and painted black and contained a 2x6x1/16 inch balsa stick, water, and a small amount of leaf litter. Traps were attached near the base of a tree and balsa sticks were removed and replaced at monthly intervals. Forty-six traps were operated in Peoria County from early June through late September and 100 were run from late June through late September in Cook County.

The 342 pools tested from Peoria County yielded 23 isolates of a California group virus closely resembling LaCrosse while 753 pools from Cook County yielded 11 similar isolates. The focal nature of LaCrosse transmission is underscored by viral isolations from three individual egg sticks in Cook County and from five of the 15 Peoria sites.

In 1980, we extended this technique to 10 state parks in Illinois, ranging geographically from near the Wisconsin state line on the north to the Ohio River on the south. In each park, 60 ovitraps will be run from mid-July to mid-August and from mid-August to mid-September. The purpose of this study is to begin delineation of LaCrosse virus distribution in the state. An extensive Culex ovitrap study was also begun this year. Ovitrap containing an alfalfa infusion were operated on a daily basis in 13 communities across the state. They appear to be effective in monitoring the gravid segment of the Culex population.

(Gary G. Clark and Harvey L. Pretula)

Table 1.

		AVIAN SEROLOGY - ILLINOIS - 1980																	
REGION	COUNTY	Collection Interval																	
		May 4-10		May 25-31		June 8-14		June 22-28		June 29-July 5		July 20-26		Aug 3-9		Aug 10-16			
		HY*	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages
NORTH	COOK		0/3					0/6	0/10	0/97	0/192			0/163	0/201				
	WILL															2/3	2/4		
CENTRAL	CHRISTIAN	0/4	0/10	0/4	0/14	0/22	1/37	0/26	0/44			0/91	0/111			0/9	0/10		
	CUMBERLAND	0/3	0/35	0/43	0/73	0/73	0/93	1/29	2/69			1/79	1/100	0/58	0/63				
	JASPER	0/14	0/14																
SOUTH	MADISON	0/4	0/5	0/8	0/9	0/7	0/36												
	ST. CLAIR	0/13	0/42	0/38	1/110	0/40	0/139	1/23	3/106			4/141	5/191			4/160	4/201		
	RICHLAND	0/25	0/25	0/84	0/116	0/138	1/158	1/61	5/110			1/91	2/108			0/94	0/104		
	WHITE	0/19	0/19	0/63	0/76	1/82	1/111	0/49	0/95			1/88	1/112			2/62	2/89		
	HAMILTON	0/17	0/17	0/9	0/21	0/10	0/20	0/2	0/2			0/100	0/117			0/5	0/9		
	SALINE			0/11	0/19			1/30	2/52			0/13	0/27			0/3	0/4		
	TOTAL (PERCENT POSITIVE)	0/99	0/170	0/260	1/438 (0.2)	1/372 (0.3)	3/594 (0.5)	4/226 (1.8)	12/ 488 (2.5)	0/97	0/192	7/603 (1.2)	9/766 (1.2)	0/221	0/264	8/336 (2.4)	8/421 (1.9)		

\*Matching year; includes older nestlings, fledglings, and juvenile birds.

\*\*Number positive (MAI antibody titer to St. Louis Encephalitis (SLE) virus of 1:20 or greater)/Number tested.

Table 2. Virus isolations from Aedes triseriatus mosquitoes collected as eggs from two Illinois counties, 1979.

COUNTY	MONTH				TOTAL	
	JUNE	JULY	AUGUST	SEPTEMBER		
	No. Tested/ Pools (Pos*)	No. Tested/ Pools (Pos.)	No. Tested/ Pools (Pos.)	No. Tested/ Pools (Pos.)	No. Tested/ Pools (Pos.)	
Cook		7665/215 (0)	7412/209 (0)	14095/329 (11)	29172/753 (11)	
Peoria	1062/32 (0)	4839/105 (3)	5798/129 (19)	3295/76 (1)	14994/342 (23)	

\* Isolations are all California group viruses closely resembling LaCrosse.

REPORT FROM THE ZOOSES RESEARCH LABORATORY, DEPARTMENT OF PREVENTIVE  
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Old automobile and tractor tires, containing transovarially infected Aedes triseriatus, are being increasingly found to be main sources of La Crosse (LAC) virus around cases of California (La Crosse) encephalitis in Wisconsin.

When discarded or used as swings or other playground equipment in shaded places, old tires often hold large amounts of rain water with tree leaves for extended periods of time and frequently become developmental sites for Aedes triseriatus. Although the natural habitat, basal tree-holes, are also found around homes of some of the cases, old tires are more often found during recent years as providing good oviposition sources closer to where children play.

During the past several years we have been studying sources of larvae and LAC virus around cases found across southern Wisconsin, including the endemic region in the western part, the central endemic edge, and the eastern non-endemic portion. Collections of larvae from tree-holes, old tires and other containers are processed for LAC virus by inoculation of pools into or feeding reared adults on new born mice. Some are held for transovarial and venereal as well as oral transmission studies of their natural maintenance mechanisms.

During 1979 our first isolations of LAC were obtained from larvae collected from an old tractor tire in the yard of the farm adjoining that of the last known fatal case of La Crosse encephalitis which had occurred in a 3-yr-old girl in western Wisconsin during July of 1978. A large number of old tires, cans, and an old boat had been removed from around the home of the fatal case by relatives, before virus studies could be done. No sources of Aedes triseriatus were found around this home when examined again during the spring of 1979, except for a tree-hole on the far edge of this farm, from which LAC was also obtained.

One of the first cases found during 1979, near Richland Center in the endemic region, was the daughter of a farmer who had a large car-crushing company and many old tires scattered about the farm, which he did not allow us to study. Studies around the farm home of a 9-yr-old boy who came down with encephalitis on August 3d south of the usual endemic region, near Blanchardville, found larvae in a dozen tires around his home, from which isolates of LAC virus were obtained.

