ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 21 November 1970

Table of Contents

Reports from (continued):

Reports from (continued): Page

Reports from (continued): Page

REPORT FROM THE CHAIRMAN OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1. Publication of 39 additional catalogued arboviruses

Facilitated by a generous grant from the Rockefeller Foundation to defray the major portion of publication costs, the November 1970 issue of the American Journal of Tropical Medicine and Hygiene carries a Supplement containing brief descriptions of 39 catalogued arboviruses which were registered after the closing date for inclusion in Catalogue of Arthropod-Borne Diseases of the World (comp. R.M. Taylor), 1st edition 1967, P.H.S. Publication No. 1760. During 1971 and subsequent years, the American Journal of Tropical Medicine and Hygiene will publish synopses of descriptions of at least 12 newly catalogued arboviruses annually. Special thanks are due to the Chairman and Members of the Subcommittee on Information Exchange for their splendid execution of this immense and important task. Reprints of the November 1970 Supplement will be mailed to all participants in the Catalogue and/or Information Exchange. Non-participants may obtain reprints through the office of the Chairman of the Subcommittee on Information Exchange.

2. Information Exchange

In order to conserve space in No. 21 (November 1970) and subsequent issues, the Executive has authorized the Editor to abbreviate lengthy submissions to occupy a total of three printed pages, including tables.

3. S.I.R.A.C.A.

A report of the work of the Subcommittee on Immunological Reactions among Catalogued Arboviruses with recommendations regarding classification of arboviruses in Group C, California, and Group A was approved by the Executive and it appears elsewhere in this issue. Written comments should be mailed to the 1971 A.C.A.V. Chairman, Dr. R.E. Shope for subsequent presentation to S.I.R.A.C.A.

4. Richard Moreland Taylor Award

At the Open Meeting on 2nd November 1970, at the San Francisco Hilton Hotel, San Francisco, California, this award was presented to Dr. William McDowell Hammon, Graduate School of Public Health, University of

Pittsburgh, Pittsburgh, Pa. by his colleague of 30 years' duration, Dr. W.C. Reeves, Dean of the School of Public Health, University of California, Berkeley, California.

5. Office Holders, 1970-71

Dr. R.E. Shope succeeded Dr. D.M. McLean, as Chairman, A.C.A.V. Dr. P.K. Russell succeeded Dr. R.W. Chamberlain as Secretary, Dr. B.E. Henderson was elected to the Executive in place of Dr. R.W. Chamberlain who rotated off. For the first time, a mail ballot was conducted among persons who attended the 1968 and/or 1969 Open Meetings to elect a new Executive member. In subsequent years, it was resolved that voting members will comprise those who attended and signed the register at any of the three preceding meetings - for 1971 this will comprise registrants at the 1968, 1969 or 1970 meetings.

Effective 2nd November 1970, the A.C.A.V. Executive comprises:

1972 Dr. R.E. Shope, Chairman
Yale Arbovirus Research Unit,
60 College Street,
New Haven, Connecticut 06510.

1973 Dr. K.M. Johnson,
Middle America Research Unit
P.O. Box 2011,
Balboa Heights, Canal Zone.

1974 Dr. P.K. Russell, Secretary,
Walter Reed Army Institute of Research,
Walter Reed Army Medical Center,
Washington D.C. 20012,

1971 Dr. D.M. McLean,
Department of Microbiology,
University of British Columbia,
Vancouver 8, B.C. Canada.

1976 Dr. B.E. Henderson,
Department of Pathology,
University of Southern California,
2025 Zonal Avenue,
Los Angeles, California 90033

1975 Dr. N. Nathanson,
Johns Hopkins University,
School of Hygiene and Public Health,
615 North Wolfe Street,
Baltimore, Md. 21205

INFORMATION EXCHANGE

Dr. T.O. Berge, Chairman
Curator, Collection of Animal Viruses and Rickettsiae,
American Type Culture Collection,
12301 Parklawn Drive,
Rockville, Md. 20852.

Dr. R.M. Taylor, Catalogue Consultant
School of Public Health,
University of California,
Berkeley, California 94720.

Dr. R.E. Shope, YARU Liaison
Yale Arbovirus Research Unit,
60 College Street,
New Haven, Conn. 06510

Dr. R.W. Chamberlain, CDC Liaison
Center for Disease Control,
1500 Clifton Road, N.E.,
Atlanta, Georgia 30333.

Dr. T.H. Work, Infoexchange Editor
University of California Medical Center,
405 Hilgard Avenue,
Los Angeles, California 90024.

S.I.R.A.C.A.

Dr. J. Casals, Chairman
Yale Arbovirus Research Unit,
60 College Street,
New Haven, Conn. 06510.

Dr. E.L. Buescher,
Walter Reed Army Institute of Research,
Walter Reed Army Medical Center,
Washington, D.C. 20012

Dr. C.H. Calisher,
Center for Disease Control,
1500 Clifton Road N.E.,
Atlanta, Georgia 30333

Miss G.E. Sather,
Graduate School of Public Health,
University of Pittsburgh,
Pittsburgh, Pa. 15213

Dr. W.F. Scherer,
Department of Microbiology,
Cornell University Medical College,
1300 York Avenue,
New York, N.Y. 10021.

LABORATORY ACCIDENTS

Dr. R.P. Hanson, Chairman
College of Veterinary Science,
University of Wisconsin,
Madison, Wisconsin 53706

Dr. S.E. Sulkin,
Department of Microbiology,
Southwestern Medical School,
University of Texas,
5323 Harry Hines Blvd.,
Dallas, Texas

TREASURER

Dr. W.C. Reeves,
School of Public Health,
University of California,
Berkeley, California 94720.

REPORT FROM CHAIRMAN OF THE SUBCOMMITTEE ON ARBOVIRUS INFORMATION EXCHANGE

A revision of the working Catalogue of Arthropod-borne Viruses is scheduled for 1971. All those who have registered viruses are being requested to review the registration cards to determine what changes need to be made or what new information should be added to bring the registrations up-to-date. The A.C.A.V. has approved a Subcommittee recommendation that registration of members of the Tacaribe-LCM group or other zoonotic agents be retained in the revised Catalogue because of the usefulness of information concerning such viruses to the arbovirologists. The name of the revised Catalogue will be changed to permit inclusion of selected non-arthropod-borne vertebrate viruses in the new edition.

A call is being sent out to submit registrations of "new" arboviruses at this time if they are to be included in the new edition. Registration forms may be requested from the Catalogue Editor.

REPORT FROM S.I.R.A.C.A. OF THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

In the course of the session an attempt was made to establish, in so far as that was possible, uniformity in the guidelines to be used by the Subcommittee for the evaluation of the degree of antigenic closeness among the viruses of a Group. In addition, a previously studied Group - California - was re-examined on the basis of some new evidence adduced as well as in view of comments and suggestions received from interested investigators, not members of SIRACA.

Our conclusions follow:

a. The members of SIRACA were unanimous in concluding that it is desirable to evolve or elaborate a scheme of classification of the registered viruses in each antigenic Group, based on their proximity - or distance - by serologic tests; the scheme should be, in so far as possible, applicable to all groups.

b. The proposed scheme is essentially the one first used in connection with Group C (see report of March 20, 1968). In the scheme now suggested the classification of an individual isolate (or strain, for example BeAn 15) within the Group requires 5 steps or levels, as shown here.

Level	Number
Group	1
Complex	2
Virus (or serotype, or type)	3
Subtype	4
Variety	5
Individual	

c. The accompanying Table gives the result of applying the system to the classification of viruses of Group C (which is very similar to the one given on March 20, 1968); it also gives the revised groupings of the California Group; and the sub-sets in Group A, the latter being the outcome of the day's deliberations.

d. For the sake of uniformity, or balance in the Table, when a virus has no close relatives, as for example EEE and Middelburg in Group A, it is still assigned in the Table to a complex, consisting at the moment of itself only.

e. The need for 5 levels or steps was unanimously and unhesitatingly adopted; however, one of the Members of the Subcommittee was of the opinion that for Group A, some of the entries under column 4 should be called "viruses" or "types" rather than "subtypes". The reason he gave was that, for example, the separation between Mucambo, Pixuna, and VEE, is far more marked by all standard tests than is, for example, the separation of any of the subtypes of California virus from the other subtypes.

f. SIRACA recommends that the Table be published in the Info Exchange, with a request that comments be sent to the Chairman, ACAV, as was done by some persons in the case of the California Group report. This Subcommittee is eager to be informed and most willing to modify its conclusion on the face of valid evidence.

New York, N. Y., 8 June, 1970

(E. L. Buescher, C. H. Calisher, J. Casals, Chairman, G. E. Sather, and W. F. Scherer)

1	2	3	4	5
Group	Complex	Virus (or type)	Subtype	Variety
C	Caraparu	Caraparu	1 (Belem) 2 (Ossa)	Two (Belem, Trinidad
		Apeu		
		Madrid		
	Marituba	Marituba	1 (Marituba) 2 (Murutucu) 3 (Restan)	
		Nepuyo	1 (Nepuyo) ?2 (Gumbo Limbo)	
	Oriboca	Oriboca		
		Itaqui		
California	California	California encephalitis	1 (California encephalitis) 2 (Tahyna-Lumbo) 3 (San Angelo) 4 (Snowshoe hare) 5 Jamestown Canyon-Jerry Slough) 6 (La Crosse) 7 (Keystone)	
	Trivittatus	Trivittatus		
	Melao	Melao		
A	EEE	EEE		Two (geographic)
	VEE	VEE	1 (VEE) 2 (Florida) 3 (Mucambo) 4 (Pixuna)	Five(geographic)
	WEE	WEE		at least three
		Sindbis	1 (Sindbis) }* 2 (Whataroa) }	several
		Aura		
	Semliki	Semliki		
		chikungunya	1 (chikungunya) }* 2 (O'nyong-nyong) }	several
		getah	1 (getah) 2 (Sagiyama) }* 3 (bebaru) 4 (Ross river)	
		Mayaro	1 (Mayaro) }* 2 (Una) }	at least two(Uruma,Trind.)
	Middelburg	Middelburg		
	Ndumu	Ndumu		

* Requires further critical serological evaluation.

LISTING OF AVAILABLE ARBOVIRUS REFERENCE REAGENTS
RESEARCH RESOURCES BRANCH
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

The Research Resources Branch of the National Institute of Allergy and Infectious Diseases, National Institutes of Health has previously announced the availability of arbovirus reagents. The list below is an updating of certified seed and ascitic fluids available as of December 10, 1970. Reagents can be obtained by completing and submitting Form NIH-381-2 to the Research Resources Branch and can usually be provided within 7 to 10 days after the request is received.

For further information on the availability of arbovirus reference reagents write to the Chief, Research Resources Branch, National Institute of Allergy and Infectious Diseases, Building 31, Room 7A-23, Bethesda, Maryland 20014.

1. Arbovirus Seed Viruses

Anopheles A
Bimiti
Buttonwillow
Bwamba
California Encephalitis
Catu
Chagres
Changuinola
Colorado Tick Fever
EEE
EHD
Guama
Guaroa
Hart Park
Hughes
Ilheus
Kern Canyon
Manzanilla
Mayaro
MML
Modoc
Naples SFF
Nepuyo
Oriboca
Oropouche
Patois
Rio Bravo
Sicilian SFF
Silverwater
Tensaw
VSV (Indiana)
VSV (New Jersey)
WEE
Wyeomyia
YF (17D)
Zegla

2. Arbovirus Immune Ascitic Fluid

Anopheles A
Buttonwillow
Bwamba
California Encephalitis
Chagres
Colorado Tick Fever
Guama
Hart Park
Ilheus
Mayaro
Melao
Modoc
Naples SFF
Oropouche
Sicilian SFF
Turlock
VSV (Indiana)
VSV (New Jersey)

3. Arbovirus Grouping Ascitic Fluid

Group A
Group B
Group C
Group Bunyamwera
Group Capim
Group Guama
Group Quaranfil
Group Simbu
Group Tacaribe
Group VSV

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
AUSTRALIAN NATIONAL UNIVERSITY
CANBERRA, AUSTRALIA

Twelve arboviruses were recovered from 8,026 mosquitoes collected at Nelson Bay, N.S.W., in April, 1969, during continuing investigations of "epidemic polyarthritis with rash" in this area. This was unexpected as there were no reports of cases of the disease during the year, and in addition the large camp of flying fox (Pteropus poliocephalus) which were regarded as a possible wildlife reservoir of the virus had been completely vacated in October 1968 after many years occupancy.

Nine of the isolates are Group A arboviruses and appear to be serologically identical to each other and to Ross River virus. The latter is currently regarded as the most likely causative agent of epidemic polyarthritis, although the virus has never been directly recovered from a patient suffering the disease. Two of the isolates are closely related to Edge Hill virus, a Group B arbovirus with no known pathogenicity in man. The remaining isolate appears to be a mixture of Ross River and Edge Hill viruses. The mosquitoes were processed in pools of approximately 100, and as virus was recovered from one out of every seven pools it is not surprising that a mixed infection was detected in one of these. Aedes vigilax was the most common mosquito in the area and this species yielded 7 of the Group A viruses and the two Group B viruses. The other two Group A viruses were recovered from Culex annulirostris and a pool of mixed Culex species. All positive pools were collected by portable light traps augmented by CO_2 bait. Despite the high virus isolation rate and mosquito attack rate, illness was not observed in sentinel infant mice exposed in the area.

Although a non-epidemic year, the recovery of Ross River virus in the area lends considerable support to the clinical and serological evidence of the presence of epidemic polyarthritis on the central coast of New South Wales, and of Ross River virus as the causative agent. An extensive serological survey of human sera from Blood Banks at Taree, Newcastle and Gosford indicated disease activity both north and south of Nelson Bay.

The identity of the wildlife reservoir remains obscure. The flying fox might yet be found to play a role either in re-introducing the virus to the area each year, or in disseminating the virus from bushland foci to human habitation during feeding forays. However it is apparent that the virus can

survive for many months in the absence of flying fox. Wallabies are a suspected host in Queensland, but although there are wallabies on the peninsula they are rare in the vicinity of the particular area from which viruses were recovered. The New Holland mouse (Pseudomys novaehollandiae) is the most commonly trapped mammal. Although there has been only one recorded specimen of this species captured during the past 90 years, Mr. Kent Keith (Division of Wildlife Research, C.S.I.R.O.) has trapped over 90 during the current investigations. The Nelson Bay strain of Ross River virus has been shown to produce viraemia with titres of up to 10^6 p.f.u./ml whole blood in laboratory investigations of this potential host.

The process of adaptation of arboviruses to laboratory hosts is poorly understood, and because of their promising characteristics the suite of Nelson Bay strains of Ross River virus has been selected for investigations of this phenomenon. Unlike the Queensland prototype, the Nelson Bay strains have not yielded haemagglutinin despite many attempts and high passage levels. Early passage material paralyzes many of the intracerebrally inoculated infant mice but rarely kills them, and most of the strains required to be serially passed between five and ten times before they would kill consistently in a predictable time period. This effect does not seem to be dose-dependent. Plaques are formed on a variety of cell lines and these show considerable heterogeneity of size and type.

A virus (NB 847) was recovered from the blood of a flying fox shot at Nelson Bay during 1968. This virus was primarily characterized as a reovirus by electron microscopy and has now been shown by fluorescent antibody staining and other serological techniques to be related to but clearly distinguishible from known mammalian reoviruses. As with some avian reoviruses, NB 847 forms syncytia in primary and continuous cell lines and it has been shown that there is a weak serological relationship between it and the Fahey-Crawley agent. Attempts to demonstrate replication in inoculated mosquitoes have failed.

