

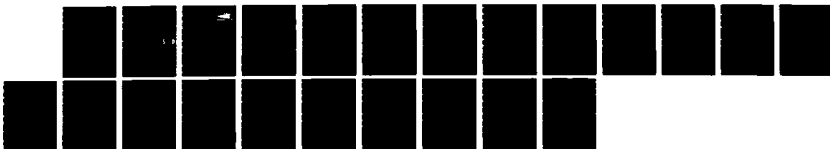
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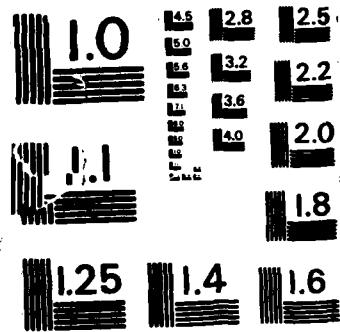
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World Reference Center for Arboviruses

FINAL REPORT

Robert E. Shope, M.D.

March, 1984

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The World Reference Center for Arboviruses identified over 800 viruses and revised the taxonomy of Bunyaviridae, Orbivirus, Rhabdoviridae, Arenaviridae, and Togaviridae. Outbreaks were diagnosed such as Rift Valley fever in Egypt in 1977. Rapid and early diagnosis methods were developed including immunofluorescence and ELISA. Some of these utilized monoclonal antibody. Virus reference strains and antibody were distributed world-wide.		

#### SUMMARY:

The World Reference Center for Arboviruses maintains on a national and an international basis: a) serologic identification and biologic characterization of arboviruses using CF, HI, neutralization, IFA, and ELISA techniques and reagents prepared in large part in mice; b) molecular characterization of viruses using PAGE, electron microscopy, and polypeptide and RNA purification; c) diagnosis of disease using sera and other specimens submitted by the military and other organizations; d) diagnosis and epidemiological study of epidemics and epizootics using submitted specimens; e) preparation and distribution of reference reagents including antibody, viruses to specific organizations, and antigens under special circumstances; f) serological survey for arboviruses on a limited scale; and g) dissemination of information through WHO and the American Committee on Arthropod-borne Viruses. The above functions of the reference center were jointly supported by these contracts and by contracts and grants from the U.S. NIH, ONR, the Australian government, and WHO.

During the 10 years of the contracts, over 800 viruses were identified, many of them new to science and/or new to a geographic region. The taxonomy of the 4 major families of arboviruses was revised. The distribution of antibody to arboviruses was determined throughout the world by serosurvey. Major epidemioepizootics were identified and diagnosed such as Rift Valley fever in Egypt and bluetongue virus from Australia; these were medically or economically major events. Techniques for rapid and early diagnosis of arbovirus diseases were developed and transferred as technology to users throughout the world. Over 6,000 ampoules of arbovirus reagents were distributed to the U.S. Military and to other laboratories throughout the world.

#### FOREWARD:

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

## BODY OF REPORT:

Introduction. The World Reference Center for Arboviruses was established at the Yale Arbovirus Research Unit in 1965 as an outgrowth of The Rockefeller Foundation program on arboviruses which was moved in 1965 to Yale University from New York City. The U.S. Army has supported this program since 1972, initially through joint Navy-Army funding, then through a separate contract. The progress over these eleven years is included in this report; it covers the work for the entire project which received support from the Navy, WHO, NIH, and Australian government, in addition to that of this contract.