An isolated focus of LAC virus activity, in eastern Wisconsin near Milwaukee, was further studied during 1979. First isolates of LAC virus from the eastern half of Wisconsin were made from Aedes triseriatus. Acquisitions of antibody were also found in chipmunks in the 20 acre woodlot around the home of a girl who had California (La Crosse) encephalitis occurring a couple of years before, indicating continuing virus activity in this isolated area; but serologic and virus isolation studies of surrounding woodlots were negative for LAC virus. Although the isolates were obtained from larvae in tree-holes, larvae were also found in about half of 35 old tires in the area. Some of the tires moved into this area in past years had a history of coming from the endemic region in western Wisconsin, but the source of LAC virus has not yet been determined. Further studies include fingerprint analysis of isolates by Dr. David Bishop of the U. of Alabama, which may provide further clues as to the origin of this LAC virus focus.

During this current 1980 season the earliest case studied, a 5-yr-old boy with onset on June 30th, had most likely exposure to mosquitoes around a town

dump in the lowlands just east of the endemic region in central Wisconsin, about 50 miles north of Madison. Isolates of LAC virus were obtained from numerous Aedes triseriatus larvae collected from hundreds of old tires in the dump and adjoining woods. No larvae were found in basal tree-holes in this woods. The numerous old tires in this dump had been discarded there from many different sources throughout the area.

A 9-yr-old boy from a small town at the south edge of the endemic region, 40 miles west of Madison, was hospitalized with convulsions and later appearance of LAC antibodies in his serum, with onset of disease on July 16th. He had been playing in a small woodlot near his home which had a tree house and also a large tractor tire filled with rain water and Aedes triseriatus larvae. When reared in the laboratory, some of the emerging females transmitted LAC virus to suckling mice.

Two other cases are presently under study. One is a 13-yr-old boy from a county-seat town of Darlington, in southern Wisconsin where cases have not been seen before, with hospitalization starting August 18th. The woodlot behind his home contained an old auto tire which had been placed there for a swing about four years ago, then it fell down into the weeds beside a wooden platform where the boys frequently play. It was full of rain water, old leaves, and several hundred Aedes triseriatus larvae--which are under virus study. No other sources were found around this home, but an old tractor tire full of similar larvae was found under some trees near the home of a playmate.

The other now under study is around the home of a 3-yr-old boy with onset of encephalitis on August 9th., and neutralizing antibodies to LAC virus. His main exposure to mosquitoes was around a farm house at the edge of Whitewater, outside the endemic region, in southeastern Wisconsin; in Waukesha county where cases have not previously been found. He had not travelled outside of the county for more than a month. His main source of exposure appears to be during play around 20 old auto tires found under trees at the edge of the front lawn and an old tractor tire under another tree on the lawn, filled with rain water and larvae which have now been collected for virus studies.

Although transovarially transmitted LAC virus is apparently widespread in Aedes triseriatus larvae in old tires as well as in tree-holes, isolation rates from most sources are much lower than filial infection rates observed in progeny of known infected females, which are usually 30-90%. Not all females depositing eggs in these sources are infected as a rule.

In summary: Old auto and tractor tires in shaded places are often filling with rain water, leaves, and Aedes triseriatus larvae in which LAC virus can be transovarially transmitted to progeny and emerging females ready to infect by bite, and males capable of spread to other females during mating. Old tires are helping to maintain LAC virus in endemic areas, and infecting man and animals. More old tires are being discarded around the country, with growing disposal problems. Some are being moved about from one part of the country to another. We are also concerned that they may be carrying along infected eggs and larvae which could be establishing foci of LAC virus activity outside of endemic regions.

(Wayne H. Thompson)

REPORT FROM THE DEPARTMENTS OF VETERINARY SCIENCE AND ENTOMOLOGY  
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The Role of Medium-sized Wild Mammals in the  
Epizootiology of LaCrosse Virus

Our previous laboratory investigations have shown that the red fox (Vulpes fulva) is an amplifying host of LaCrosse virus (LACV). The studies have shown all of 5 red foxes became viremic following the bite of a single LACV-infected Aedes triseriatus mosquito. Four of the 5 red foxes developed a 1-3 day viremia that exceeded the threshold of infection for A. triseriatus.

The goals of our latest research are to evaluate 1) the susceptibility of other medium-sized wild mammals [raccoon (Procyon lotor) and opossums (Didelphis virginiana)] to LACV infection transmitted by mosquitoes, and the capability of these mammals to serve as sources of infectious blood meals under simulated natural conditions in the laboratory; 2) the prevalence of LACV infection in free-ranging red foxes, gray foxes (Urocyon cinereoargenteus), raccoons and opossums; 3) the susceptibility of sentinel red foxes to LACV infection in the field and 4) the epizootiological relationship of a natural predator such as the red fox to a natural prey species such as a chipmunk (Tamias striatus), both of which are involved in the LACV amplification cycle.

Susceptibility to infection, resulting viremia and antibody responses and potential to provide infectious blood meals to A. triseriatus were determined and compared for raccoons and opossums. Neither raccoons nor opossums were as susceptible to LACV infection as were red foxes. Only 1 of 5 raccoons became viremic following the bite of a single LACV infected A. triseriatus. Viremia titer was low and persisted only 1 day. Four of 5 raccoons developed LACV neutralizing antibody titers. Biological transmission of LACV from the infected raccoons to A. triseriatus did not occur. None of the opossums engorged upon by infected A. triseriatus became viremic and only 2 developed LACV neutralizing antibody titers.