(I.D. Marshall, G. Gard, G. Woodroofe and W.P. Taylor)

REPORT FROM THE VETERINARY PREVENTIVE MEDICINE UNIVERSITY OF QUEENSLAND MOGGILL, AUSTRALIA

Several strains of bovine ephemeral fever virus, isolated in Queensland during the 1967-68 outbreak, have been compared using the neutralization tests in suckling mice. No antigenic differences between strains have been demonstrated. Plaques have been produced under agar overlay in BHK21 cells, and a neutralization test based on plaque reduction is being developed.

Direct evidence of arbovirus infection in sheep in western Queensland was sought in March 1970. Blood samples were taken from 60 sheep and returned to Brisbane in liquid nitrogen. The samples were passaged in cultures of the PS line of pig kidney cells and in suckling mice inoculated intracerebrally. No mouse pathogenic agents were isolated. However on prolonged passage in PS cells approximately half the samples yielded cytopathogenic agents, which have not yet been identified.

(P.B. Spradbrow)

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

Studies were performed on the structural proteins of Chikungunya virus (ChV), some components derived from it and an intracellular component associated with ChV -specific RNA (the "X" -component), by electrophoresis in polyacrylamide gels. The results, supported by morphological and biochemical evidences, showed that ChV contains 2 major structural proteins. One of them is associated with hemagglutinin (HAnin), and the other with the core of ChV. The molecular weights of these proteins, estimated from their mobilities in polyacrylamide gels containing sodium dodecylsulfate, were 53,000 for the HAnin protein and 30,000 for the core protein.

Pronase treatment of ChV (density 1.24 g/cc) yielded smooth-surfaced particles (density 1.20 g/cc), which seemed to have an intact core surrounded by a membrane containing phospholipid, but which were devoid of HAnin. Further digestion seemed to remove the membrane. On treatment with Tween 80 and ether, small but heavy HAnin (density 1.28 g/cc) was released from ChV. The fundamental structure of the HAnin seemed to be a hollow circular or hexagonal cylinder with a diameter of 3 mμ. Treatment of ChV with Nonidet P40 yielded small, light HAnin (density 1.19 g/cc), which seemed to retain part of the membrane containing phospholipid.

The "X"-component was shown to contain the core protein of ChV as its major protein component, but it was not found to have the phospholipid membrane. The results are compatible with the idea that the "X"-component is a nucleoprotein core of ChV accumulating in ChV-infected BHK21 cells.

Sera from rabbits immunized with the "X"-component yielded no detectable antibodies as measured by hemagglutination-inhibition (HI), neutralization (N) or complement-fixation (CF) tests using HAnin (density 1.28 g/cc) as antigen, but they gave a positive CF reaction when antigen from infected suckling mouse brain or "X" was used. The sera from rabbits immunized with Tween 80 and ether extracted HAnin (density 1.28 g/cc) showed a significant positive reaction by the HI, N and CF tests with HAnin or antigen from infected suckling mouse brain, but their response against "X" was not so high in the CF test. Similar results were obtained when rabbits were immunized with formalin-treated purified ChV.

(A. Igarashi, T. Fukuoka and K. Fukai)

REPORT FROM THE DEPARTMENT OF VIROLOGY
SEATO MEDICAL RESEARCH LABORATORY
BANGKOK, THAILAND

Japanese Encephalitis in Northern Thailand

During the period of June-September, 1969, an epidemic of Japanese encephalitis was reported from Chiengmai Province in northern Thailand. The epidemic affected nearly three hundred individuals, mostly children. In 1970 the epidemic re-occurred and is now being intensively studied by SMRL. The specific objectives of this study include: an estimation of the extent of human infection and its seasonal variation; observations of the clinical manifestations of human infection, emphasizing the post-encephalitic sequelae; determination of the mosquito vectors and animal reservoirs; elucidation of environmental factors bearing on the incidence of human infection; attempts to improve virus detection and isolation techniques in the field and in the laboratory; elucidation of the inter-relationships existing between many co-existing group B arboviruses in Chiengmai, with reference to competition, antagonism, or synergism in mosquito vectors and immune systems of their human and animal hosts.

The first isolation of JEV from mosquitoes in Chiengmai Province occurred in April, 1970. The isolation was followed in several weeks by serological conversion of sentinel pigs, which in turn preceeded by several weeks the first human case of encephalitis. To date (Oct. 1970) there have been 90 serologically confirmed human cases of encephalitis, and the death rate is 23%. Thus far about one-half of the total number of mosquitoes collected in the field have been processed in the laboratory for virus isolation. From 3,963 mosquito pools processed (340,000 mosquitoes) we have isolated 44 infectious agents in MK_2 cell monolayer cultures and in suckling mice. We have 9 confirmed JEV isolates, 7 from Culex tritaeniorhynchus, and 2 from Culex fuscocephala, and 1 Tembusu virus, isolated from Culex gelidus. Thirty-four more isolates from C. tritaeniorhynchus, C. fuscocephala, C. gelidus, C. vishnui complex, A. lineatopennis, A. mediolineatus and A. vexans, have not yet been identified.

We have accumulated indirect evidence that Thai water buffalo (Bubalus bubalus) in addition to pigs may represent an important amplifying host for JEV in Northern Thailand. Several smaller vertebrates, particularly chickens, have been excluded as important reservoirs hosts by sentinel

studies, determination of vector mosquito biting habits, and serological surveys.

Our interpretation of human and animal serological HI test results has been limited by the inability to consistently and reliably differentiate between repeated infections produced by JEV, dengue and probably other group B arboviruses, all of which circulate in Northern Thailand. The plaque reduction neutralization test, which is purported to be more viral specific than HI and CF tests for recent infection, has not provided increased specificity in our hands. Work is underway to improve serological methods for the specific identification of a recent group B arbovirus infection in individuals who have been sensitized by previous group B infections.

Dengue in Thailand

Surveillance by this laboratory of Thai hemorrhagic fever and shock syndrome patients admitted to Thonburi and Bangkok Hospitals has been carried out every month since January 1962. For the first 9 months of 1970, 376 cases were admitted to Bangkok area hospitals. These low incidence figures stand in marked contrast to the 2526 patients admitted during the first 9 months of last year, and to the 1128 cases admitted in the first 9 months of 1968. Except for 2 or 3 focal outbreaks, the striking reduction in admissions for dengue hemorrhagic fever and shock has been noted this year in hospitals throughout Thailand. Of those cases admitted, the percentage that have died has remained about the same for the past 3 years, namely 3.6% in 1968, 3.5% in 1969, and 4.5% in 1970. Thus the severity of the illness has probably not lessened. If the trend continues, every month of 1970 will have experienced the lowest incidence of dengue-associated illness since the beginning of surveillance in 1962. There is as yet no satisfactory epidemiological explanation for this.

In other dengue studies we are attempting to determine if cellular immunity to dengue virus appears after dengue infection in humans. We are employing the techniques of macrophage inhibition and lymphocyte-mediated cytotoxicity to demonstrate cell immunity; work is in progress. Dr. Dumrong Chiewsilp is searching for dengue, type-specific, soluble complement fixing antigen in the serums of patients with dengue fever and T.H.F./shock syndrome.

(R. Edelman and T. J. Smith)

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

Serological survey of the same areas after six years

A serological survey for arbovirus antibody was carried out in 1960 with 400 human sera collected from four areas in West Bengal. The four areas were: the city of Calcutta; Darjeeling (a hill station, about 7000 feet above sea level), Belpukur (a village 50 miles south of Calcutta and about 20 miles from the sea coast) and Parbatpur (a village 40 miles West of Calcutta). The virus antigens used were: Dengue types 1 & 2, WN, JE, KFD, & Sindbis. In the year 1963, Calcutta experienced dengue haemorrhagic fever and chikungunya fever epidemic which persisted till 1965. In 1966, it was thought worthwhile to examine sera collected from the same four areas to see if there had been any change in the pattern of antibody, as compared to that of 1960. For obvious reason, it was planned to include chikungunya virus in the list of antigens for testing 1966 sera, as well as to check the available 1960 sera against this virus antigen.

Results are shown in the table.

REPORT FROM THE VIRUS RESEARCH CENTRE POONA, INDIA

Presence of Haemagglutinating Activity in Normal Cell Culture Fluid of Singh's Aedes Albopictus Cell Line

While studying haemagglutinating (HA) and complement fixing (CF) antigens in Aedes albopictus cell culture infected with different arboviruses it was noted that culture fluids from noninfected A. albopictus cells also agglutinated goose RBC. Some of the characteristics of this haemagglutinin are described here.

Table

	Calcutta		Belpukur		Parbatpur		Darjeeling	
	1960	1966	1960	1966	1960	1966	1960	1966
Group B	86/106* (81%)	107/143 (75%)	9/98 (9%)	118/133 (88.7%)	12/98 (12%)	93/117 (79%)	6/97 (6%)	30/117 (25.6%)
Chikungunya	13/105 (13%)	28/143 (19.6%)	6/56 (10.7)	19/133 (14%)	1/50 (2%)	27/117 (23%)	0/50 (0%)	0/117 (0%)

*Numerator indicates number positive, denominator indicates total number tested.

RBC from different species including goose, rooster, human 'O' group, guinea pig and sheep were tested and it was found that only goose, human 'O' group and to a certain extent guinea pig RBC gave agglutination. Optimum pH for agglutination was 6.0. Culture fluids from freshly seeded A. albopictus (137th. passage) when tested on second or third day showed low HA titres of 1 in 4 or 1 in 8. However, in the older cultures usually 14 days after seeding there was an increase and HA titres of 1 in 32 or 1 in 64 were recorded. The HA titres were not very consistent and varied in different batches of culture. Treatment with protamine sulphate or acetone did not significantly affect the HA, except for an occasional improvement in the pattern of HA. The HA activity was heat labile. Antisera of JE, WN, DEN-1, 2, 3, 4, chikungunya and Batai viruses did not inhibit the haemagglutination. Differential centrifugation of the normal culture fluids at 6,000 G for one hour, and 105,400 G for 2 hours showed that the haemagglutinin could be sedimented only at 105,400 G. No HA activity was observed in the intracellular material; low grade activity was however detected in the sediment after centrifugation at 105,400 G for 2 hours and at a concentration ten times the original volume. Haemadsorption was also observed with goose cells but the optimum pH for this phenomenon was 6.2 or 6.4.

It was thought that this culture which was at 137th passage level might have been contaminated by some extraneous virus. A. albopictus cell cultures stored in liquid nitrogen at earlier passage levels (14th and 34th passage) were therefore revived and tested for HA. It was found that all the cultures contained such HA activity. Three sets of A. albopictus primary cell cultures prepared on different days also developed HA activity 14-21 days later. Infant mice inoculated with the extracellular media or intracellular material did not show any signs of sickness or mortality. The extracellular fluid from normal noninfected cell cultures did not react in CF test with antisera prepared against chikungunya or polyvalent group A serum (obtained from CDC, Atlanta). Cell cultures prepared from larvae of two other species of mosquitoes, namely, Aedes w-albus and Aedes vittatus did not show any HA activity at any time, although occasional HA was present in cell cultures prepared from larvae of A. aegypti.

These findings indicate that the haemagglutinin in normal noninfected A. albopictus cell cultures is not due to presence of any extraneous viral agent but is probably due to some inherent property of the culture or due to some latent virus present in the larvae.

REPORT FROM UNITED STATES NAVAL MEDICAL
RESEARCH UNIT NUMBER THREE
CAIRO, EGYPT, U.A.R.

Bibliography of ticks and tickborne diseases

During October, 1500 copies of volume 1 were mailed. Volume 2 is expected to be ready for mailing in November. Volumes 3 and 4 are planned for 1971. An equal number of copies are available for distribution upon request. References to papers that may have been overlooked will be included as an appendix in volume 4. We shall be grateful for corrections and additions, and for reprints on all aspects of tickborne viruses.

Virus from ticks parasitizing birds and humans Zirqa Island, Arabian Gulf

In a paper to be published in the November 1970 issue of the Annals of the Entomological Society of America, the ecological features of the locality where this virus (see Information Exchange No. 20, page 124) infects Ornithodoros (Alectorobius) muesebecki are described, as well as clinical symptoms in humans bitten by this tick and the larval and nymphal stages and life cycle of the tick. We deeply regret to have to report that Dr. R.M. Oliver, Chief Medical Officer of the British Petroleum Company, who collected the infected tick material and furnished the clinical data, died on 6 October.

Virus from ticks parasitizing doves in Africa and Cyprus

Argas (Persicargas) streptopelia Kaiser, Hoogstraal, and Horner, described in the Annals of the Entomological Society of America (62(3):799-807; 1970), infests virus-infected doves. The virus is being characterized at YARU, and virus-tick-dove interrelationships are under investigation at NAMRU-3.

Viruses from Afghanistan

In Information Exchange number 20 we reported 19 virus strains isolated from Argas (A.) hermanni collected in Afghanistan. Sixteen have been related by CFT to Quaranfil. Further work by Casals at YARU on 1 of the 16 strains confirms its close relationship to Quaranfil and distant relationship to Johnston Atoll virus. This is the first record of the presence of this human disease agent in Afghanistan.

Two of the Afghanistan isolates were related by CFT to Group B. Work by Casals indicated that antigenically 1 differs significantly from 39 Group B agents he tested. These 2 strains have been sent to the Institut Pasteur in Dakar for further tests against several other Group B agents. Another isolate unrelated to any reference stocks maintained in this laboratory was shown by Casals to be related by CFT to Grand Arbaud and Uukuniemi, more closely to the former than to the latter.

Viruses from ticks from Egypt, Sudan, Thailand, and Taiwan

A virus isolate from Hyalomma a. anatolicum collected in Egypt, and 3 from Argas (Persicargas) robertsi collected in Thailand, give reactions by CFT to Manawa (T-461) but not to Eg An 1835-61, another member of the Uukuniemi group isolated from ticks from migrating birds in Egypt. However, we have noted some non-specific positives with our Manawa immune serum. A fourth isolate from A. (P.) robertsi of Thailand, and 1 from Hyalomma impeltatum of Egypt, are unrelated to any maintained as reference stocks in this laboratory. These, and 2 from Argas (P.) persicus from an Egyptian oasis, have been sent to YARU for identification. The agents from A. (P.) persicus are the first recorded from this species since structural differences to separate the taxons persicus and arboreus were shown. Three other strains now under study were isolated from Hyalomma dromedarii infesting camels driven from the Sudan to Egypt. Two other strains were isolated from a new Argas species parasitizing night herons in Taiwan.

Infection/transmission studies

For the past year we have attempted to validate the assumption that Wad Medani and Qalyub viruses are true arboviruses through infection/transmission studies using the tick species from which they were isolated, as vectors, and laboratory mammals as hosts. Efforts to find a susceptible vertebrate host continue to be unsuccessful. Varying doses of virus inoculated by various routes into adult mice, white rats, hamsters, guinea pigs, rabbits, and several wild-caught rodent species have failed to produce a detectable viremia. Wad Medani did produce illness and death when inoculated I. C. into suckling hamsters and white rats. Current studies employ wet chicks as possible susceptible hosts. The possibility that Argas (Argas) hermanni ticks may serve as the overwintering host for West Nile virus has been mentioned in papers by Taylor, Hoogstraal, and Schmidt. This assumption was based on WN isolation from these ticks during winter months in the Nile Delta. Subsequent experiments by Schmidt indicated that the ticks could be infected and that the virus persisted up to 5 months in the

nymphal and adult stages. Trans-stadial passage of the virus occurred but no evidence of transsovarial passage was obtained. Attempts to demonstrate transmission to susceptible hosts were consistently negative. It was concluded from this work that birds may become infected through ingestion of infected ticks (Schmidt, personal communication). We have initiated studies to elucidate this possibility. To date, doses of WN up to 6,000 $SMICLD_{50}$ have been administered orally in 4.5% BAPS diluent. Symptoms of illness and viremia have not been observed.