Virus identification. A primary function of the reference center is to receive viruses from all parts of the world for identification. The most resounding identifications were bluetongue virus from Australia and Rift Valley fever virus from Egypt in 1977. Details of these and other identifications are given in Annual Reports from 1973 to 1983. New viruses identified included Aroa virus from Venezuela and Tamana virus from bats in Trinidad, both new flaviviruses, as well as other new flaviviruses from Australia, and France. Twenty-seven new viruses from Brazil were identified. Among them was a new Anopheles A group virus; 2 new ungrouped viruses from Sabethes and from Aedes mosquitoes; a new flavivirus; 4 new ungrouped viruses in the Bunyamwera group from Sabethes and Wyeomyia mosquitoes and a bat; a new bunyavirus from a sentinel mouse; 4 new phleboviruses -- one from a spiny rat and 3 from sandflies; 2 new rhabdoviruses from a lizard and a toad and a new vesiculovirus from male sandflies; a new Bunyamwera group virus from a fatal case of hepatitis; 5 new Changuinola group viruses from a sloth, an armadillo, and sandflies respectively; 2 new Corriparta group viruses from mosquitoes; a new Pacora group virus from a bird; and a new Bunyamwera supergroup virus from a bat. A new Yogue group virus was recognized from febrile patients and from bats in Uganda, and a new Ornithodoros virus and a new Tete group virus from India. Tilligery was a new Eubenangee group orbivirus from Australia. Other new viruses included the orbivirus, GG668, the tick bunyavirus MI 19334, Termeil, Yacaaba, and PK886 viruses from mosquitoes, CSIRO-25 virus from Culicoides, and a new virus from Argas ticks -- all isolated in Australia; a new virus from Tanzanian Argas, a new virus from Ornithodoros ticks collected in the U.S., and Connecticut virus, a new rhabdovirus from ticks in New England.

Previously described viruses were identified from new geographic regions, including Thogoto from ticks, Sango from Culicoides, Dugbe from birds, Arumowot from rodents, and Germiston from sentinel mice -- all from Ethiopia; Umbre and Thimiri for the first time in Australia; Arumowot from South Africa; Wad Medani, Wanowrie, and Quarafil from Iran; Soldado Rock virus from France and the Seychelles; Chenuda virus in Morocco; Tataguine virus from the Gambia; Kemerovo group viruses from the U.K., Finland, and France; Salehabad, a Sakhalin group virus and Eyach virus from France, a Kemerovo group virus recovered from anal swabs of Thai bats, Sindbis from bats of Zimbabwe, and Tyuleniy from ticks of the North Atlantic. Crimean-Congo hemorrhagic fever virus was confirmed from China. Rift Valley fever (Zinga) virus was recognized in Central African Republic, Madagascar, Guinea, and Senegal. Keystone virus was recognized for the first time in New England, and Japanese encephalitis for the first time in the Philippines. An isolate from a patient in the Netherlands was Colorado

tick fever virus. The patient had vacationed in the western United States and returned sick to Holland where he removed a tick from himself. This is an example of long distance transport of a human viral pathogen.

Over 150 strains isolated from mosquitoes in Indonesia were referred by the NAMRU-2 Field Facility in Jakarta. Although these strains are not yet completely identified, initial studies indicate that over half of these are dsRNA viruses, probably orbiviruses. The dsRNA segments were studied by polyacrylamide gel electrophoresis (PAGE). The PAGE patterns indicated that there were multiple genotypes among these isolates. These viruses were isolated in the C6/36 clone of Aedes albopictus cells; they illustrated the power of the PAGE technique in identification of strains which were not mouse-pathogenic, and in some cases did not cause CPE in vertebrate tissue culture. Other similar mosquito cell isolates were studied from China, Hawaii, Thailand, and Israel.

The center was called upon frequently to confirm the identity of viruses used by other researchers. The identity of two strains of SLE virus isolated by U.S. Army researchers in overwintering Culex was confirmed; the identity of Junin vaccine virus was certified; and a plaque-clone of Dhori virus was identified for researchers at the University of Alabama who had shown it to contain 8 segments of RNA. In all, 850 viruses were identified between 1973 and 1983.

Virus classification. The vast majority of arboviruses pertain to 4 major virus families -- Togaviridae (genera Alphavirus and Flavivirus), Rhabdoviridae, Reoviridae (genus Orbivirus), and Bunyaviridae (genera Bunyavirus, Nairovirus, Phlebovirus, and Uukuvirus). Reference sera and viruses were produced and distributed for viruses in each of these families. These reagents formed the basis for a collaborative study by plaque reduction neutralization carried out at CDC, Fort Collins and USAMRIID, Fort Detrick to classify the viruses in the genus Flavivirus. A major revision of the family Rhabdoviridae was done by complement fixation, immunofluorescence, and plaque reduction neutralization involving 51 viruses. A similar revision of the genus Phlebovirus was completed with 35 viruses. The finding that Rift Valley fever belonged to this genus had a major impact on the progress of research with this virus. The serological relationships among viruses of the genus Nairovirus were delineated, and these agents were supplied to the Department of Microbiology at the University of Alabama where biochemical studies were done. Another major revision was done in the genus Orbivirus using complement fixation, plaque reduction neutralization, RNA hybridization and PAGE of dsRNA. It was established that the serotype differences within some serogroups represented hybridization differences in only one or two of the 10 genes comprising each virus in the serogroup.