Natural infection of sentinel red foxes and free-ranging red foxes, gray foxes and raccoons with LACV was demonstrated. One isolate of LACV was obtained from a sentinel red fox in an enzootic area. The viremia titer of the LACV infected red fox was above the threshold of infection for A. triseriatus mosquitoes. Antibody responses were measured by the microneutralization test employing four California group viruses: LAC, snowshoe hare (SSH), trivittatus (TVT), and Jamestown Canyon (JC). Four of 6 sentinel red foxes developed LACV neutralizing antibody. Antibody titers peaked between day 7 and 34 and were still detectable by day 345 post-infection. Homologous LACV antibody titers were consistently 4-fold or greater than heterologous titers.

Serological evidence for natural infection of LACV in 33 of 57 (58%) free-ranging red foxes, 18 of 32 (57%) gray foxes, and 4 of 16 (25%) raccoons was demonstrated. Antibody titers in free-ranging and experimental mosquito-transmitted virus infected red fox were comparable. The prevalence of infection was significantly different ( $P < .001$ ) for foxes trapped in 3 distinct areas within the enzootic region. Prevalence of LACV infection ranged from 17% to 100% in red and gray foxes. Rates of infection in foxes by area coincided with LACV antibody prevalence observed in free-ranging chipmunks and with reported cases of human LAC encephalitis in Wisconsin.

Oral transmission of LACV from chipmunks to red foxes was demonstrated by natural predation in the laboratory. Susceptibility to infection, and resulting viremia and antibody responses of red foxes were determined. Four of 7 red foxes became viremic following consumption of LACV infected chipmunks (Table 1). One fox developed LACV neutralizing antibody but no viremia. Maximum viremia titers in all orally infected fox were comparable to maximum titers observed in A. triseriatus infected red fox and were sufficient to exceed the threshold of infection for A. triseriatus. Duration of viremia ranged from 2 to 4 days. LACV was isolated from the oro-pharyngeal secretions of 2 of 6 red foxes. Five of 7 red foxes developed LACV neutralizing antibody by day 14-30 post infection. This is the first reported case of arbovirus transmission from an infected natural vertebrate prey species to a natural vertebrate predator species via the oral route, through predation.

Oral transmission of LACV to chipmunks was also demonstrated in the laboratory. Susceptibility to infection and resulting viremia and antibody responses of chipmunks were determined. Four of 12 chipmunks given 1 ml of a serial 10 fold dilution of a LACV infected A. triseriatus titrated suspension became viremic. All of 3 chipmunks given  $10^{3.8}$  SMICLD<sub>50</sub> for virus in 1 ml and 1 of 3 chipmunks given  $10^{2.8}$  SMICLD<sub>50</sub> of virus in 1 ml became viremic. Viremia persisted from 1 to 3 days. Maximum titers were  $\geq 10^{3.6}$  SMICLD<sub>50</sub> of virus per ml of blood. Oropharyngeal secretions, urine and feces were collected daily but have not yet been examined for virus isolation.

Oral transmission of LACV to chipmunks by consumption of a single LACV infected A. triseriatus will be attempted in the near future.

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TABLE 1. Occurrence of LACV in red foxes following consumption of LAC virus infected chipmunks

Fox No.	Chipmunk Titer	Maximum Titer in Fox		Duration of Viremia
		Throat*	Blood <sup>tt</sup>	Days
013	5.5 <sup>tt</sup>	-	3.7	4
070	4.1	NT <sup>++</sup>	3.3	2
052	6.7	2.7	3.3	3
087	6.3	-	3.1	4
086	1.2	1.5	-	-
038	4.3	-	-	-
065	3.6	-	-	-

\*)  $\log_{10}$  suckling mouse intracerebral median lethal dose SMICLD<sub>50</sub>

+)  $\log_{10}$  SMICLD<sub>50</sub> of virus per ml of blood

++) throat swabs not taken for virus isolation

Vector efficiencies of Aedes triseriatus with  
several LaCrosse (LAC) virus isolates

The objective is to determine whether the vector efficiency of Aedes (A). triseriatus differs with several LAC virus isolates from different geographic areas. A. triseriatus New Glarus F strain in the second laboratory generation, was used. Three LAC virus strains used were isolated from mosquitoes collected in Wisconsin, Minnesota and Ohio. Each of these three strains were passed once in vero cell culture. Groups of mosquitoes fed through a membrane on one of four different serial ten fold dilutions of virus. LAC virus infection of the mosquitoes was determined by the immunofluorescence technique. Infection rates and  $ID_{50}$ 's were calculated for each virus strain. The transmission rates were determined by feeding mosquitoes individually on suckling mice. Suckling mice that died during seven days observation were examined for the presence of LAC antigen in the brain by immunofluorescence.

Table 2 summarizes the infection rates of the three different LAC strains in A. triseriatus. There were no obvious differences in  $ID_{50}$ 's between three LAC virus strains. Table 2 also shows the transmission rates. LAC isolates from Minnesota (Min-78-J3) had low transmission rates judged by  $ID_{50}$  and also transmission rates at  $10^{-1}$  and  $10^{-2}$  dilutions compared to those of the other two strains. This discrepancy in infection rate vs transmission rates of LAC Min-78-J3 virus suggests that this strain may not be as able to pass the barriers and reach the salivary glands as are the other two strains tested.

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Table 2. Infection and transmission rates of three LAC virus isolates in Aedes triseriatus  
New Glarus F Strain