Immunodiffusion studies

Effort has been directed to developing a relatively standardized immunodiffusion methodology as a supplementary procedure for identifying Egyptian arboviruses strains. Two findings of some value have resulted from these studies. A cross reaction was noted (precipitation line) between Quaranfil serum and Sindbis antigen in agar gel precipitation (AGP) tests. Treatment of the Quaranfil serum by bubbling dry ice as reported by Imam et al (J. Egypt Publ. Hlth Ass., 41(1):33-36 (1966)) removed this non-specific reaction. Three homologous systems, Sindbis, Quaranfil, and Bunyamwera, failed to give positive reactions either in AGP or immunoelectrophoresis (IE) systems, although homologous CF titers were 128/64, 256/512, 256/512 (serum/antigen) respectively. Antigens of these viruses prepared by sucrose-extraction and lyophilized in 0.5 ml aliquots, when rehydrated in 0.2 ml amounts, gave clear, positive precipitation lines with their homologous sera.

Medical Zoology Department (Medical Zoology and Virus-Vector Divisions):

(H. Hoogstraal and R.E. Williams)

REPORT FROM THE ARBOVIRUS LABORATORY INSTITUT PASTEUR AND ORSTOM BANGUI, REPUBLIC OF CENTRAL AFRICA

Isolations from human cases

During the year 1969, five strains of arbovirus have been isolated. One Nyando virus was isolated from a patient exhibiting a febrile syndrome with vomiting and stiffness which lasted for four days without any other clinical symptoms. Isolation was possible from serum collected on the day following onset of the disease. Authenticity of the strain was confirmed by the presence of antibodies in the late serum. The neutralizing index of the late serum is 2.5 if considering as a negative control the serum from which virus isolation was made.

Four Ilesha viruses from adult patients exhibiting fever, in some cases a generalized rash, and in one case erythematous spots on the anterior surface of the legs. Headache and stiffness were found in every case. During the same epidemic which lasted until 1970 we isolated Ilesha in March 1970 from a batch of 38 Anopheles gambiae.

Isolations from vertebrates

Two hundred birds were captured during the year; a strain of Uganda S was isolated from Saxicola rubetra.

Isolations from Arthropods

During the year, 73,414 mosquitoes were collected, 36,798 females were distributed in 1,010 pools. 21 strains were isolated:

1 Zika from Aedes africanus,
2 Usutu from Culex perfuscus,
1 Ntaya (sub type A 209) from Culex guiarti
4 Mossuril from Culex pruina, Culex perfuscus, and Culex weschei,
1 Tataguine from Anopheles gambiae,
1 Simbu from Aedes cumminsi,
1 Sindbis from Culex pruina,
10 are different from the strains to which they were compared in the WHO regional center.

Ar B 1569 isolated from Culex tigripes related but not identical to Olovantsvlei,

Ar B 1976 and Ar B 1986 isolated from Mansonia africana and Aedes gr palpalis identical between each other but showing no antigenic relationship with the other strains,

Ar B 2053 isolated from Culex perfuscus,

Ar B 2076 and Ar B 2078 resisting to chloroform isolated from Culex perfuscus,

Ar B 2181 isolated from Mansonia uniformis,

Ar B 2198, Ar B 2202, Ar B 2239 isolated from Anopheles pharoensis and Anopheles squamosus. Those three strains are very related to each other, belong to Bunyamwera group, but are different from Bunyamwera, Ilesha, Shokwe and Germiston.

REPORT FROM THE VIRUS RESEARCH LABORATORY UNIVERSITY OF IBADAN, NIGERIA

Lassa Fever

Data regarding Lassa virus were included in the last issue of the Information Exchange. That report dealt with the initial isolates of 1969 plus laboratory infection. We now report a short, sharp outbreak of Lassa fever on the Jos plateau, Nigeria in January and February, 1970. A total of 28 cases occurred during the period 25 December through 7 February. There were 13 deaths. Infection was detected by CF tests done at Ibadan, and virus isolation was performed at the Arbovirology Unit, Center for Disease Control, Atlanta.

Laboratory confirmation of Lassa was available for 19 patients; 7 by virus isolation alone, 7 by CF alone, and 5 by both methods. Of the remaining 9 patients; 6 died, while 3 could not be located.

Epidemiologic investigation revealed that a presumptive index case (so far not located) had somehow, while hospitalized at a Mission hospital in Jos, transmitted the infection to 18 persons. These secondary cases, the majority of whom became ill within a one week period, consisted of patients, hospital personnel, and visitors. The mode of transmission has not been ascertained. There were subsequently 8 third generation cases comprising close family contacts of those infected at the hospital. One of the fatal infections occurred as a result of an autopsy accident.

Serologic study by CF of family contacts and hospital personnel failed to reveal evidence for sub-clinical infection with Lassa virus. Neutralization tests at C. D. C., however, have shown the presence of antibody in the sera of up to 10 percent of certain groups of plateau residents and others.

No cases of Lassa fever have been diagnosed since early February, 1970.

Entomological Observations Related to Yellow Fever

The last number of the Information Exchange (No. 20, March, 1970) carried information of the Yellow Fever epidemic in Jos Plateau area in October - November, 1969. Since our observations were made after the peak of the epidemic, and we were unable to incriminate a vector mosquito, follow-up studies were planned and are being carried out during the 1970 rainy season. The follow-up studies were deemed desirable to elucidate yellow fever vector/s and also as a possible source of viruses. The following isolates were obtained from mosquito collections carried out at the time of the epidemic in 1969: Zika - 2 strains; Dengue - 1 strain; and YF - 1 strain, all from Aedes (Stegomyia) spp.; Ofoumselek - 1 strain; and Bwamba - 1 strain from Anopheles gambiae and funestus, respectively.

Bimonthly investigations in the rural areas of the previous epidemic were carried out in April, June, August and October, 1970. These studies have shown the following stegomias present in those areas. A. aegypti, A. africanus, A. luteocephalus, A. simpsoni, and A. vittatus.

Changes in the species composition throughout the rainy season have not yet been plotted, but rough data show that the human-bait landing/biting rates are in the following order:

1) A. luteocephalus
2) A. aegypti
3) A. africanus
4) A. vittatus
5) A. simpsoni

A. luteocephalus is by far the predominant mosquito in the captures, and is present in greatest numbers in each of 3 areas studied intensively. A. africanus is present in significant numbers in but one of the areas. A. simpsoni has been found in each of the areas but has not been taken in condition indicating that of actually having taken a blood meal. That simpsoni is in fact anthropomorphic in this area has yet to be demonstrated.

Surveys for larval development have revealed that A. luteocephalus, A. aegypti and A. africanus commonly utilize sites in Euphorbia Kamerunica hedges so abundant around settlements and small farming plots. Again A. luteocephalus is predominant in these collections and present information indicates sizeable populations being produced in these sites close to human activities.

Field Studies

Over the past several years numerous field trips have been conducted in Nigeria and on one occasion each to Dahomey and Togo. Such trips are ordinarily planned as multipurpose Safaris as distances are great, and roads are frequently difficult. Depending on personnel available at the particular time the efforts may be directed towards collecting specimens of small mammals, domestic livestock, wild birds, ticks, mosquitoes, human serum, or a combination of activities.

One of these multipurpose trips was recently conducted to Kware in the north-west corner of Nigeria. Kware is a medium sized village about 13 miles north of Sokoto on the Rima river. The duration of the trip was from May 19, 1970 to June 1, 1970. This trip was planned to coincide with the beginning of the rains. It was hoped that arrival would post-date the rains by two or three weeks. Unfortunately, the rains were late and the first rain came only on the day of arrival at Kware. The countryside presented the appearance of parched earth. The usual bright green appearance of a larger fadama (flood plain grassland) had changed to brown. Over-grazing had occurred to such an extent that only roots remained. Livestock was attempting to regraze these roots. Goats were attempting to climb thorn trees in an effort to reach small green leaves. The usual water holes in the fadama were devoid of water, and the soil was cracked. The only water source was the Rima river which still had a narrow margin of green reeds. This collecting expedition is reported as an example of the surprising amount of viral activity which may be detected in what would appear to be unlikely circumstances.

Trapping and mist netting were conducted along the river. A total of 551 small mammals of 15 species and 304 birds of 31 species were sampled during the period. The most common mammals taken were the Nile rat Arvicanthis niloticus, (339); and hedge hog Atelerix albiventris (121). The most common birds collected were the Senegal Combassou H. chalybeata (118), and the little bishop E. orix (42).

Preliminary testing indicates more than 40 virus strains were isolated. Twenty six of these strains were identified as Ib AN 10065, formerly referred to as Fika, and now identified as a member of the Phlebotomus fever group by Robt. E. Shope at YARU. This virus was isolated frequently from Arvicanthis niloticus and Atelerix albiventris and Taterillus spp.

Potiscum virus (related to Uganda S) was isolated from Mastomys natalensis and Arvicanthis niloticus.

Semliki forest virus was isolated from Atelerix albiventris and Quelea erythrops. These are thought to represent the first isolations of this agent in Nigeria.

Tete virus was identified from the little bishop, Euplectes orix.

Ingwavuma (Simbu group) was isolated from Quelea, and West Nile was isolated from Arvicanthis.

Virus Ib AN 28946, isolated on several occasions in Ibadan and not as yet identified was isolated from H. chalybeata and Quelea erythrops.

A number of strains from birds and mammals remain unidentified.

REPORT FROM THE ARBOVIRUS LABORATORY PASTEUR INSTITUTE AND ORSTOM DAKAR, SENEGAL

This report reviews field and laboratory studies on arboviruses in Senegal during the period January 1, 1970 through June 30, 1970.

Virological Studies

The arbovirus laboratory processed 2769 specimens obtained from various sources (table 1).

A. Human blood samples.

30 from 34 human blood samples have been collected at the Bandia Dispensary: they yielded one arbovirus strain identified as Ilesha. This isolation is of great interest as it takes place in February, during the dry season when the mosquito population is rather scattered. During the 1969 rainy season 16 strains of human origin had been isolated from febrile children, and identified: 7 strains of Tataguine, 7 strains of Ilesha and 2 strains of Zika. The chronology of isolations is shown in table 2.

B. Wild vertebrate samples.

Most of the material processed (584/605) was collected at the Bandia and Saboya field stations. 4 arbovirus strains have been identified.

1. Bandia: 1/347 vertebrate specimens yielded a strain of Bandia virus.

2. Saboya: Dak An D 5314 (Keuraliba) virus, a new arbovirus isolated for the first time in 1968, has been isolated again from organs of three Tatera.

This isolation points out the importance of this rodent as arbovirus host in the Saboya area.

C. Arthropods.

2130 pools of arthropods were processed for virus isolation and 26 viruses were isolated.

Table 1

MATERIALS PROCESSED FOR ISOLATION ATTEMPTS

	ORIGIN			TOTAL
	BANDIA	SABOYA	DIVERS	
Human sera	30	-	4	34
Wild vertebrates	347	237	21	605
Mosquito pools	596	375	-	971
Tick pools	115	27	840	982
Other arthropods	76	101	-	177
T O T A L	1164	740	865	2769

Table 2

CHRONOLOGY OF ISOLATES

	Inoculated	Positive	Identification		
			Tataguine	Ilesha	Zika
January-July	74	0	0	0	0
August	47	7	6	1	0
September	83	2	1	1	0
October	73	4	0	4	0
November	37	2	0	1	1
December	18	1	0	0	1
T O T A L	332	16	7	7	2

From mosquitoes there were 10 isolations from 101.767 specimens and from ticks there were 16 isolations from 17.446 specimens. There were no isolations from Culicoides (39.578) Phlebotomine flies (3599) or tse-tse flies (75).

1. Bandia: Bandia virus was isolated from 9 pools of Ornithodoros erraticus sonrai. Thus, after an "eclipse", this virus seems to be again very active in the Bandia forest.

Adult mosquitoes were caught in the forest and in the village, around the dispensary: 8 viruses have been isolated.

- 4 strains appear to be Ilesha virus: 3 obtained from Anopheles gambiae and one from Culex thalassius.

- 1 isolated from Anopheles gambiae has been identified as Tataguine. These findings point out the ability of Anopheles gambiae to carry Ilesha and Tataguine viruses. The potential role of Anopheles gambiae as a vector of these arboviruses has to be tested in transmission experiments in the laboratory.

- 4 strains of Zika virus were isolated from Aedes furcifer taylori (2) and Aedes luteocephalus (2).

2. Saboya.

- 2 strains of Zika virus have been isolated 1 from Aedes luteocephalus and one from Anopheles gambiae.

3. Dakar.

- 7 virus strains have been recovered from tick samples collected at the slaughter house (table 3) 4 isolates: 2 from H. truncatum 2 from H. rufipes appear to be strains of the same virus but show no relationships to reference material maintained in our laboratory.

- 2 from A. variegatum appear to be strains of Jos virus (Ib Ar 18735).

- 1 from A. variegatum was found to be identical to Bhanja virus.

Table 3

TICKS

S P E C I E S	POOLS	NBRE	POSITIVE	IDENTIFICATION
Amblyomma variegatum	248	449	3	Jos virus (2) , Bhanja (1)
Hyalomma truncatum	328	6637	2	Unidentified - New ?
Hyalomma rufipes	53	519	2	Unidentified - New ?

Serological Studies

A. Human sera.

1. Dakar: Serological study of paired sera from a severe case of encephalitis with coma showed a pattern suggesting multiple group B exposure and accurate diagnosis of the current illness was impossible, even by neutralization test.

2. Bandia: 176 sera collected from febrile children seen at the Dispensary were tested in CF tests using Zika, Ilesha, Bandia and Tataguine antigens.

Results are summarized in table 4.

3. Wild vertebrate specimens: Sera obtained from 793 vertebrates were tested for HI antibodies against 9 viruses. Results are shown in table 5. As used, virus circulation appear to be most active in Saboya area than in Bandia forest.

(Y. Robin, P. Bres, Institut Pasteur; R. Taufflieb, M. Cornet and J.L. Camicas, ORSTOM)

Table 4

HUMAN SERA - CF TEST

DATE OF COLLECTION	Nbre SERA STUDIED	POSITIVE FOR FOLLOWING ANTIGENS							
		ZIKA		ILESHA		BANDIA		TATAGUINE	
		Nbre	%	Nbre	%	Nbre	%	Nbre	%
January - June	74	2	2.7	0	0	5	6.7	0	0
July - December	102	0	0	21	20.6	1	1	23	22.5
T O T A L	176	2	1	21	12	6	3.4	23	13

Table 5

WILD VERTEBRATE SEROLOGICAL STUDIES

HI TEST

ANTIGENS	BANDIA		SABOYA	
	Sera tested : 443		Sera tested : 350	
	POSITIVE		POSITIVE	
	Nbre	%	Nbre	%
Group A Chikungunya	38	8.6	34	9.7
Group B Yellow fever	47	10.6	78	22.3
Uganda S	67	15.1	85	24.3
Dakar Bat	51	11.5	65	18.6
West-Nile	97	21.9	88	25.1
Zika	55	12.4	52	14.8
Bukalasa Bat	24	5.4	46	13.1
Wesselsbron	61	13.8	73	20.8
Bunyamwera group	1	0.2	0	0

REPORT FROM THE VIRUS DEPARTMENT
ANDRIJA ŠTAMPAR SCHOOL OF PUBLIC HEALTH
MEDICAL FACULTY, UNIVERSITY OF ZAGREB
ZAGREB, YUGOSLAVIA

Isolation of Čalovo Virus from Mosquitoes

The Virus Department, Andrija Štampar School of Public Health, Medical Faculty, Zagreb, and the Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, have carried out a field study in the north-east of Croatia with a view to investigating the appearance of Arboviruses (excluding TB virus which has been known to exist there for several years).