An attempt to place Hantaan virus into one of the existing serogroups of arboviruses was negative, in spite of extensive serological testing, especially among the members of the family Bunyaviridae.

Serologic surveys. Broadly based arbovirus serological surveys were carried out with sera from Brazil, Guam, U.S.A., Colombia, West Irian, Papua, New Guinea, Liberia, Yugoslavia, Greece, China, Ghana, Cameroon, Israel, Sinai, Egypt, Turkey, Sudan, Australia, and Indonesia. The distribution of antibody to alphaviruses, flaviviruses, and several



hemorrhagic fever viruses such as Lassa, Rift Valley fever, Marburg/Ebola, and Crimean-Congo hemorrhagic fever were determined.

Diagnosis of disease. The outbreak of Rift Valley fever in Egypt was diagnosed. An outbreak of Crimean hemorrhagic fever was diagnosed in Pakistan, chikungunya infection was detected in an American working in West Africa, as was Japanese encephalitis in a Canadian exposed in China, eastern encephalitis in a child from Rhode Island, western encephalitis in a teen-ager exposed in the western U.S.A., Tacaribe virus infection in a laboratory worker, LaCrosse encephalitis in a child from Westchester County, N.Y., and Ross River infections in Canadians returning from the Western Pacific. The cause of fevers in Indonesians was determined in a special fever study. In addition, many outbreaks (apparently not of arbovirus origin) were investigated without obtaining a diagnosis.

Development of techniques and rapid diagnosis. The following were investigated or developed: 1) arbovirus attachment to neural and non-neural cells, 2) demonstration of high salt HA with bunyaviruses and with dengue virus, 3) demonstration of HA in mosquito tissues with WEE virus, 4) animal models for demonstrating pathogenicity of bunyavirus reassortant viruses, 5) ELISA for alphaviruses, flaviviruses, and bunyaviruses, 6) Aedes pseudoscutellaris cells for primary isolation of arboviruses in the field, 7) PAGE analysis of dsRNA viruses for classification, 8) trypsinization and mass culture of Rhipicephalus appendiculatus tick cell line, 9) feeding ticks on capillary tubes, 10) high titered JE virus from persistently infected C6/36 cell line, 11) antigen detection ELISA applied to infected mosquitoes, 12) monoclonal antibodies to Rift Valley fever and Crimean-Congo hemorrhagic fever viruses, 13) IgM antibody detection using ELISA, 14) latex beads for rapid antibody and antigen detection, 15) modified Southern blotting to determine genetic relatedness of RNA viruses, 16) glutaraldehyde fixation of goose cells for use in HI tests, 17) the use of CER cells for Crimean-Congo hemorrhagic fever virus propagation, 18) type-specific identification of California group viruses using 1-injection hamster sera, and 19) ELISA on filterpaper blood collections.

Collection of low passage arbovirus strains. A large collection of low passage arbovirus strains has been developed and maintained. Priority was given to yellow fever, dengue, chikungunya, western encephalitis, eastern encephalitis, Japanese encephalitis, and other human disease arboviruses. The original (or as close to original as was available) material was passaged once in C6/36 mosquito cells and the progeny lyophilized in aliquots. These were stored and distributed to any and all persons requesting material for study.

Distribution of reagents. Reference antibody and virus was maintained as lyophilized stock for distribution to U.S. military users and to others on a world-wide basis. Since 1973, 7,341 ampoules of arbovirus reagents were distributed to laboratories in more than 40 countries. This total consisted of 2,790 ampoules of virus stock, 1,939 ampoules of virus antigen, and 2,612 ampoules of antibody.

Cell lines were also distributed. These included 96 Aedes albopictus, 23 Aedes aegypti, 2 Aedes novalbopictus, 2 Aedes w-albus, 7 LLC-MK2, 4 Rhipicephalus, 8 toad, 20 Aedes pseudoscutellaris, 18 Vero, 2 MA-111, 2 BSC-1, 12 BHK-21, 7 RNL-15 and 16 CER lines.

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