LAC strain	feeding period	titer of virus in blood meal(X1) (log SMLD <sub>50</sub> )	Dilutions of virus				Dose <sub>50</sub> (log SMLD <sub>50</sub> )
			X1	X10 <sup>-1</sup>	X10 <sup>-2</sup>	X10 <sup>-3</sup>	
INFECTION							
Wis-78-40	0 to 4 hr	6.7	17/19*	7/9	3/13	0/21	ID <sub>50</sub> ≥ 5.4 <sup>§</sup>
			(89) <sup>+</sup>	(78)	(23)	(0)	
Min-78-J3	0 to 4 hr	6.7	9/11	7/9	3/9	0/7	ID <sub>50</sub> ≥ 5.3
			(81)	(77)	(33)	(0)	
Ohio-77-37-35	0 to 2 hr	6.8	4/4	3/5	1/4	0/16	ID <sub>50</sub> 5.5
	(100)	(60)	(25)	(0)			
	2 to 4 hr	5.8	11/11	1/3	0/11	0/7	ID <sub>50</sub> 5.0
	(100)	(33)	(0)	(0)			
TRANSMISSION							
Wis-78-40	0 to 4 hr	6.7	19/19**	6/8	4/11	0/9	TD <sub>50</sub> 5.1 <sup>++</sup>
			(100) <sup>++</sup>	(75)	(36)	(0)	
Min-78-J3	0 to 4 hr	6.7	9/10	2/7	0/7	0/11	TD <sub>50</sub> ≥ 6.1
			(90)	(29)	(0)	(0)	
Ohio-77-37-35	0 to 2 hr	6.8	2/2	3/6	1/4	0/4	TD <sub>50</sub> 5.6
	(100)	(50)	(25)	(0)			
	2 to 4 hr	5.8	4/6	0/2	0/9	0/5	TD <sub>50</sub> ≥ 5.6
	(67)	(0)	(0)	(0)			

\* positive mosquitoes/total by immunofluorescence  
 \*\* positive mosquitoes/total by feeding on suckling mice  
 + % of positive mosquitoes  
 ++ dose<sub>50</sub> for transmission by bite to suckling mice  
 § infectious dose<sub>50</sub>

REPORT FROM THE HORMEL INSTITUTE

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Previous experiments in our lab have shown that BHK-21/13 cells can be serially cultivated in a serum-free Waymouth shaker culture system (1) and these cells can be successfully challenged with the lipid containing togavirus, Japanese Encephalitis Virus (JEV) (unpublished results). It has been shown that the addition of different isomers of the 18:1 polyunsaturated fatty acid to various growth media containing serum, inhibits the growth of JEV (2,3,4). We chose two cis isomers of 18:1,  $\Delta 6$  and  $\Delta 9$  which have inhibitory and enhancing effects on JEV growth, respectively, to examine their effects on JEV in a serum-free cell system.

Using our cell system, 60  $\mu\text{g}$  of 6-18:1/ml (noncytotoxic concentration) was added to growth medium before, after, or both before and after, the absorption period to determine if treatment had any effect on JEV growth. Virus was quantitated by the methyl cellulose plaque assay and cell growth and cell death was monitored on the cytograf. After 72 h incubation, JEV yields in cells treated with 60  $\mu\text{g}$  of 6-18:1/ml were lower than in untreated cells ( $p < 0.05$ ). The cis isomer  $\Delta 9$  was previously observed to enhance viral yield in serum supplemented systems (3,4). We observed that treatment of cells with 60  $\mu\text{g}$  of either 6-18:1 or 9-18:1/ml 24 h before absorption did not markedly affect JEV yields at 48 h after incubation when comparisons were made with untreated infected controls.

The presence of 60  $\mu\text{g}$  of either  $\Delta 6$  or  $\Delta 9$ , 18:1 fatty acid/ml to cell-free growth medium inactivated JEV ~ 3.3 logs in 6 h. The addition of 30  $\mu\text{g}$  of 6-18:1/ml inactivated JEV titers 75% and 30  $\mu\text{g}$  of 9-18:1/ml inactivated JEV titers 40% when comparisons were made to viral titers in unsupplemented Waymouth medium 36 h after incubation at 37°C. The cis isomers of 18:1 appeared to differentially affect JEV on direct contact rather than in the intracellular environment in the absence of serum.

To determine if combinations of either fatty acid affected cytotoxicity in our cell system or if the addition of 2.5% newborn calf serum (NBCS) would affect cytotoxicity, we examined different combinations of the 6,9-18:1 fatty acids and NBCS. Using the noncytotoxic concentration of 6-18:1 as our base (60  $\mu\text{g}/\text{ml}$ ), we observed that different combinations of 6-18:1 and 9-18:1 with or without the addition of NBCS did not significantly affect cell growth.

In determining the effects of different lipid additives on lipid containing viruses in cell systems, it is imperative to also consider what effects, secondary or primary, existing lipids in the system may have on virus maturation.

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S. I. Reed and H. M. Jenkin

REPORT FROM THE VECTOR-BORNE DISEASES DIVISION, CENTER FOR DISEASE CONTROL,  
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Experimental transmission of Ross River virus by Aedes (Stegomyia) polynesiensis and Aedes (S) aegypti

Ross River virus (RRV) is an alphavirus which until recently, was limited geographically to the Australia-New Guinea region. It has been associated with a disease in man known as epidemic polyarthrititis. The principal mosquito vectors in Australia are Culex annulirostris and Aedes vigilax, although the virus has been isolated from 5 genera of mosquitoes.

In early 1979, epidemic polyarthrititis was reported for the first time in the South Pacific. Outbreaks occurred in Fiji and subsequently in American Samoa. In Fiji both Ae. vigilax and Cx. annulirostris were present and suspected of being epidemic vectors, but in American Samoa, epidemiological evidence suggested that Ae. oceanicus and Ae. polynesiensis were involved.

In March 1980, another epidemic occurred on Raratonga, Cooks Islands. Although Cx. annulirostris was present, the limited distribution and density of this species suggested that it was not the epidemic vector. Again epidemiological evidence incriminated Ae. polynesiensis. The purpose of this investigation was to document transmission of RRV by this species.

The Raratongan strain of Ae. polynesiensis was colonized from eggs collected during the epidemic in March 1980. Ae. aegypti were the Jakarta, Indonesia strain colonized in 1975. The virus was the Raratongan strain isolated by Dr. L. Rosen in March 1980 from an early case.

Newly emerged mosquitoes were infected by intrathoracic inoculation of RRV. They were held at 30°C for 11 to 15 days and transmission was attempted using the hanging drop technique of Gubler and Rosen (1976). Infected mosquitoes were also allowed to feed on two day old suckling mice.