In August 1969, following serological investigation, the attempts were made to isolate virus from about 20.486 mosquitoes of which 10.320 were identified as Anopheles maculipenis and the rest as Aëdes, Culex and other species. The isolations were performed in pools of each 20-200 mosquitoes per species inoculated intracerebrally into baby mice.

On 52 tested Anopheles maculipenis pools 10 strains have been isolated so far. All the strains have been verified by re-isolation and further passages. The attempts of isolating virus from other mosquito species are in progress.

The neutralization test with Čalovo and Tahyna antisera has shown that the first isolated strain NS3 is antigenically similar to the original Čalovo virus 134 (Table 1). The results of the cross-neutralization test with Čalovo virus are shown in Table 2.

Further field and laboratory studies, as well as the study of the relation of sheep between the isolated virus and human infections are under way.

Table 1. The neutralisation test with Calovo and Tahyna antisera

Virus	Serum	
	anti Calovo 184	anti Tahyna 181
MS3	3,84[+]	0
Calovo 134	>5,oo	-[++]

Table 2. The cross-neutralisation test with Calovo strain 184

Virus	Serum	
	anti MS3	anti Calovo 184
MS3	≥ 5,o5	4,84
Calovo 184	4,83	5,83

\+ in log NI

++ not done

REPORT FROM THE INSTITUTE OF HYGIENE
UNIVERSITY OF PALERMO
ITALY

Isolation of viral agents from arthropods in Western Sicily

During October and November 1968 and May through August 1969 tick and mosquito specimens collected in different areas of Western Sicily have been processed and inoculated into baby mice for arbovirus isolation attempts.

5100 Rhipicephalus bursa, 1948 Hyalomma marginatum, 550 Rhipicephalus sanguineus, 87 Haemaphysalis punctata, 77 Boophilus calcaratus and 26 Ixodes exagonus specimens were tested. Males and females were equally represented. 6 isolates have been obtained from pools of adult male Rhipicephalus ticks captured on goats in July; their identification is in course.

No isolates have been obtained from 5400 Aedes caspius, 2100 Aedes detritus, 200 Teobaldia annulata and 100 Culex pipiens specimens tested.

Further isolation attempts from arthropods collected in different periods are in course.

(M. Albanese, C. Bruno, L. Dardanoni, and A. Lavagnino)

REPORT FROM THE MICROBIOLOGY DEPARTMENT
ISTITUTO SUPERIORE DI SANITA'
ROME, ITALY

Arbovirus Studies in Birds in Italy, 1967-1970

A study on the role of migratory birds on the dissemination of arboviruses in nature has been done in Italy in the years from 1967 to 1970.

The field studies were restricted to the area, close to the Eastern Alps, where are the lowest mountain-passes, which act to funnel some bird species migrating from Northern Europe to the South. In Fig. 1 the frequencies of recapture, in the study area, of birds banded in some European countries are reported. It is evident that bird migration from Eastern-Europe is prevalent.

Serologic examinations. A serologic survey, performed by HI test, on 660 birds of 25 species gave evidence of previous contacts with group A, B and Bhanja arboviruses (Table 1). Antibodies to West Nile or strictly related viruses were found mainly in winter visitors species. No antibodies to Sindbis antigen were found, although birds have been demonstrated to have a role in the circulation of this virus in Eastern Mediterranean area.

Virus isolations. From a total of 216 birds of 20 species, captured during the fall migration in 1968, three birds (Fringilla coelebs, Fringilla montifringilla, Passer domesticus) were found viremic. The three viruses share a common CF antigen and, at least, two distinct serotypes were demonstrated by HI test.

The study of one of these viruses, performed by Dr. R.E. Shope at YARU, indicates that it is a close serological relative of Bahig virus, a member of the Tete group of arboviruses.

From the analysis of the fall migration between Europe and Africa, it was supposed that the as yet undetected "foci" of Bahig and relative viruses exist somewhere in Europe or Asia. Our findings seem to exclude that infection of these birds originated in Italy and this observations is

BIRDS BANDED IN EUROPE AND CAPTURED IN ITALY (Gorizia Province) DURING THEIR FALL MIGRATION, 1966 - 1969.

Fig. 1

HEMAGGLUTINATION - INHIBITING ANTIBODIES TO 5 ARBOVIRUSES IN SERA OF 660 BIRDS CAPTURED BY NETS IN ITALY, 1967 - 1969.

MIGRATORY GROUP AND SPECIES	Totol no of samples	WEE		SINDBIS		TBE		WEST NILE		BHANJA		TOTALS	
		No positive	%	No positive	%	No positive	%	No. positive	%	No positive	%	No positive	%
WILD AVIAN SPECIES													
Permanent resident													
House sparrow	17	0	-	0	-	0	-	1	5 9	0	-	1	5 9
White owl	1	0	-	0	-	0	-	0	-	0	-	0	-
Buzzard	1	0	-	0	-	0	-	0	-	0	-	0	-
Kestrel	1	0	-	0	-	0	-	0	-	0	-	0	-
Owlet	1	0	-	0	-	0	-	0	-	0	-	0	-
Jay	2	0	-	0	-	1	50 0	1	50.0	1	50 0	1	50 0
Subtotals	23	0	-	0	-	1	4 3	2	8 7	1	4 3	2	8 7
Winter visitor													
Gold-finch	1	0	-	0	-	0	-	0	-	0	-	0	-
Blackcap	3	0	-	0	-	0	-	1	33 3	0	-	1	33 3
Siskin	37	0	-	0	-	0	-	3	8 1	0	-	3	8 1
Chaffinch	62	0	-	0	-	0	-	1	1 6	0	-	1	1 6
Brambling finch	311	5	1 6	0	-	0	-	32	10 3	7	2 2	37	11 8
Green finch	3	0	-	0	-	0	-	0	-	0	-	0	-
Haw finch	74	0	-	0	-	0	-	9	12 2	1	1 3	8	10 8
Black bird	92	1	1 0	0	-	0	-	14	15.2	3	3 2	15	16 2
European redwing	4	0	-	0	-	0	-	0	-	0	-	0	-
Thrastle	5	2	40 0	0	-	0	-	0	-	0	-	2	40.0
Hedge-sparrow	5	0	-	0	-	0	-	1	20.0	0	-	1	20 0
Mountain-sparrow	6	0	-	0	-	0	-	0	-	0	-	0	-
Cirl bunting	1	0	-	0	-	0	-	0	-	0	-	0	-
Rock bunting	3	0	-	0	-	0	-	0	-	0	-	0	-
Starling	3	0	-	0	-	0	-	0	-	0	-	0	-
Subtotals	610	8	1 3	0	-	0	-	61	10 0	11	1 8	68	11 1
Summer visitor													
Golden oriole	1	0	-	0	-	0	-	0	-	0	-	0	-
Nightingale	1	0	-	0	-	0	-	0	-	0	-	0	-
Subtotals	2	0	-	0	-	0	-	0	-	0	-	0	-
DOMESTIC AVIAN SPECIES													
Pigeon	12	0	-	0	-	0	-	2	16 6	1	8 3	3	25 0
Chicken	13	0	-	0	-	0	-	1	7 7	1	7.7	2	15 4
Subtotals	25	0	-	0	-	0	-	3	12.0	2	8 0	5	20 0
GRAND TOTALS	660	8	1 2	0	-	1	0 1	66	10 0	14	2 1	75	11 3

supported by the fact that no isolations were obtained from mosquitoes and ticks collected in associated sites.

(M. Balducci, P. Verani and M. C. Lopes)

REPORT FROM THE WHO REGIONAL REFERENCE LABORATORY FOR ARBOVIRUSES INSTITUTE OF VIROLOGY SLOVAK ACADEMY OF SCIENCES BRATISLAVA, CZECHOSLOVAKIA

Experimental infection of rhesus monkeys with Uukuniemi virus

The experimental pathogenicity of Uukuniemi virus for Macaca rhesus monkeys has been studied. Five rhesus monkeys were inoculated intrathalamically and four monkeys intraperitoneally with $5 \times 10^{10.5}$ mouse LD_{50} of a strain of Uukuniemi virus isolated in Slovakia. The clinical symptoms, the cytological changes in the cerebrospinal fluid, the presence of the virus in organs, and the histopathological changes in CNS were examined.

For viraemia tests, blood was taken every day from 1st through 10th/p.i./. Blood coagulation was prevented by the addition of heparin; thereafter the plasma was obtained by centrifugation. Viraemia was detected in ic inoculated monkeys from 1st to 7th days p.i. viraemia titers varied in the blood cells from $10^{1.5}$ to $10^{5.0}$; in plasma from $10^{3.0}$ to $10^{5.4}$ /Table 3/. In ip inoculated monkeys viraemia occurred between the 1st to 7th days p.i. in a titre ranging from $10^{1.5}$ to $10^{5.0}$ in the blood cells and in a titre ranging from $10^{2.0}$ to $10^{3.5}$ in the plasma /Table 1/.

Symptoms of meningitis with marked pleocytosis in the spinal fluid were found in ic infected monkeys on the 4th and 9th days after virus inoculation /Table 2/. The virus was isolated from the spinal fluid on 4th and 14th days p.i. In ip inoculated monkeys symptoms of meningitis in the spinal fluid were marked in one case on the 9th day after virus inoculation, although the virus was reisolated on the 4th day after infection.

Table 1

Viraemia in rhesus monkeys following infection with Bukuniemi virus

Days after infection	ic inoculated monkey		ip incculated monkey	
	virus titre+ in blood cells	in plasma	virus titre in blood cells	in plasma
1	$10^{1.5}$	$10^{5.4}$	$10^{4.7}$	$10^{2.0}$
2	$10^{3.5}$	$10^{3.0}$	$10^{2.5}$	$10^{2.0}$
3	$10^{3.5}$	$10^{4.0}$	$10^{5.0}$	$10^{3.5}$
4	$10^{5.0}$	$10^{3.0}$	$10^{1.5}$	$10^{3.5}$
5	$10^{3.6}$	0	$10^{3.5}$	$10^{3.0}$
6	$10^{3.0}$	$10^{4.0}$	$10^{1.5}$	$10^{2.0}$
7	0	$10^{3.6}$	0	$10^{3.5}$
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0

+ ic mouse LD_{50} per 0.01 ml

0 = no virus isolated

Table 2

Results of laboratory examination of liquor from monkeys ic infected with Uukuniemi virus

Days after inoculation	Pándy	Protein /mg %/	Leukocytes	Lymphocytes	Glykorrhachia	Virus in liquor /titre in LD_{50} per 0.01 ml/
4	+	-	275	270	78	$10^{4.7}$
9	+	35	2605	2540	50	0
14	0	10	70	68	38	$10^{3.0}$
18	0	20	16	16	38	0
Control	Opalescence	20	9	17	52	-

+ = Results positive, 0 = results negative

- = not tested

(Table 3 not submitted)

A very low level of infective virus was detected on 9th and 14th days in the medula spinalis of monkeys ic inoculated with Uukuniemi virus. In ip inoculated monkeys the virus was detected at low levels in the brain cortex on 14th and 19th days after infection. Positive virus isolation was demonstrated in medulla spinalis on 14th day after virus inoculation.

All monkeys infected with Uukuniemi virus were examined for the presence of HI antibodies. No animal had detectable circulating HI antibodies to Uukuniemi virus.

Histological examination: in ic infected monkeys the histological examination revealed a picture of meningitis with lymphocytic and mononuclear infiltration between the 9th and 14th postinfection days. On the 4th day p.i. perivascular cuffings and mononuclear infiltrations were found in the vicinity of the needle track. The meningeal infiltrates were situated mainly on the cerebral bases, and mainly in the fissures on the convexity of the hemispheres. On the 14th day a few perivascular cuffings around the lateral ventricles were observed. On the 19th day the histological findings remained negative. The results of the immunohistological examination of the CNS tissues by the means of fluorescent antibodies remained negative. The histological changes and the results of the liquor examination were similar to those observed in ic infected monkeys with the Tribeč virus.

No marked inflammatory changes could have been observed in the meninges of ip inoculated monkeys, contrary to ip infected suckling mice, in which a marked meningoencephalomyelitis developed.

Studies on haemagglutination-inhibition antibodies against some arboviruses in human population of selected regions in Yugoslavia

Arthropod-borne viruses from different serological groups are known to be active in Yugoslavia. Epidemics of tickborne encephalitis, Phlebotomus fever and haemorrhagic fever have been reported. From the Bunyamwera supergroup Ťahyňa virus was isolated in Yugoslavia. In the present communication HI antibodies against A, B, Bunyamwera groups and ungrouped viruses were investigated in human population.

Blood samples were collected in May and June 1969 from persons living in rural area. Sera were stored frozen at -20°C.

Antigens for haemagglutination-inhibition /HI/ tests were prepared from the brains of infected suckling mice by sucrose acetone extraction /Clarke and Casals 1958/. Sera were extracted by acetone and adsorbed with goose

erythrocytes prior to testing. The HI test was carried out by the method of Clarke and Casals, using 4-8 units of antigens. The sera were tested with following antigens: Sindbis, tick-borne encephalitis /TBE/, Omsk haemorrhagic fever /OHH/, Kyasanur Forest disease /KFD/, West Nile /WN/, dengue type, 1, 3 and 4; Sicilian phlebotomus fever, Ťahyňa, Čalovo and Uukuniemi.

Results of the HI tests for arboviruses on 173 human sera are presented in Table 1. In the community Kesovská Mitrovice no HI antibodies to Sindbis, TBE, OHH, KFD, WN, dengue, Ťahyňa and Čalovo antigens were detected. In one serum sample from 16 tested, antibodies to Sicilian Phlebotomus virus were detected in a titre 1:20.

In the community Priština, 30 serum samples were examined. One serum sample contained antibodies to Uukuniemi virus and the other to Čalovo virus. No antibodies to Sindbis, TBE, OHH, KFD, WN, dengue and Ťahyňa antigens have been found.

In the locality Péč, HI antibodies were detected to Ťahyňa virus /2 sera were positive from 37 tested/ to Čalovo virus /2 samples positive from 37/ and to Uukuniemi virus /1 serum positive/.

In the locality Titograd, antibodies to TBE virus were detected in one serum sample from 45 tested. The cross reactions to OHH, KFD, WN and dengue type 3 were observed. In 1 case antibodies to Ťahyňa virus were found in a titre 1:160. Two serum samples reacted with Čalovo antigens and 3 samples with Uukuniemi antigen /Table 1/.

In the locality Ulcinje, no antibodies to the viruses belonging to A, B, Phlebotomus and ungrouped arboviruses have been found. The only positive reaction was found to Čalovo virus antigen in one serum sample.

In the locality Šavnik-Milošovec, HI antibodies were found to OHH virus /1 being positive from 17/ and to Čalovo virus /2 being positive from 17/. No antibodies to other arboviruses tested have been found /Table 1/.

It is of interest, that antibodies to Čalovo virus have been found in human sera. The titres of antibodies were reaching the values from 1:20 up to 1:320. Further studies are required to elucidate the existence of natural foci of Čalovo virus in the area investigated.