Table 1 shows the results of transmission attempts. Ae. polynesiensis refused to feed on suckling mice. Of 17 individual mosquitoes exposed to mice in 1/2 pint cardboard cartons, only one fed, with negative results. None of the others even probed the mice. By contrast, most of the Ae. aegypti either probed or fed to repletion on the suckling mice. Three

litters were used for this species and all mice developed hindleg paralysis by day 8 post feeding. The brains of a sample of mice from each litter which were fed upon by Ae. aegypti were harvested and confirmed as RRV infection by neutralization test.

Both species transmitted RRV to measured hanging drop suspensions of serum, washed human red blood cells and 10% sucrose. A higher rate of transmission was observed among mosquitoes taking more than one half a blood meal, than among those which took only small amounts of blood or only probed the drops.

A high titered virus suspension has been prepared and experiments are now under way to repeat the transmission attempts after oral infection.

These preliminary results support the epidemiological data implicating Ae. polynesiensis as the principal vector in the Raratonga RRV epidemic. The results have important implications since this species or closely related members of the scutellaris group are the dominant mosquitoes on most islands of the South Pacific. Furthermore, Ae. aegypti is present on many of the same islands of the Pacific as well as having widespread distribution in Asia, the Caribbean and Africa.

(D. J. Gubler)

EXPERIMENTAL TRANSMISSION OF ROSS RIVER VIRUS  
BY Aedes polynesiensis AND Ae. aegypti\*

Method of Transmission	Number Mosquitoes	Number Fed	Number Transmitting	Percent Transmission
<u>Ae. polynesiensis</u>				
Suckling mice	17	1	0	0
Hanging drop	18			
engorged		5	2	40
trace		11	1	9
<u>Ae. aegypti</u>				
Suckling mice	17	10	All mice died	
Hanging drop	17			
engorged		8	4	50
trace		2	1	50

\*-Ten to fifteen days post infection.

Comparative St. Louis encephalitis virus transmission studies  
and Culex pipiens complex mosquitoes

Early studies by Chamberlain and coworkers with Culex pipiens complex mosquitoes and SLE virus established that there was a difference in transmission rates at a given temperature between the North American subtypes, Cx. p. pipiens and Cx. p. quinquefasciatus. The present study was initiated to evaluate the hypothesis that time related transmission rates are consistent within a subspecies, and that this characteristic is genetically determined.

Studies on the time-related transmission efficiency of hybrid forms of this complex are of interest because of the occurrence of an interbreeding population of Cx. p. pipiens and Cx. p. quinquefasciatus in Memphis, Tennessee where SLE epidemics occurred in 1974-76, and long-term studies on the ecology of SLE virus are being conducted in collaboration with the Memphis Shelby County Health Department. The seasonal distribution and annual variation in the composition of the field populations of Cx. pipiens complex in Memphis are being studied. The current transmission experiments were initiated to complement these field studies. The hypothesis being explored is that annual variations in SLE virus amplification in Memphis may vary as the composition of the Cx. pipiens complex changes. The ability to transmit virus efficiently with a shortened extrinsic incubation period is an important variable in the epidemiology and ecology of SLE virus.

Colony mosquitoes have been used for the experiments. The Cx. p. pipiens were started with field material collected in Dayton, Ohio in 1975, and the Cx. p. quinquefasciatus with specimens collected in Tampa Bay, Florida in 1977. The virus strain used (76V-15838) was isolated from Cx. pipiens complex mosquitoes in Memphis, Tennessee in 1976 and had been passed once in Cx. pipiens to prepare a seed pool. Preliminary results are summarized in Table 1. The ratios are for transmission on day 11 after feeding on viremic chicks. Each replicate represents groups of each kind of mosquito fed simultaneously on the same chick, so that the groups being compared for transmission efficiency received identical infecting doses of SLE virus. The mosquitoes were held at 25°C during extrinsic incubation.

Data from the experiments completed to date clearly established two points: 1.) there is a consistent and reproducible difference in time-related transmission efficiency between the Cx. pipiens subtypes, with Cx. p. pipiens transmitting significantly earlier than Cx. p. quinquefasciatus, 2.) hybrids of both types demonstrate transmission ratios that are intermediate between the parental types.

The study will be continued by looking at additional crosses to elucidate possible genetic mechanisms controlling transmission efficiency. Experiments will also be extended to study the sequential development of SLE virus in the subtypes by organ titration and FA; we hope to determine thereby the basis for the difference in time-related transmission efficiency.

(W. A. Rush and D. B. Francy)



Comparison of SLE virus transmission rates between strain of Culex p. pipiens, Cx. p. quinquefasciatus and hybrids.

<u>Transmission ratios - Parental strains</u>		
<u>Replicates</u>	<u>Cx. p. pipiens</u>	<u>Cx. p. quinquefasciatus</u>
a	4/18 (22) <sup>a</sup>	2/19 (11)
b	10/20 (50)	2/20 (10)
c	14/22 (64)	2/21 (10)
d	15/20 (75)	2/18 (11)
e	12/19 (63)	1/16 ( 6)
f	15/19 (79)	1/17 ( 6)
TOTALS	70/118(59.3)	10/111( 9)

<u>Transmission ratios - hybrid strains</u>		
	<u>Cx. p. pipiens</u> $\frac{o^+o^+x}{o^+o^+}$	<u>Cx. p. quinque.</u> $\frac{o^+o^+x}{o^+o^+}$
	<u>Cx. p. quinque.</u> $\frac{o^+o^+}{o^+o^+}$	<u>Cx. p. pipiens</u> $\frac{o^+o^+}{o^+o^+}$
a	0/17	1/14 ( 7)
b	9/24 (38)	5/22 (23)
c	9/24 (38)	6/24 (35)
d	7/24 (29)	5/23 (22)
e	7/24 (29)	13/19 (68)
f	6/22 (27)	6/19 (32)
TOTALS	38/135(28.1)	36/121(29.8)

<sup>a</sup>No. mosquitoes transmitting/no. infected mosquitoes feeding (% transmission).