Table 1

HI antibodies in 173 human sera from Yugoslavia against 12 arboviruses

Localities	No.of sera tested	No. of positives and titres with arbovirus antigens											
		Sindbis	TBE	OHH	KFD	WN	Dengue type 1	Dengue type 3	Dengue type 4	Tahyňa	Čalovo	Phlebotomus	Uukuniemi
Kesovské Mitrovice	16	0	0	0	0	0	0	0	0	0	0	1x1:20	0
Priština	30	0	0	0	0	0	0	0	0	0	1x1:20	0	1x1:20
Péč	37	0	0	0	0	0	0	0	0	2x1:20	2x1:20	0	1x1:20
Titograd	45	0	1x1:80	1x1:20	1x1:20	1x1:20	0	1x1:20	0	1x1:160	2x1:20 1x1:80	0	2x1:20 1x1:40
Ulcinje	28	0	0	0	0	0	0	0	0	0	1x1:40	0	0
Šavník Milošovec	17	0	0	1x1:20	0	0	0	0	0	0	1x1:80 1x1:320	0	0

TBE = Tick-borne encephalitis
OHH = Omsk haemorrhagic fever
KFD = Kyasanur forest disease
WN = West Nile

REPORT FROM THE D. I. IVANOVSKY INSTITUTE OF VIROLOGY MOSCOW, USSR

Arboviruses in Azerbajan

The investigations on the isolation of arboviruses in nature were started in 1967 in south-east Azerbajan. The work was carried out within a rather limited area, which included two landscape regions:

1) river flooding on a semideserted plain with rich aquatic vegetation;

2) subtropical forests of Lenkorane lowland and foothills of Talishsky mountains with considerable area of cultivated land on the plain.

Lenkorane lowland is situated to the South of Baku at latitude 39° N and longitude 48°30' E. The temperature in July is 21° to 29° C; the temperature in January is +3.5°C. Annual precipitation averages are about 200 to 300 mm for the desert regions and more than 1.000 mm for the subtropics. According to the climatic conditions, fauna and flora, Lenkorane lowland belongs to subtropics. This region is of great interest for the investigation of arboviruses, as the fly ways of migratory birds from Africa and India cross here, and there is a nesting place of the birds on the sea coast of the Caspian Sea. All these facts allow us to suppose that in this place arboviruses may be discovered. Our investigations are carried out in collaboration with the arbovirus laboratory of the Institute of Virology, Microbiology and Hygiene in Azerbajan.

The material was collected yearly, from April to September. In 1967 and 1968, 615 small mammals of 22 species, 1113 birds of 111 species, 11200 mosquitoes of 5 species and 800 ticks were collected and tested. From this year we started the investigation of ticks more intensively. Besides the isolation of the virus, serological investigations were carried out. The methods of work were as follows: the material was primarily treated in the field laboratory: blood, visceral organs and brain were asceptically taken, placed into glass bottles and frozen at -70°C in CO_2 cabinet. Mosquitoes were first identified and then frozen under the same conditions. For 24 hours after the time of the collection, the ticks were kept at 4°C.

For virus isolation 1 to 2 litters of 1-3 day-old mice were infected i/c. The material from 3 to 5 animals of the same species was collected simultaneously on the same territory and pooled. The infected animals were kept under observation for 14-15 days. Blind passages were not done. In the case of positive results reisolation was carried out from the initial

material. For reisolation not only suckling mice were infected, but also tissue culture and chick embryo.

During 2 years, from 1967 to 1968, 4 strains of arboviruses were isolated, all of them from birds. Two strains of arboviruses were isolated from Turdus merulla: the strain 1628 of West Nile (WN) virus was isolated in 1967 and the strain 540 in 1968. The latter has not been identified thus far. The strain 1640 of WN virus was also isolated from Sitta europaea in 1967. The strain 574, identical or closely related to Sindbis virus, was isolated from Ardeola ralloides. The isolated strains were studied by the routine methods. We determined the type of nucleic acid by indirect method in the test with bromdeoxyuridine, sensitivity to ether and deoxycholate, the size by filtration through the "Millipore" membranes. Both strains of WN virus were antigenically identical. In HI test they were neutralized by both homologous sera and sera against strain Eg 101.

It is quite possible, that we shall study the antigenic structure by agar immunodiffusion method and by the method of electrophoresis in a greater detail. These experiments have been recently started.

As tested in HI, human sera, the blood sera of birds and mammals collected in Azerbajan, showed antibodies to WN virus and Sindbis virus. In 1969 we gained valid data supporting the role of WN virus in human pathology. During the collection of the material in a colony of birds a member of the team, Dr. G., experienced a febrile disease. The onset of the disease was sudden with headache and muscle pains. The temperature was above 39^{o} and lasted 3 days. Doctor G. was in the place of the collection of the material from August 11, and fell ill on August 16. Consequently the incubation period ranged within 4 to 5 days. There were very many mosquitoes there at that time. Such species as Aedes caspius and Culex molestus predominated, and there were small numbers of Anopheles hyrcanus and Mansonia richardi. Unfortunately we could not make a detailed follow up of the course of illness, since Doctor G., his technical assistant and hunters were in a very isolated place. The study of the blood in HI test with several arbovirus antigens revealed a pronounced serum conversion to WN virus. No antibodies to WN virus were demonstrable on the day of the onset of the disease; on the 10-th day antibodies were in titer of 1:40 and on the 30-th day - in titer of 1:80. This is the only case of WN fever disease, observed thus far in man which provided final proof of the role of WN virus in human pathology in the region studied.

We did not succeed in the identification of strain 540 yet, but according to the preliminary data, it is an arbovirus. The virus has been named Sumakh

according to the place of isolation. As was demonstrated by the very fact of isolation, the virus was pathogenic for suckling white mice. On intracerebral inoculation the incubation period at the time of isolation was 11 days but in subsequent passages was shortened to 96 hours. The virus titers in the brain reached lg 6.5-7.0 LD_{50}. After i/p and s/cut inoculation 1-6 day-old mice became ill after 9-10 day incubation period. Intracerebral inoculation of 3-4 week-old mice resulted in irregular death of occasional animals. After inoculation of adult mice intraperitoneally, subcutaneously, intranasally and intracutaneously into the plantar area of the leg the animals remained normal and showed no pathological changes in the lungs or in the skin respectively.

The course of infection in suckling mice was characterized by viremia which at the height of the disease reached a titer of lg 3.0 LD_{50}. After inoculation of chick embryos into the yolk sac hemorrhages were observed and some of the embryos died between 3 and 6 days. According to the results of titration in mice, the virus titer in embryos was not high -lg 2 LD_{50}.

The virus multiplied in chick embryo fibroblast cultures producing cytopathic effect in three passages and titers of lg 5.5-6.0 TCD_{50}/ml.

The type of nucleic acid was determined by an indirect method using the effect of BUDR on virus reproduction. The presence of BUDR in chick embryo fibroblast cultures inoculated with the virus not only did not inhibit but even slightly increased the titer of the virus, whereas multiplication of DNA-containing herpes virus under similar conditions was inhibited. The virus passed freely through Millipore membranes with a pore size of 100 mμ, but was retained by 50 mμ filter. Both ether and sodium deoxycholate caused a drop in virus titer by 3 and more lg LD_{50} indicating the presence of supercapsid lipoprotein envelope.

Hemagglutination of goose erythrocytes with borate-saline, freon and Tween-ether antigen from suckling mice brain using Clarke and Casals (1958) methods was negative. Since the Sumakh virus showed no hemagglutinating properties, the main identification method was complement fixation test. For this the sucrose-acetone brain antigen of the Sumakh virus with a titer of 1:320 and IAF with a titer of 1:160 were used. In order to exclude relationship of the virus under study with some murine and avian viruses most probably by the conditions of isolation despite the absence of common biological and physico-chemical properties, complement fixation tests were performed with the antigens and IAF of ornithosis, Newcastle disease, Corona I a. Corona II, Sendai, encephalomyocarditis, Reo 3 and ectromelia viruses. They were all negative.

Complement fixation tests were performed with the Sumakh antigen and a large set of polytypic and monospecific IAFs for arboviruses. IAF for the Sumakh virus was also tested in the CFT with a set of arbovirus antigens. Moreover, the IAF for the Sumahk virus was used in the HI test with different hemagglutinating arbovirus antigens.

The Sumakh antigen did not react with polytypic arbovirus sera of Groups A, B, Bunyamwera, Guama, California. In cross-tests no antigenic relationships were detected with Group A viruses: Aura, Venezuelan equine encephalomyelitis, Getah, Western and Eastern equine encephalomyelitis, Mayaro, Middelburg, Mucambo, O'nyong-nyong, Una, Semliki Forest, Sindbis, Pixuna, Chikungunya; Group B: West Nile, tick-borne encephalitis, St. -Louis, Ilheus, Wesselsbron, Usutu, Ntaya, yellow fever; Group C: Oriboca and Apeu; California group: California, Lumbo, Tahyna; Kemerovo group: Kemerovo, Chenuda, Tribec; Guama, Bunyamwera, Bwamba, Simbu, Sicilian and Naples sandfly fever, Congo, Bhanja, Kaisodi, Quaranfil, Uukuniemi, Colorado tick fever viruses.

Some tests with the IAF for the Sumakh virus were carried out at the WHO International reference center for arboviruses by Dr. Jordi Casals using 11 ungrouped viruses: Acara, Aurac, Kali, Hart-Park, Palyam, Urona, Mapputa, Mirim, Mossuril, Nyando and Triniti. No antigenic relationships with these viruses were found. Thus, the Sumakh virus was differentiated serologically from 53 arboviruses.

Further investigations are in progress.

(S. Ya Gaidamovich, L.P. Nikiforov, V.L. Gromashevsky, G.A. Klisenko, E.E. Melnikova, V.R. Obukhova, and V.I. Chervonsky)

REPORT FROM THE LABORATOIRE DES ARBOVIRUS INSTITUT PASTEUR PARIS, FRANCE

At the end of 1969, a survey on sera from patients in the eastern part of France showed evidence for a case of tick borne encephalitis. This case had happened in September 1968 with typical clinical signs and typical serological pattern in CF, NT and IHA tests. It was possible to interview the patient and to obtain informations on the possible sites of contamination.

On these grounds, a program was set up for visiting a forest south of Strasbourg and collecting each month small mammals and arthropods for virus isolation and for ecologic studies. The project started in February and each month 5 to 15 trapping nights were organized. As a result, 1,300 small mammals and 5,000 ticks were obtained from dragging the vegetation.

A first strain of tick borne encephalitis was obtained from a pool of Ixodes ricinus in July, another in August, seven in September and one in October. In addition, during the September expedition, a member of the collecting team was contaminated and had to be sent to the hospital for a month with a severe meningo-encephalitis.

In the same region, Tahyna virus is known to be present from human serological data, but virus has not been isolated yet. On the other hand, strains of LCM or LCM-like virus have been isolated from the brains of Cricetus cricetus in April and several other strains of viruses have been obtained from rodents and from ticks, and are under study.

REPORT FROM THE LABORATOIRE DE
MICROBIOLOGIE GÉNÉRALE ET MÉDICALE
UNIVERSITÉ DE LIÈGE, BELGIUM

After nine cycles of heating (5 min 60°) of SF virus grown in BHK21 cells and inoculating the surviving virus to the same cells, it was observed that the virus population, which was homogeneous as regards to the plaque size (5 mm) on chick embryo cells at the beginning of the experiment, had become highly heterogeneous. The final virus suspension formed 90% of small plaques (diametre <1 mm) and 10% of larger plaques (3.5 mm diam. <6 mm).

The small plaque "mutant" and the original large plaque strain were cloned three times and stock virus was prepared on BHK21 cells for further studies. The small plaque strain and the large plaque strain were equally neutralized by an antiserum prepared in rabbits with the original uncloned laboratory strain. The following tables summarize the results of our experiments with these two strains.

I. General characteristics		SF_{16} small p.	SF_0 large p.
Titre of the stocks in pfu/ml	at 27°	$1.0\ 10^9$	$1.3\ 10^8$
	at 38.5°	$9.0\ 10^8$	$1.2\ 10^8$
Plaque diam. CE cells "Noble" agar		<1 mm	>5 mm
Virus synthesis in BHK21 cells. Infection multiplicity 7 pfu/cell	24h 27° RNA(1)	27.1	14.7
	pfu(2)	$3.5\ 10^8$	$8.0\ 10^7$
	6h 38.5° RNA	22.8	18.0
	pfu	$4.5\ 10^8$	$1.2\ 10^8$
Buoyant density in (3) CsCl	complete particles	1.233	1.233
	empty envelopes	1.200	1.200
Diameter of empty envelopes (4)		± 101 nm	± 102 nm

(1) in per cent of the incorporated Uridine -2-^{14}C

(2) Total virus produced in the supernatant

(3) complete particles: infectious and haemagglutinating empty envelopes: haemagglutinating not infectious (Osterrieth 1968)

(4) No other difference in morphology. No data available for complete particles.

II. Sensitivity to physical and chemical agents		SF_{16}	SF_0
Heat inactivation in Gey's solution + bovine serum albumin			
inactivation constant k(1)	at 2°	$4.2\ 10^{-5}$	$1.9\ 10^{-5}$
	at 37°	$1.4\ 10^{-4}$	$1.1\ 10^{-4}$
	at 43°	$1.4\ 10^{-4}$	$1.7\ 10^{-4}$
	at 56°	$1.4\ 10^{-2}$	$1.9\ 10^{-2}$
ΔH^{++} (2) (calorie/mole)	T<43°	6,300	8,300
	T>43°	58,800	48,000
ΔS^{++} (calorie/mole/degree)	T<43°	-49	-43
	T>43°	+117	+85
Dialysis in Gey's solution + bovine serum albumin overnight at 4°	V/V°	0.01	1.00
Action of chemicals(3)			
Ethylmethanesulfonate (1/100 V/V) 6 hr 27°	V/V°	0.0007	0.005
$NaNO_2$ 2M 2 hr 27°	V/V°	0.2	0.01
N methyl N nitro N' nitrosoguanidine 0.1 mg/ml 30 min 20°	V/V°	1.0	1.0

(1) $V/V^o = e^{-kt}$

(2) Eyring absolute rate equation

(3) 0.3 M phosphate buffer pH 7.0 as diluent
0.25 M phosphate buffer pH 6.7 as diluent for $NaNO_2$

Thus, although the general characteristics of the two strains are quite similar, their resistance to physical and chemical agents is quite different. SF_{16} is more resistant to high temperatures and to $NaNO_2$ than SF_0, but more sensitive to low temperature, dialysis and ethylmethanesulfonate. It is to be noted that SF_{16} gave small plaques under overlays containing "Noble" agar, Bacto agar or carboxymethyl cellulose.

The SF_{16} strain although cloned three times still gave a small percentage of large plaques (± 1 % at 27° and ± 0.1 % at 38.5°). Some of these "revertant" large plaques were cloned and surveyed for temperature sensitivity. Five Ts strains were obtained. Two of them called 1- gave large plaques at 27° and one thousand time less large plaques at 38.5°. Three others gave large plaques at 27° and ten times less small plaques at 38.5°; they

were called 1s strains. The following table gives the results obtained with two of these strains:

		1-	1s
grown at 27°	plaque	large	large
	RNA(1)	12.5	26.3
	pfu (1)	$8.1\ 10^7$	$3.4\ 10^8$
grown at 38.5°	plaque	large	small
	RNA	5.2	24.2
	pfu	$1.8\ 10^4$	$1.5\ 10^7$

(1) CF. Above

Thus in both cases we deal with RNA+ Ts mutants. The 1- strains appear to be "ordinary" Ts mutants, the 1s strains are plaque size Ts mutants.

The details of these results are to be found in the "Mémoire de licence en zoologie" de Françoise Delrue.