Comparision of the genomes of various isolates of Colorado Tick Fever virus by SDS-polyacrylamide gel electrophoresis.

Access to a large number of isolates of Colorado Tick Fever (CTF) virus strains from humans, rodents, and ticks (*Dermacentor andersoni*) from Colorado has encouraged us to evaluate several questions about the genetic variation of CTF using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The strain designation, source, location, year of isolation, and passage history for the 22 isolates used in this study are given (Table 1).

Vero cells were infected at a multiplicity of 10 p.f.u./cell, and were labeled with either  $^3\text{H}$ -uridine (50 uCi/ml) or  $^{32}\text{P}$  (200 uCi/ml) in the presence of 0.25 ug/ml actinomycin D. The cells were incubated for 48 h, lysed with Triton X-100, and centrifuged to remove nuclei. The RNA was extracted with phenol-chloroform-isoamyl alcohol, and precipitated with 2.5 volumes of ethanol. Double-stranded RNA was purified by precipitation with LiCl and chromatography on a CF-11 column. The purified RNA was analyzed on 5-20% SDS-PAGE gradient gels. The genome of CTF contains 12 dsRNA species (Figure 1). To be consistent with the current nomenclature, we have sequentially numbered the segments from large to small in each of the size classes. The dsRNA consists of three size classes; four large RNA species, six medium RNA species, and two small species which electrophorese faster than the smallest Reovirus type 1 dsRNA species. The estimated molecular weights of each of the dsRNAs of CTF are given in Table 2. This pattern of genomic RNA is unique among the family of Reoviridae.

The CTF isolates from various animal species were subsequently grown in Vero cells, the genomes were extracted, and the RNA analyzed by 10% SDS-PAGE. The electropherograms of the dsRNA from four of the isolates are presented to demonstrate the heterogeneity accompanying this family of viruses (Figure 2). The major heterogeneity was noted in the migration of the six M segments.

The electrophoretic pattern of the dsRNA of the isolate S6-14-03 was the most distinct of those examined. This isolate is serologically related to Eyach virus which can be related to the CTF group by complement fixation but not by neutralization.

The complex relationships among the dsRNA electropherograms were analyzed for correlations using computerized

numerical taxonomy. The migration distances for each dsRNA species were measured in comparison to the dsRNA of Reovirus type 1, and the data were subjected to similarity matrix analysis. It was apparent from the resultant dendrogram that no set pattern of variation was detectable. Samples isolated over a wide geographic area and different hosts showed many different patterns. Virus isolates from a single species from the same area over a relatively short time period showed more limited variation. Therefore, in comparing the RNA patterns of CTF virus isolates from different origins, the dsRNA patterns do not identify the virus, host of origin, or geographic locale.

( J.A. Grant, D.W. Trent, J.T. Roehrig)

Table 1. Colorado Tick Fever Isolates Under Study, Their Isolation and Passage History

Virus Designation	Isolation Source	Year	Area	Passage
76-1-807	<u>I. hudsonicus</u>	1976	CO	SM <sub>4</sub>
76-1-1810	<u>S. nuttallii</u>	1976	Co	SM <sub>4</sub>
76-1-392	<u>S. lateralis</u>	1976	Co	SM <sub>4</sub>
76-1-1078	<u>E. minimus</u>	1976	CO	SM <sub>4</sub>
76-1-1007	<u>S. lateralis</u>	1976	CO	SM <sub>4</sub>
S6-14-03	<u>L. californicus</u>	1976	CA	SM <sub>4</sub>
75V3863	<u>D. andersoni</u>	1975	CO	SM <sub>3</sub>
75V1843	<u>D. andersoni</u>	1975	CO	SM <sub>3</sub>
75V1906	<u>D. andersoni</u>	1975	CO	V <sub>2</sub> SM <sub>3</sub>
75V1841	<u>D. andersoni</u>	1975	CO	SM <sub>4</sub>
77V5270	<u>D. andersoni</u>	1977	CO	SM <sub>1</sub> V <sub>1</sub> SM <sub>1</sub>
64V37	<u>D. andersoni</u>	1964	CO	SM <sub>6</sub>
Eyach	<u>I. ricinus</u>	1972	W.Ger.	P <sub>35</sub> SM <sub>3</sub>
Florio	man	1943	CO	P <sub>60</sub> SM <sub>12</sub>
69V28	man	1969	CO	SM <sub>3</sub>
71V11	man	1971	CO	SM <sub>5</sub>
R-1575	man	1974	CO	SM <sub>4</sub>
R-6225	man	1975	CO	SM <sub>4</sub>
R-11515	man	1976	CO	SM <sub>3</sub>
R-15823	man	1977	CO	SM <sub>2</sub>
R-19372	man	1977	NM	SM <sub>4</sub>
R-19420	man	1977	CO	SM <sub>4</sub>

Table 2. Molecular Weights of Colorado Tick Fever R-1575 ds RNA Species

Species	Molecular Weight <sup>a</sup>
L1	2.40
L2	2.32
L3	2.30
L4	2.20
M1	1.62
M2	1.50
M3	1.38
M4	1.30
M5	1.21
M6	1.18
S1	0.5
S2	0.2

<sup>a</sup> Molecular weight calculated using dsRNA segments of Reovirus type 3 as standard.

FIGURE 1. ds RNA PROFILES

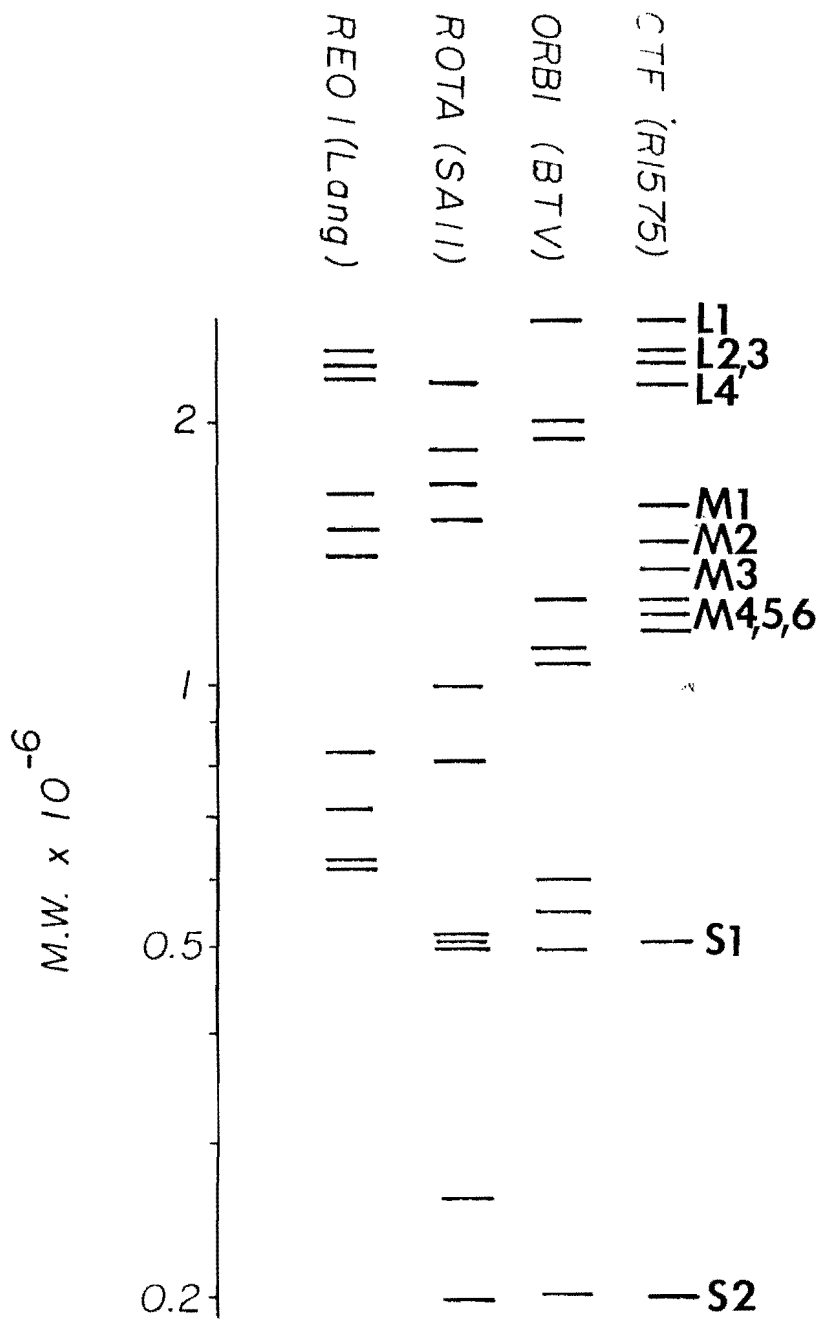
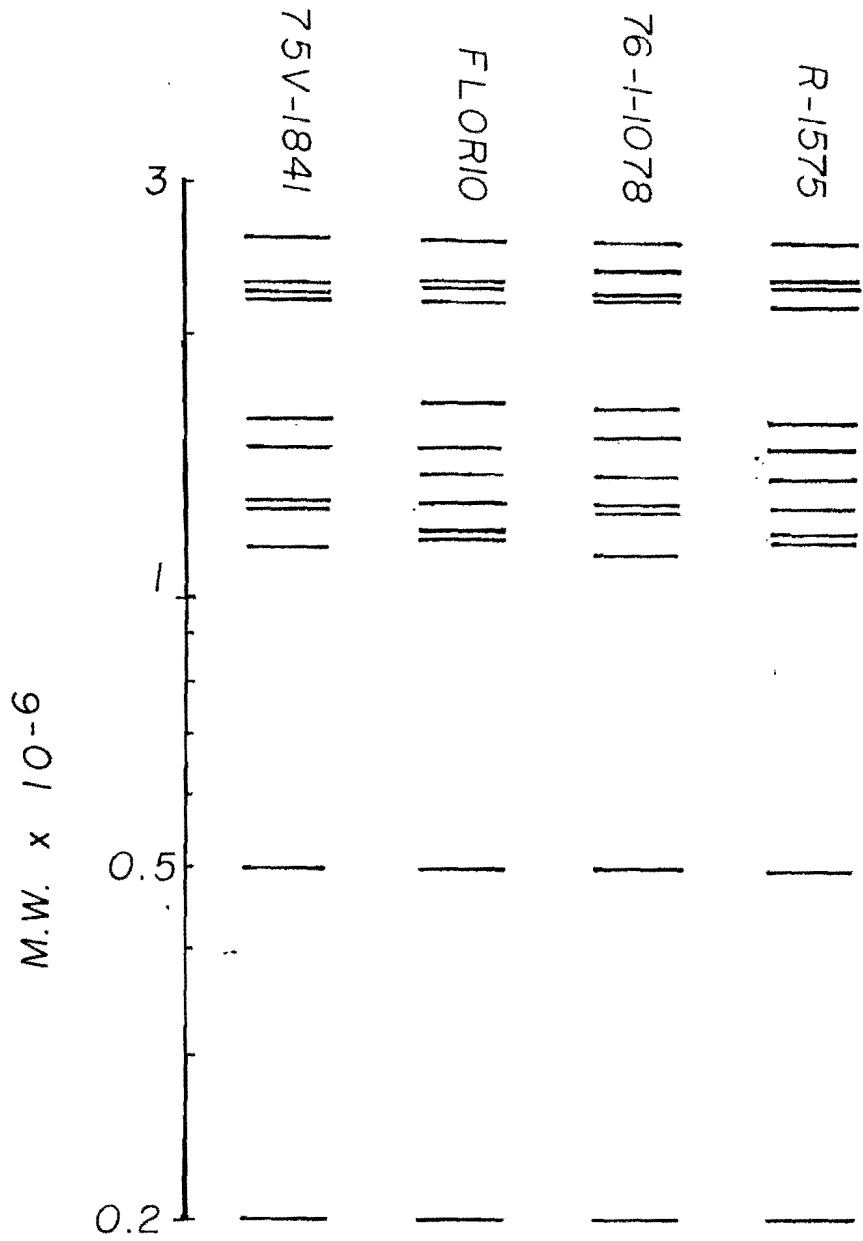