(P.M. Osterrieth)

REPORT FROM THE MICROBIOLOGICAL RESEARCH ESTABLISHMENT, PORTON DOWN, SALISBURY WILTSHIRE, ENGLAND

Epidemiology of Japanese encephalitis in Sarawak

Previous short-term studies in Sarawak, carried out between 1961 and 1966, suggested that Japanese encephalitis virus is maintained throughout the year mainly by a cycle involving Culex gelidus and domestic pigs. Towards the end of the year the population of C. tritaeniorhynchus increases both in absolute numbers of mosquitoes and in the number of infected individuals and there is a greater risk of virus transmission to man. An investigation was carried out between September 1968 and February 1970 to test this hypothesis and to study the factors which influence seasonal fluctuations among mosquito populations in a single ricefield area and the infection rate in pigs over at least a 12 month period. Kampong Tijirak, a Land-Dyak village situated 19 miles south of Kuching, was the principal study area and was typical of most rural Dyak villages. A large commercial piggery 6-1/2 miles south of Kuching was used for the infection rate estimations.

Culex tritaeniorhynchus was the predominant mosquito species at Kampong Tijirak making up 58% of human bait catches and 89% of light-trap collections, C. gelidus was much less common. At the 6-1/2 mile piggery C. gelidus, a pond breeding mosquito, made up about 80% of the total catch, of which 17% were C. tritaeniorhynchus. In a transect across the ricefield habitat, the largest numbers of C. tritaeniorhynchus biting man were collected in the village and the least in the padi fields. 24-hour biting catches on human bait showed that C. tritaeniorhynchus bit mainly at night and out of doors. This reduces the risk of bites to man as the villagers retire indoors shortly after dusk and often sleep under nets. The low incidence of human biting was reflected in precipitin test results which showed 90% of blood meals came from pigs even though there were never more than 18 pigs (as compared with about 500 humans) in the village at any one time.

There were marked seasonal fluctuations in C. tritaeniorhynchus numbers during the year with a very substantial increase in numbers during October and November. There was a very sharp increase in egg laying and larval growth in September and October due entirely to agricultural practice. This increase occurred when the vegetation of fallow ricefields was cut down prior to digging, irrigation and planting; the resultant rotting vegetation produced an infusion which encouraged egg laying and larval growth with a

subsequent increase in the number of adults. The monsoon rains which occurred soon after planting had little effect on this population increase.

3102 pools of approximately 100 mosquitoes each, collected between September 1968 and January 1970 almost exclusively from Kampong Tijirak and the 6-1/2 mile piggery were processed at the Microbiological Research Establishment and the Institute for Medical Research, Kuala Lumpur, for virus isolation. 121 isolations were made in suckling mice (73 from 1697 pools of Culex tritaeniorhynchus and 14 from 189 pools of C. gelidus) and a further 36 isolates were obtained in primary chick embryo cell cultures. 18 of the suckling mouse isolates have been identified as strains of Japanese encephalitis virus and 15 as Tembusu while a further 36 have been provisionally identified as members of group B and 5 more as Group A. The 36 tissue culture isolates are still under study but no relationship to other viruses has yet been established.

Of 268 sera from inhabitants of all ages from K. Tijirak 169 (63%) were shown to have antibodies against Japanese encephalitis virus. The annual infection rate has been calculated to be approximately 6% per annum. The results of Japanese encephalitis neutralization tests on the sera of birds, bats, domestic animals and small mammals collected at K. Tijirak are shown in Table 1.

Table 1: Results of a Japanese Encephalitis Virus Neutralization Test on Various Animal and Bird Sera Collected at K. Tijirak

Animal Species	Number of sera tested	Number of sera positive	Percentage positive
Dogs	19	16	84
Fowls	100	0	0
Ducks	31	6	19
Geese	3	0	0
Wild birds	188	34	18
Bats	192	4	2
Rodents	313	0	0

Domestic pigs at the 6-1/2 mile piggery were serially bled at monthly intervals throughout 1969. Pigs were marked with numbers during the first month of life; blood was obtained from the tail vein and collected on filter paper discs. After drying these were sent by air mail to the United Kingdom where they were eluted in pH 9.0 borate buffered saline to a dilution of 1 in 5. 1211 blood samples from 447 pigs were tested for Japanese encephalitis virus neutralizing antibody. Table 2 shows the proportion of pigs with antibody according to age. Maternal antibody present during the first month of life declined until at 3 months of age only 0.6% had antibody. Thereafter the proportion of pigs acquiring antibody increased steadily at a rate of approximately 17% per month. Table 3 indicates that pigs do not acquire antibody at a constant rate but appear to become infected more readily as they get older and bigger, possibly because a larger pig attracts more mosquitoes to bite. Table 4 shows the antibody conversions during the months of 1969 in pigs of 3 months of age or older. Between February and October the proportion of pigs infected remained fairly constant but during the period from November to January there was a significant increase in the infection rate. This corresponds to the time when C. tritaeniorhynchus was most abundant.

(D.I.H. Simpson and M.N. Hill)

Susceptibility of monkeys to experimental arbovirus infections

The following viruses were used: (1) Semliki Forest virus (SFV) - (6th hamster passage, L10 - London School of Hygiene); (2) Venezuelan Equine Encephalitis (VEE); a. Trinidad strain, and b. VEE-9 Colombian strain; (3) Western Equine Encephalitis (WEE + 4 strain); (4) Quaranfil virus (Egar 1095 strain, 27th mouse passage; (5) Louping ill virus (Moredun sheep isolate - 6th mouse passage).

1. SFV

When virus was inoculated i.c. into rhesus monkeys (Macaca mulatta), no clinical signs became evident within 30 days, but when the monkeys were destroyed CNS lesions were found. These were confined to the brain stem, cerebellum and the whole of the spinal cord and consisted of perivascular cuffings and microglial infiltrations. In addition the cerebellum had a marked meningeal cellular exudate and glial shrubs.

TABLE 2. Showing results of Japanese encephalitis neutralization tests on the sera of 447 pigs serially bled at monthly intervals after birth

Age in months	Number of sera tested	Number with JE neutralizing antibody	Percentage with JE antibody
1	218	38	17·4
2	191	5	2·6
3	168	1	0·6
4	184	7	3·8
5	142	27	19·0
6	118	36	30·5
7	59	18	30·5
8	60	30	50
9	44	25	56·8
10	20	13	65
11	7	5	71·4

TABLE 3. Showing Japanese encephalitis antibody conversions at different age levels in pigs at 3 months of age and above

	Age in months								
	3	4	5	6	7	8	9	10	11
Sera tested	212	199	136	94	56	30	12	5	2
Antibody conversions	1	3	16	11	11	8	4	2	2
Percentage	0·5	1·5	11·8	11·7	19·6	26·7	33·3	40	100

TABLE 4. Showing the number of Japanese encephalitis antibody conversions in pigs at 3 months of age and above during the months of the year 1969 at 6½ mile piggery

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Number of antibody conversions	18	5	3	2	1	0	4	1	3	3	12	6
Number of sera from previously susceptible pigs tested	87	82	57	61	51	26	55	47	45	48	60	70
Percentage of antibody conversions	20·7	6·1	5·3	3·3	2·0	0	7·3	2·1	6·7	6·3	20	8·6

2. VEE

(a) The Trinidad strain infected rhesus monkeys only by the intracerebral route, giving rise to severe clinical signs and death. When virus was given by the respiratory or intranasal routes, monkeys developed neither clinical signs nor histological brain lesions. (b) The Colombian strain of VEE proved highly lethal for rhesus monkeys, not only by the i.c. route but also by the respiratory and intranasal routes.

3. WEE

Rhesus and vervet (Cercopithecus aethiops) monkeys were susceptible to only i.c. infection, not by the intranasal, respiratory or subcutaneous routes. After i.c. infection the disease was invariably fatal and the brain showed widespread encephalitis. The other methods of infection produced neither clinical signs nor CNS lesions.

4. Quaranfil virus

Rhesus monkeys proved completely resistant to i.c. or respiratory infection with this virus, both with regard to clinical signs and CNS lesions.

5. Louping ill

Four species of monkeys were tested: patas (Erythrocebus patas), vervet, rhesus and capucin (Sebus apella). All monkeys proved susceptible to virus when infected i.c.: clinical signs and CNS lesions were observed, but mortality was rare and seen only in one or two vervets. Clinical signs appeared between days 5-8 and were most severe in patas monkeys, least severe in capucins, vervets and rhesus taking 2nd and 3rd place. As a rule most monkeys recovered after an illness of 1-4 weeks, but histological lesions usually persisted for up to nine weeks. The intranasal route gave similar results, but with less severe disease, while subcutaneous and respiratory infections produced neither signed or CNS lesions.

(I. Zlotnik)

shallow intramuscular route. Blood samples were taken at intervals for virus and antibody titration. Virus in blood was titrated by counting plaques produced on duck embryo cells maintained under agar, and sera were titrated for plaque-neutralizing antibody.

A total of 65 birds of six species were infected. The majority of the birds responded with a viraemia which began on the first or second day after injection and reached a maximum level on the third or fourth day. At its peak level the viraemia in individual birds ranged from $10^{7.1}$ to $10^{8.2}$ plaque-forming units of virus per ml. of blood. The disappearance of virus from the blood between the fifth and seventh day was associated with the appearance of serum neutralizing antibodies. Antibody titres reached their highest levels during the first two weeks after infection. Some birds have been tested for as long as 10 months after infection and although the antibody titres have dropped, in no case has antibody disappeared.

A feature of the plaque-neutralizing antibody titrations was the presence of a prozone, particularly in sera collected immediately after the end of viraemia. Attempts to identify the component responsible for the prozone by separation of serum proteins have not yet been entirely successful as the component appears to be rather labile.

(F.J. Austin)

REPORT FROM THE
DEPARTMENT OF TROPICAL MEDICINE AND MEDICAL MICROBIOLOGY
UNIVERSITY OF HAWAII SCHOOL OF MEDICINE
HONOLULU, HAWAII

Studies on dengue virus isolation methods from tissue samples

Dengue virus has proven to be very difficult to isolate from human autopsy material of fatal dengue hemorrhagic fever cases. The reason for this difficulty is not known, but preliminary experiments in dengue susceptible rhesus monkeys have clearly shown that many tissues contain potent dengue virus inhibitors when triturated. There is suggestive evidence that dengue virus

inhibitors are also released when infected tissues are stored for even short periods at -70°C prior to trituration or explant culture. These "inhibitors" are particularly effective in preventing multiplication of dengue virus in the tissue culture plaque assay system, less so in infant mouse brain.

Since studies on the pathogenesis of dengue virus in rhesus monkeys required a sensitive assay system free from the effect of normal viral inhibitors, it was necessary to investigate new techniques of virus recovery from tissues. An assay system involving the co-cultivation of trypsinized, washed cells from infected monkey tissues on LLC-MK2 cell monolayers showed some promise. This technique resulted in a higher rate of recovery of virus from monkey tissues than the inoculation of triturates onto susceptible LLC-MK2 monolayers. However, the method was rather laborious, requiring many hours to process tissues and resulted in a considerable loss of cell mass.

Consequently the Tissue Fragment Culture Technique was devised. In this method small samples of tissue are minced with scissors and placed in a 1 oz. prescription bottle containing tissue culture growth medium (BME - Earles with 10% calf serum, 1% glutamine and 200 units each of penicillin and streptomycin per ml and 3 ml of 7.5% $NaHCO_3$ per 100 ml). Minced tissues are cultured at 37°C for up to 13 days with periodic changes of growth medium. The fluid overlay is tested for dengue virus using the LLC-MK2 plaque method. The metabolic activity of tissue fragments is readily monitored by observing pH changes in the culture medium. The optimum interval between medium changes has not been established. In the system currently employed, the original growth medium is discarded after 3 days incubation at 37°C; two to three additional medium changes are made at 3 day intervals and the removed medium assayed for dengue virus.

The tissue fragment culture method has been used for detection of dengue virus in the tissues of rhesus monkeys experimentally infected with low tissue culture passage dengue types 1, 2, 3 and 4. The results vary with the virus type used and the time of sacrifice after inoculation. Preliminary results suggest that after subcutaneous inoculation, dengue virus replicates in skin at the site of inoculation then in the regional lymph nodes and eventually spreads to other lymphatic tissue and the digestive tract and skin.

The tissue fragment culture technique so far has been used mostly for dengue virus isolation from tissues of experimentally infected monkeys taken immediately after death, whereas tissue samples from fatal cases of human dengue hemorrhagic fever usually cannot be obtained until 24-48 hours or longer after death. It is not known how long dengue virus can remain viable in tissues of fatal hemorrhagic cases, but rapid loss of viability of virus particles after host death could explain the difficulty of recovering dengue from human autopsy material. Experiments with tissues from experimentally infected monkeys, however, suggest that dengue virus may remain viable in tissue samples for long periods. Virus was readily recovered by culture of tissue fragments stored at 4°C for 1, 2, 3 and 4 days. Periods longer than 4 days were not tested.

On the other hand, dengue virus could not be recovered from tissue samples stored for 2 weeks at -70°C even though virus was readily recovered from duplicate samples of the same tissue when tested fresh or after 1 to 4 days storage at 4°C.

(Scott B. Halstead)

REPORT FROM THE INSTITUTO DE VIROLOGIA, CORDOBA, ARGENTINA

LCM virus in the endemic area of Argentinian hemorrhagic fever

In March, 1969, a virus strain closely related to LCM virus was isolated from the brain and blood of one Mus musculus rodent, captured in the field of the endemic area of AHF in the province of Cordoba, Argentina. The brains of two Calomys musculinus rodents caught simultaneously with this M. musculus yielded Junin virus.

The reisolation attempts from the brain and blood of the M. musculus were again positive. In addition, serological evidence of LCM activity was obtained in blood from Mus also caught in the same area.

These results meet the requirement for the validity of the isolation. Furthermore, it was also shown that the colony of laboratory mice used for the isolation attempt was free of infection with LCM virus.

This is the first time that two different members of the recently proposed "Arenovirus" family are demonstrated in the same geographic area. This is not unexpected because the host of one of them is a cosmopolite species but it presents an interesting situation in the ecological investigations.

These studies have been carried out with the participation of Dr. J. Barrera Oro and Dr. J. Maiztegui.

(M.S. Sabattini)

REPORT FROM THE VIRUS LABORATORY
FACULTAD DE MEDICINA, UNIVERSIDAD DEL VALLE
CALI, COLOMBIA

Following the widespread 1967-68 epizoodemic of Venezuelan equine encephalomyelitis (VEE) in areas of low and moderate seasonal rainfall in Colombia, a strain of VEE virus was sought in the high-rainfall region of the Pacific lowlands where previous serologic studies had shown past activity of VEE virus in humans without indication of overt clinical illness in epidemic form. A total of 20 sentinel hamsters, in two groups, were exposed for two 2-week periods in July and August-September 1969 along the margins of a grass-overgrown freshwater swampy area 50 km inland from the port of Tumaco near the Ecuadorian border. One hamster yielded VEE virus and two others yielded Eastern equine encephalomyelitis (EEE) virus. Virus was not isolated from 7,199 mosquitoes captured in the vicinity of the exposed hamsters. This isolation of VEE virus confirms the suspected presence of the agent in the Pacific lowlands of Colombia and also the utility of sentinel hamsters for detecting endemic VEE virus activity in the American tropics. The isolations of EEE virus are the first from Colombia and the first from the west coast of South America.

In 1969, two strains of virus, not WE, were isolated from Saskatchewan mosquitoes. One of these, from a pools of Culex tarsalis taken at Weyburn on July 24, was identified by Dr. C.H. Calisher of the NCDC, Atlanta, as belonging to the Flanders-Hart Park group. The other, from a pool of Aedes campestris taken at Delisle on June 5, has not yet been identified.