FIGURE 2. dsRNA Heterogeneity of CTF Strains



REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY, STATE OF CALIFORNIA

DEPARTMENT OF HEALTH SERVICES, BERKELEY, CALIFORNIA

STUDIES OF A PERSISTENT FOCUS OF MODOC VIRUS (Dr. Harald N. Johnson)

Modoc virus is a group B virus isolated from *Peromyscus maniculatus* mice trapped at Hackamore Station, Modoc National Forest, Modoc County, California, July 9, 1958. This is a field study site for the investigation of Colorado Tick Fever. At the time of the original isolation of this virus we were snap trapping *Peromyscus* mice to test liver and mammary gland specimens for CTF virus because laboratory studies had shown that this virus had a tropism for these organs. Twenty *Peromyscus* mice were trapped. None were positive for CTF virus but the liver specimen of one mouse contained a virus later identified as Modoc virus. Only one mouse was lactating and Modoc virus was isolated from the mammary gland tissue. The liver test was negative. We could not reisolate the Modoc virus from the liver specimen. The virus does produce a viremia so it was probably present in the blood associated with the liver and there was inactivation of the virus by the bile present in the liver tissue. The mammary gland contained an adequate amount of virus for reisolation in mice and hamsters by peripheral inoculation and harvesting of infected blood so that non-neuroadapted lines of the virus could be obtained.

Modoc virus exhibits a tropism for salivary gland, lung and kidney tissue. In 1970 cell cultures were prepared from a pool of salivary glands, lungs and kidneys of three *Peromyscus m.* mice (M2873,80,82) collected at Hackamore Station, June 8th. The cell culture yielded Modoc virus and furnished a stock of virus obtained directly from the organs of the naturally infected animals. On April 25th, 1980 nine *Peromyscus m.* mice were trapped at Hackamore Station and tested for Modoc virus. Four were direct tests of pools of salivary glands, lungs and kidneys. These were negative. Four



were tested by cell culture of the kidneys. The entire kidneys of each mouse were minced and trypsinized and the tubule cells were planted in bottles and tubes. The cell cultures of one of these mice has yielded Modoc virus and the cultures continue to produce virus. One of the mice was pregnant and had five young. These will be tested later.

Modoc virus has been isolated at two other study sites in California. No subsequent tests were made at these sites. The isolation of Modoc virus on two occasions, ten years apart, at Hackamore Station, using primary cell cultures of wild caught Peromyscus mice as the source of the test material illustrates the value of this system for identifying the presence of Modoc virus in wildlife. This test system should prove to be valuable for the study of the ecology of wildlife viruses, especially those associated with small mammals such as mice, bats and shrews.

#### ARBOVIRUS SURVEILLANCE IN CALIFORNIA, PRELIMINARY REPORT FOR 1980

A high potential for mosquito population increase, coupled with cuts in tax revenue finding for local mosquito abatement district control efforts this year, increased our concern about possible epidemic WEE and SLE. Surveillance and control efforts were augmented by help from the University of California School of Public Health Arbovirus Research Unit and by a special emergency California legislative appropriation. As of August 15, 2160 mosquito pools were tested, yielding 32 isolates of WEE virus, 4 of SLE virus, 6 of California encephalitis virus, and 71 other (Turlock, Hart Park). We continue to have no evidence of human disease due to CE Virus in this State, and neither Turlock nor Hart Park viruses have been implicated as human disease agents. Monthly

sampling of 30 chicken flocks in selected areas (1881 samples since May) showed WEE antibody conversion in only 7 chickens from 4 flocks in the Sacramento Valley so far. No cases of WEE or SLE have been confirmed in either equines or humans. Telephone reports of positive findings in mosquitoes and chickens, and a weekly mailed bulletin to participating agencies have been used to disseminate the surveillance findings.

(R. W. Emmons)

Snowshoe hare (SSH) virus (California encephalitis group) was isolated from 1:382 Aedes communis mosquitoes collected at Km 197 Dempster Highway (66°N, 138°W), on 16 July 1980. This comprises the first Bunyavirus isolate from our summer 1980 collection of mosquitoes throughout the Yukon Territory within the Western Canadian Arctic. Mosquito catches were less numerous than usual, associated with a particularly cool dry spring. Snowshoe hare isolates were achieved from the same location in 1972, 1973, 1974, 1978.

After intrathoracic injection of Aedes aegypti mosquitoes with 10,000 PFU snowshoe hare virus (78-Y-133 strain), viral antigen was detected by immunofluorescence in head squash and salivary gland preparations as frequently as by infectivity titrations using plaque counts in BHK tissue culture monolayers. Similar good correlations were observed after feeding of SSH virus to mosquitoes. Furthermore, immunofluorescence and infectivity titrations yielded virtually identical results for detection of Northway (NOR) virus (78-Y-284 strain) after intrathoracic injection of 1000 PFU but only 3 of 39 A. aegypti supported multiplication of NOR virus after feeding and incubation at 23 or 32°C.

(Donald M. McLean)