The Saskatchewan WE Project continued in 1970 in three areas of the Province, viz. at Weyburn in an area of dryland farming in the WE endemic southeastern corner of the Province; at Outlook, in an area where the incidence of WE in man and horses has been comparatively low in the past and where irrigation farming is at present being introduced; and at Saskatoon, the headquarters area of the Project. No epidemic of WE occurred in Saskatchewan during the 1970 season and again no human cases of the disease were reported. Seventeen equine cases were reported but to date only one of these has received laboratory confirmation. Examination of all the mosquito pools and vertebrate tissue specimens collected during the 1970 season has not yet been completed. To date, one isolation of WE virus has been obtained from the brain of a ground squirrel collected in the Saskatoon district and one isolation from a pool of Culex tarsalis taken at Outlook from August 18-24. Testing of the blood samples from four sentinel flocks has been completed. At Weyburn, 14 of the 25 birds in the flock contracted WE infections during the summer. In the Outlook flock, 4 of the 25 birds contracted infections. In the Saskatoon flock, 1 of the 25 birds became infected; this is the first WE infection found in the Saskatoon flock since 1966. At St. Walburg, none of 25 birds became infected.

(J. McLintock and A.N. Burton)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER 8, B.C., CANADA

Between 5th May and 27th August 1970, sera which were collected from 1018 vertebrates at Penticton (49° 30'N, 120'W), Williams Lake (52°N, 118°W) and Dawson Creek (56°N, 120°W) were examined for hemagglutination inhibition (HI) antibodies to Powassan (POW), St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) virus. To date, intracerebral mouse neutralization tests against POW, SLE and the Grey Sage strain of California encephalitis (CE) virus have been completed on 511 sera collected during May and June, and the CE antibody status of 52 sera collected near Williams Lake in July has also been investigated. HI antibodies to POW and/or SLE viruses were detected in 144 of 756 mammals and 15 of 203 birds (total 159 of 959 vertebrates, 17%) at Penticton throughout the summer, none of 7 animals at Dawson Creek during June, and 6 of 52 (12%) of vertebrates at Williams Lake during July. Principal positive reactors at Penticton were 61 of 252 (24%) marmots (Marmota flaviventris), 21 of 74 (28%) red squirrels (Tamiasciurus hudsonicus), 20 of 262 (9%) chipmunks (Eutamias amoenus) and 38 of 141 (27%) columbian ground squirrels (Citellus columbianus). No WEE antibodies were detected. The 24 sera with CE antibodies were derived from 8 of 184 M. flaviventris and 1 of 7 T. hudsonicus at Penticton, 14 of 23 snowshoe hares (Lepus americanus) at Williams Lake and the sole L. americanus collected at Dawson Creek. No HI antibodies to group A or group B arboviruses were detected in 52 sentinel chickens bled in June, 33 in July or 50 in August. No virus was isolated from 33 pools of Aedes sp. mosquitoes collected at Penticton between 22nd June and 10th August. No virus was recovered from 9 pools of flagged Dermacentor andersoni adult ticks and 35 pools of D. andersoni nymphs or adults removed from rodents at Penticton during May and June. These results provide serological evidence that the range of prevalence of CE virus in British Columbia extends northwards from 49°N to 56°N, and group B arbovirus activity was detected at 52°N.

(D.M. McLean)

REPORT FROM THE CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH: VIRAL AND RICKETTSIAL DISEASE LABORATORY AND ROCKEFELLER FOUNDATION COOPERATIVE ARBOVIRUS STUDY (IN COOPERATION WITH THE BUREAU OF VECTOR CONTROL AND SOLID WASTE MANAGEMENT)

There were 3 cases of Colorado tick fever in humans this year: a 9 year old girl exposed in Lassen County (onset 9 April); a 37 year old man exposed at Prosser Lake, Truckee area, Nevada County, onset 17 June; and a 36 year old man exposed in Idaho (onset 16 July). Virus was isolated in suckling mice from the blood clot and also demonstrated by fluorescent antibody staining of acute or convalescent-phase blood smears in these cases. Despite the rather low incidence of the disease, a field study area in Siskiyou County was productive of the virus. Nine strains were isolated in suckling mice from blood clots taken from live-trapped (and released) Citellus or Neotoma species from 9 May through 21 August (out of 92 blood specimens tested), and 12 isolates in suckling mice were made from pools of Dermacentor andersoni and D. occidentalis flagged from brush, removed from trapped animals, or attracted by dry ice from 9 May through 9 July. Fluorescent antibody staining was also successful in identifying virus-positive blood clots from the rodents. Previous tests have shown this can also detect infected individual ticks, but we have not used the method routinely for ticks.

In conjunction with a field study for Rocky Mountain Spotted Fever in Monterey County, 9 pools of Haemaphysalis spp. and Ixodes spp. ticks (131 ticks) were tested in suckling mice, but no isolates of rickettsiae or arboviruses were made.

Mosquito-transmitted encephalitis remained at a low level in California during 1970. No human cases of western encephalitis (WEE) were detected and only 1 case of St. Louis encephalitis (SLE) has been confirmed; a 63 year old woman from Colusa County, with onset 19 July. A large number of aseptic meningitis and encephalitis cases occurred this year, and all cases were routinely screened for WEE, SLE, and California encephalitis antibodies, with negative results. Enteroviruses, predominantly echovirus types 3 and 4, were implicated as the etiologic agents for the majority of these cases. Suspect horse cases of encephalitis were also screened for WEE, SLE, and CEV, but only 1 presumptive positive case of WEE has been detected: an unvaccinated horse from Turlock, Stanislaus County, with onset the last part of August (high stationary CF titer 1:128/1:64).

A total of over 9,367 mosquitoes or Culicoides were tested in 209 pools including: 144 pools of C. tarsalis (7,880); 3 pools of C. variipennis (320); 6 pools of A. nigromaculis (72); 3 pools of C. inornata (9); 5 pools of A. punctipennis (66); 12 pools of A. melanimon (420); 16 pools of C. pipiens (439); 2 pools of A. vexans (23); 7 pools of A. freeborni (106); 3 pools of A. franciscanus (21); 2 pools of C. incidens (7); 1 pool of C. erythrothorax (2); 2 pools of A. occidentalis (2); pools of C. peus (total unknown); and 1 pool of C. quinquefasciatus (total unknown). One isolate of SLE virus was obtained from a pool of 50 C. tarsalis collected 1 September in Sutter County. Seven isolates of Turlock virus were made from pools of C. tarsalis and 1 isolate of Turlock virus from C. pipiens collected 30 June to 1 September in Yolo, Placer, Sutter, and Fresno Counties. Fluorescent antibody staining was useful in the rapid preliminary identification of the viral isolates.

Brain tissue specimens (or in some instances other tissues) from 63 sick wild animals (largely tree and ground squirrels) were tested in suckling mice for arboviruses. In contrast to previous years, no viral isolates were made. Distemper virus was identified in one sick raccoon by fluorescent antibody staining. Future efforts will be directed more towards culturing fresh tissues from selected feral animals in vivo in an attempt to uncover "latent" viruses.

A virus isolated from a spotted skunk (Spilogale putorius) trapped at Windsor, Sonoma County, on 1 April, 1969, has been identified as a strain of Powassan virus. This isolation is the first reported west of the Rocky Mountains in the United States. Direct tests in suckling mice of the throat swab, salivary glands, lung, and kidney tissue from the skunk were negative for viruses. Fresh lung and kidney from the skunk were used to establish primary cell cultures. From the kidney culture a cytopathogenic agent was isolated, subpassaged in mice, and proven to be Powassan virus by neutralization with specific immune serum. Serum from the skunk contained neutralizing antibodies to the homologous virus and to a standard laboratory strain of Powassan virus.

Serologic surveillance of human cases of encephalitis or meningoencephalitis, or of persons with significant illness following tick bites, during the past 6 years has revealed no evidence of Powassan virus infection in humans in California. However, significant levels of Powassan virus HAI antibody in certain wild animal species have been found during field studies in Kern County (School of Public Health, U.C., Berkeley). Further studies to clarify the epizootiology of Powassan virus in California are in progress. Experience elsewhere in North America suggests that the virus may be widely

present in wildlife cycles but affect humans rarely. Nevertheless, because of the known pathogenicity of the virus, discovery of its presence in an area is ample justification for additional field and epidemiological studies.

A limited study of the pathogenesis of Turlock virus in mice was conducted by visiting Fellow, Mr. Alan Watson. Groups of young adult and suckling mice were infected i.p. with 1,000 suckling mouse i.c. LD_{50}/0.03 ml (mouse passage 3). At intervals of 2, 3, 4, 5, 6, 8, 10, 14, 18, and 25 days, 3 inoculated mice and 1 uninoculated control mouse were sacrificed, and tissues were examined for Turlock virus by fluorescent antibody staining (direct method). Adult mice also were inoculated i.c. with 10,000 suckling mouse i.c. LD_{50}/0.03 ml and were sacrificed and examined when moribund. The i.p. inoculated adult mice did not become ill, and no antigen could be found in tissues except suggestive evidence in the kidneys. Turlock antibody (indirect FA method) showed a greater than 4-fold rise in the mouse sera. The i.c. inoculated adult mice showed extensive fluorescence in the brains only. In the suckling mice, fluorescence was noted first on day 3 in the brains, increasing in intensity up to day 7 when all mice had died. Specific fluorescence was not detected with certainty in any of the other tissues. In vivo cultures of the kidneys of infected adult mice were prepared on days 8 and 25 and were maintained for several weeks. Inoculation of suckling mice with the cell culture harvest at various intervals did not yield virus. Plaquing studies were done with Turlock virus using both methyl cellulose and Ion agar as overlay media (10^5 to 10^7 p.f.u./ml of virus suspension). These studies should be repeated, using non-neuroadapted virus strains for the pathogenesis studies, since this may affect the tropism of the virus.

Comparative titrations of sera from 40 cases of St. Louis encephalitis have been completed, using CF, Indirect FA, and plaque-reduction methods (and in some cases mouse neutralization tests). The IFA method is rapid, accurate, and useful for screening sera from suspected cases or for a confirmatory test. Cross-reactions by the IFA method for group B arboviruses are being compared with the CF, HI, and PR test results. Homologous titers for SLE, Yellow fever (17D), Rio Bravo, and West Nile immune sera are 4-fold or more higher than the heterologous titers against a dozen other group B viruses. The FA method thus appears useful as a supplementary serologic test in differentiating group B arbovirus infections as well as in identifying viral isolates in mice or cell cultures. Plaquing of Rio Bravo, SLE, YF (17D), Ilheus, Uganda S, Dengue (NGC), JBE, Modoc, Powassan, Zika, West Nile, MVE, and Bussuquara viruses has been successful using a line of BHK cells developed in this laboratory. Two distinct plaque sizes were obtained for Uganda S virus. The same plaquing methods have been

successful for LCM virus titration and antibody assay, and appear promising for rabiesvirus.

REPORT FROM THE BACTERIOLOGY DEPARTMENT SOUTH DAKOTA STATE UNIVERSITY BROOKINGS, SOUTH DAKOTA

Arbovirus Isolations from South Dakota Mosquitoes Collected During the Summer of 1969

In the past there have been no arbovirus epidemiological studies in the state of South Dakota pertaining to virus isolation from mosquitoes. This report summarizes the data on mosquitoes collected and processed for virus isolation in 1969. Mosquitoes were collected on 40 different trap nights during the period July 20 to September 9 in Brookings County, located in eastern South Dakota. Approximately 42,000 mosquitoes were caught during this period but of these only 22,000 were identified and processed for virus isolation. On three different trap nights from one of the sites extremely high catches of mosquitoes were obtained. The largest catch of 12,000 mosquitoes in one trap was caught on August 7. Aedes vexans comprised the majority of these large catches, therefore, only a small number of the mosquitoes from each of these catches were identified. Ninety-three percent of the mosquitoes identified consisted of three mosquito species; these were Aedes trivittatus, Aedes vexans, and Culex tarsalis. (Table 1). C. Tarsalis comprized 31% of the total mosquitoes identified with most of this species being caught during mid August.

Brookings collections were made at three permanent sites, all of these were farmsteads, but they differed with respect to the flora and fauna present. Viruses were isolated from mosquitoes from two of these sites but mosquitoes from the third site, a farmstead, which ecologically seemed to be the best site, yielded no virus. This third site also had high populations of C. tarsalis. One of the sites from which viruses were isolated was a dairy farm located near a residential district. Over 60% of the total mosquitoes trapped from this site were identified as C. tarsalis. A total of nine mosquito pools of 100 pools tested from this site yielded arboviruses. The arboviruses isolated were WEE, Turlock, and Cache Valley (CVV) viruses.

TABLE 1. WEEKLY CATCHES BY SITE OF THE MAJOR MOSQUITOES IDENTIFIED AND PROCESSED FOR VIRUS ISOLATIONS FROM BROOKINGS COUNTY IN 1969

Week of	Culex tarsalis			Aedes trivittatus			Aedes vexans		
	Farmstead	Horse Ranch	Dairy Farm	Farmstead	Horse Ranch	Dairy Farm	Farmstead	Horse Ranch	Dairy Farm
7-27 to 8-2	- *	67	1013	-	347	154	-	430	513
8-3 to 8-9	546	321	251	2480	705	234	571	1030	164
8-10 to 8-16	523	263	657	1076	1835	48	701	474	83
8-17 to 8-23	1379	444	832	56	729	27	205	606	444
8-24 to 8-30	-	11	-	-	209	-	-	138	-
8-31 to 9-5	-	-	458	-	-	58	-	-	168
TOTAL	2248	1106	3211	3612	3825	521	1477	2678	1412

KEY:

*Mosquito traps were set at these sites during these weeks, but few, if any, mosquitoes were caught; this was due to faulty traps, bad weather or no mosquitoes.

(Table 2). Seven of these nine isolates were WEE virus from C. tarsalis with a peak infection rate of 7.39/1000 occurring during the third week in August. (Table 2). Isolations of Turlock virus from C. tarsalis and A. vexans were also made from this same site. The other trap site from which we obtained four virus isolates was a horse ranch. About 50% of the total catches from this site consisted of A. trivittatus. Two isolates of the California group of viruses were obtained from A. trivittatus at this site in late July. (Table 2). Both of these isolates were identified as trivittatus virus by the serum neutralization test. Other isolates from this site included one WEE isolate from C. tarsalis and one isolate of CVV from A. trivittatus.

In all, 13 identified virus isolates were obtained from the 320 pools of mosquitoes tested. One other isolation was made from C. tarsalis but it has not as yet been identified. Virus isolates were identified at the Arbovirus Ecology Laboratory, Fort Collins, Colorado. Mosquitoes were identified by Dr. John E. Rowe, Regional Program Director, DHEW, Kansas City, Missouri.

The isolations of WEE, CVV, trivittatus and Turlock viruses were the first reported arbovirus isolates from mosquitoes in the state of South Dakota. The study in 1969 was a preliminary study to determine the presence of different arboviruses in South Dakota. In 1970 the study was expanded to include the use of sentinel chicken flocks as well as mosquito light traps at each of the sites used the previous year. Other sentinel chicken flocks were also located at new sites within 150 miles in radius of Brookings. A sentinel flock of pheasants was employed at Canton, South Dakota, the site where an EEE outbreak occurred in a commercial pheasant flock in 1967. Results from the mosquito collections and the sentinel flocks for 1970 have not been completed. In late September and early October serum samples of approximately 600 pheasants were taken from 300 chickens, 300 ducks, and 100 geese from various counties in the state. These will be used in a projected arbovirus antibody survey. Also in the summer of 1970 a human serum bank was started. This bank contains 10,000 human sera from all counties of South Dakota. About 20% of these serum samples are from the different Indian reservation in South Dakota. These serum samples will be tested for arbovirus antibodies against different arboviruses to study the epidemiology of these viruses in South Dakota.

(G. C. Parikh)

TABLE 2.

VIRUS ISOLATIONS BY DATE AND SITE

WITH WEEKLY INFECTION RATES

Date by Week	Site	Virus - no. of isolates	Mosquito Species	Infection Rate (per 1,000 mosq.)
7-27 to 8-2	Dairy farm	WEE-1	C. tarsalis	1.03*
	"	CVV-1	A. vexans	2.87
	Horse ranch	CE-2	A. trivittatus	10.73
8-3 to 8-9	Dairy farm	No isolations	C. tarsalis	--
	Horse ranch	"	A. trivittatus	--
8-10 to 8-16	Dairy farm	WEE-2	C. tarsalis	3.39*
	Horse ranch	WEE-1	C. tarsalis	4.45
	"	CVV-1	A. trivittatus	0.73
8-17 to 8-23	Dairy farm	WEE-4	C. tarsalis	7.39*
	"	Turlock-1	C. tarsalis	1.03
	Horse ranch	No isolations	--	--

Key:

* showing increased infection rate of C. tarsalis with WEE at dairy farm.

REPORT FROM THE DIAGNOSTIC VIROLOGY UNIT
ANIMAL HEALTH DIVISION
NATIONAL ANIMAL DISEASE LABORATORY
AMES, IOWA

Virus isolations from cotton rats (Sigmodon hispidus) inoculated with two types of vesicular stomatitits virus

Cotton rats were inoculated to determine their potential role as vesicular stomatitis (VS) hosts in wildlife. Ground tongue vesicle suspensions from cattle infected with New Jersey (NJ) and Indiana I types of vesicular stomatitis virus (VSV) were used to inoculate laboratory propagated cotton rats orally, intranasally and subcutaneously. Uninoculated rats served as contact controls in many cages in hopes of elucidating information on the transmission of VSV. Virus isolation was attempted from selected animal tissues. Survivors were checked for homologous neutralizing antibodies. One to three month old cotton rats were used except in the pregnant female studies where breeders were inoculated.

By oral inoculation 25 cotton rats were refractory to VSV and no antibodies were produced. All cotton rats were susceptible to intranasal inoculation (Table 1) resulting in death with the NJ type (24/25) and survival with the Indiana type (0 deaths/42). With the NJ type, virus was isolated from the brain (18/18) and often the lungs (7/17) for six days following intranasal inoculation (Table 2); neutralizing antibodies were produced in survivors. Subcutaneous inoculation resulted in many deaths with NJ (13/16), survival with Indiana type (0 deaths/16) and antibody production. Following subcutaneous inoculation virus was frequently recovered from the brain with New Jersey VSV (9/9) but not with the Indiana (0/7) type. Occasional isolations of Indiana VSV from the spleen of cotton rats (4/18) inoculated subcutaneously as compared to isolations from the brain of those inoculated subcutaneously (9/9) with New Jersey VSV suggests blood clearance of Indiana VSV by the reticuloendothelial system as found by Brunner.

Virus was recovered from oral swabs (5/9) on the second and third days following intranasal exposure with Indiana VSV but only 1 of 10 attempts was positive with the NJ type. Virus was isolated from the brains of Indiana VSV inoculated animals, 8/10 of the intranasally inoculated cotton rats sacrificed at 2-6 days, but no deaths occurred among 23 allowed to recover and proven to have been infected by subsequent tests for antibodies. The presence of virus in all tissues listed as positive has been determined

Table 1.

Summary of Deaths Following Inoculation of Cotton Rats by Various Routes With Either of Two Types of Vesicular Stomatitis Viruses

Type	Method Inoc.	Date Inoc.	No. Inoc.	Survival Range (Days)	Deaths	Total
New Jersey	IN*	10/8/69	10	5-6	10/10	
		2/28/70	9	4-6	9/9	
		3/3/70	3	5-6	3/3	
		3/11/70	3	4-5	2/3	24/25
	SC**	10/8/69	10	6-13	9/10	
		3/3/70	3	5-6	2/3	
		3/11/70	3	5-6	2/3	13/16
	Oral	2/26/70	10		0	
Indiana	IN	10/8/69	10		0/10	
		11/26/69	11		0/11***	
		12/23/69	6		0/6	
		2/26/70	9		0/9	
		3/3/70	3		0/3	
		3/11/70	3		0/3	0/42
	SC	10/8/69	13		0/13	
		3/11/70	3		0/3	0/16
	Oral	2/26/70	10		0	
		12/23/69	5		0	0/15

* Intranasal
** Subcutaneous
*** Besides those killed before 7 DPI

Table 2.

Summary of Virus Isolations from Cotton Rats Inoculated with New Jersey and Indiana Types of Vesicular Stomatitis by Three Routes

Route	Date Inoc.	Disposition PI*	Tissue: Buffy Coat	Plasma	Brain	Lung	Kidney	Bladder	Liver	Spleen	Feces	Testicles	Oral Swabs	No. with Titers/ No. Inoc.
New Jersey														
Oral	2/26/70												0/2	0/10
Intra-nasal	10/8/69	D** 5-6 da			10/10	2/10	2/10			0/10				
	11/26/69	K***2-5 da	0	1/8	8/8	5/7	3/8	0/8		1/8	0/8	0/2		
	2/26/70	A+ 2 da											1/6	
	2/26/70	A 3-4 da											0/13	
Subcu-taneous	10/8/69	D			9/9	0/9	1/9	0/9		0/9				3/3
Indiana														
Oral	2/26/70	K 3 wk												0/10
	12/23/69	K 1 mo												0/5
Intra-nasal	10/8/69	K 1 mo												10/10
	11/26/69	K 2-6 da		0/10	8/10	6/10	1/10	0/10		1/10	0/10	1/10		1/2 @ 6 da
	11/26/69	K 2-6 wk												9/9
	12/23/69	K 1 mo												6/6
	2/26/70	A 2-3 da											5/9	
	2/26/70	A 4-5 da											0/8	
Subcu-taneous	10/8/69	K 1 mo												10/10
	11/26/69	K 2-6 da		0/10	0/10	0/10	0/10	0/10		2/10	0/10			1/2 @ 6 da
	11/26/69	K 7 da		0/2	0/2	0/2	0/2	0/2		0/2	0/2	0/1		0/1
	11/26/69	K 2-6 wk												11/11
	2/26/70	K 1-3 da	0/7	0/6	0/7	1/6	1/6	0/7	0/7	2/6				

* Indicates the number of days postinoculation that specimen was taken and the status of the animal as:
+Alive **Dead ***Killed

In all cases the number of positive virus isolations is listed over the number of virus isolations attempted.

by embryo death following inoculation of 8 day embryonated eggs and complement fixation tests on pooled allantoic-amnionic fluids harvested from each dead egg. Four titrations indicated that 50 percent lethal egg infectivity titers in the brain of New Jersey VSV inoculated cotton rats reached 10^{-7} per ml. Although six eggs were inoculated per tissue all eggs were generally killed in brain suspensions and in some lung suspensions; titers generally appeared to be low in other tissues with only a few embryos dying.

Most contacts became infected when exposed to animals inoculated (Table 3) by the intranasal route (13/16 NJ and 10/18 Indiana), but rarely with subcutaneously inoculated animals (1/11 NJ and 0/8 Indiana). The high incidence of VS among controls placed with intranasally infected cotton rats contrasts to the resistance to oral exposure and suggests exposure through the respiratory system.

Efforts to detect carriers or spreaders by direct isolation from urine, kidney, and bladder or feces were negative except for occasional isolations from the kidneys. Pregnant females were inoculated by similar routes (Table 4) with similar results plus one isolation from an embryo-uterus pool.

Although there may be early virus shedding we have not detected any chronic virus discharge from infected animals which would be of epidemiological significance. Kidneys could be cultured as described by Johnson to determine if the virus is protected in kidney tissues. Transmission through the respiratory tract needs further study. Cotton rats are one of the most common wild mammals in vesicular stomatitis areas of the southern United States, Central and South America. The vesicular stomatitis viruses used for inoculation were from natural bovine cases passaged only intradermal-lingually in cattle but showed a marked neurotropic tendency evidenced by many isolations from the brains of cotton rats.

References

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(E. Jenney and C. Brown)

Table 3.

Transmission of Vesicular Stomatitis Virus to Contact Controls - Following Inoculation of Cotton Rats by Two Routes

Virus Type	Method of Inoc.	Date of Inoc.	No. Deaths/ No. Exposed	Av. Survival Time (Days)	No. Infected*/ No. Exposed
New Jersey	Intranasal (IN)	10/8	4/5	7	5/5
		2/26	5/5	5	5/5**
		3/3	2/3	5	2/3
		3/11	1/3	6	1/3
	Total IN		12/16		13/16
	Subcutaneous (SC)	10/8	0/5	No deaths	0/5
		3/3	0/3	do.	0/3
		3/11	0/3	do.	1/3
	Total SC		0/11		1/11
Indiana	IN	10/8	0/5	No deaths	3/5
		2/26	0/5	do.	3/5
		3/3	0/3	do.	3/3
		3/11	0/2	do.	0/2
		12/23	0/3	do.	1/3
	Total IN		0/18		10/18
	SC	10/8	0/5	No deaths	0/5
		3/11	0/3	do.	0/3
	Total SC		0/8		0/8

* Number infected as indicated by either death or antibody

** Added 1 day postinoculation; most other controls present from time of inoculation.

Table 4.

Summary of Vesicular Stomatitis in Pregnant Cotton Rats

Type	Method of Inoc.	Virus Isolation Attempted: Amnionic Fluid	Brain	Embryo-Uterus	Kidney-Bladder	Non Gravid Lung	Non Gravid Uterus	Non Gravid Spleen	Survival Time Range	Titer	Interval Post Inoc.
Dead											
New Jersey	IN*		4/5**	1/4	1/5	0/5	0/1	0/5	4-8 da		
	SC***		3/3	0/1	0/3	0/3	0/2	0/3	5-6 da		
Survivors (killed 1-3 weeks postinoculation)											
New Jersey	IN	0/1	0/1	0/1	0/1	0/1				1/1	K+ 7 da
	SC	0/1	0/3	0/2	0/1	0/1	0/1			1/1	K 15 da
Indiana	IN	0/5	0/6	0/3	0/6	0/6	0/3	0/2		4/4	K 7-20 da
	SC		0/3	0/2	0/3	0/3	0/1	0/1		2/2	K 15 da

* Intranasal
** Number of animals positive/number of animals tested
*** Subcutaneous
+ Killed

REPORT FROM THE HORMEL INSTITUTE
UNIVERSITY OF MINNESOTA
AUSTIN, MINNESOTA

Effects of fatty acids on JEV growth

In order to study lipid metabolism of Japanese encephalitis virus (JEV), the organism was cultivated in BHK-21 cells using the defined media containing added supplements of different fatty acids. Addition of the fatty acids after absorption for 90 min. at 37° permitted the highest yield of virus ($10^{7.5}$ pfu/ml). The longer the chain length of the saturated fatty acid, the higher the virus yield. The effect of isomeric cis octadecenoic acids 2-18:1 through 17-18:1 on viral growth was variable with oleic acid giving the highest yield of virus whereas the 6-18:1 isomer reduced viral growth. Polyunsaturated fatty acids were inhibitory, particularly when the unsaturation was in the $\Delta 12$ position.

Effect of Japanese encephalitis virus infection on plasma membranes of BHK-21 cells

Lipid composition of purified JEV, normal and infected plasma membranes BHK-21 has been completed. There was little difference between purified JEV lipids and infected plasma membranes when gross parameters were measured. There was a reversal in the ratio of neutral lipid to phospholipid between normal and infected cells. The major neutral lipids were free sterol and free fatty acid. An increase in the free sterol was noted in the infected BHK-21 cells. The major phospholipids were phosphatidyl choline, sphingo-myelin, lysophosphatidyl choline (LPC) and phosphatidyl ethanolamine (PE). A difference was observed between the PE and LPC ratios between infected cells and the purified JEV. Fatty acid composition of neutral lipid and phospholipid classes was determined and some major differences in particular lipid classes were noted, particularly in the 18:0, 18:1 and 18:2 fatty acids between infected plasma membranes and purified JEV. These studies will serve as a baseline for future studies in lipid metabolism of arboviruses.

Incorporation of labeled Oleic acid into normal and JEV infected BHK-21 cells

A major portion of ^{14}C oleic acid was incorporated intact into BHK-21 cells as evidenced by the low recovery of $^{14}CO_2$ activity throughout the length of the experiment. Radioactivity of phosphotidyl choline dropped within 12-24

hrs after virus inoculation with a concommitant increase in synthesis of phosphatidyl ethanolamine. The specific activity of PE remained low in the control cells. The percentage of 18:1 in PC in control cells increased markedly compared to that in infected cells. The 16:0 and 18:1 fatty acids of PC had the highest rate of activity throughout the experiment. Marked decrease in the radioactivity of fatty acids was observed 12-24 hrs after virus inoculation in the infected cells compared to the control cells. A high percentage of polyunsaturated fatty acids with high radioactivity was observed in phosphatidyl ethanolamine in infected and control cells but this activity decreased during virus infection. Results from these studies suggested that phospholipid metabolism of infected cells markedly changed with altered fatty acid metabolism when using labeled oleic acid as a marker.

These studies will be published shortly in the Journal of Virology.

(H. M. Jenkin and S. Makino)

REPORT FROM THE ECOLOGICAL INVESTIGATIONS PROGRAM LABORATORIES CENTER FOR DISEASE CONTROL FORT COLLINS, COLORADO

Colorado

There were 12 confirmed WE and three confirmed SLE human cases in 1969. In 1970, there was no confirmed WE in humans and one confirmed SLE case. Of the 12 WE and three SLE cases in 1969, seven cases of WE and one SLE case were in residents in northern Jefferson County, a suburban area adjacent to Denver. Jefferson County is a rapidly expanding residential area which also reportedly has one of the largest populations of pleasure horses of any county in the U. S.

Testing of C. tarsalis in 1969 yielded 21 isolates of WE virus, while in 1970 there were only three WE isolations and no SLE virus was isolated. Limited C. tarsalis collections were made in Jefferson County in 1970, and it is of interest that while there was only one WE isolation from 332 pools of

C. tarsalis collected in Weld and Larimer Counties, there were two WE recoveries from 17 pools of C. tarsalis collected in Jefferson County.

A study was initiated during 1968 to determine the number of WE, SLE, and CE virus infections occurring in a rural community, Timnath, Colorado. Initial testing in 1968 showed 14% of 161 persons with WE neutralizing antibody. Highest prevalence rates were in the 0-4 (13%) and 10-19 (33%) age groups with five years or less residence in the area. The population was again bled in 1970 with one enzootic season intervening between the two samples. There were two WE conversions among 90 susceptibles, no conversions among 73 SLE susceptibles, and two CEV conversions among 95 susceptibles. The prevalence of CEV antibody in the 1970 sample was 9.6%.

A preliminary study on Colorado tick fever in Boulder and Larimer Counties to evaluate diagnostic methods and locate areas of maximum human risk of infection was completed during 1969 and 1970. Forty-five cases of CTF were confirmed in the two counties in 1969. Testing of blood clots using the fluorescent antibody technique (FA) proved to be a very reliable and sensitive method of detecting infections. The timing of collection of blood for FA testing was important. Of 27 specimens for FA testing taken in the first six days of illness from subsequently confirmed cases, 14 were FA negative. Second clots taken after day 6 from 12 of these 14 cases were FA positive. The two FA negatives were not retested. Five of the positive FA specimens were taken 4-7 weeks after onset of illness, and one was FA positive eight months after onset of illness. The plaque reduction neutralization test performed in Vero cell culture proved to be an excellent serologic test while the CF test was relatively insensitive compared with the other diagnostic methods. In 1970, two human blood specimens were tested in Vero cell cultures for CTF virus isolation. CTF virus was recovered from both specimens.

25/27

During 1969, twenty isolations were made in duck embryo cell culture (DECC) of an agent producing floc-like plaques. Two additional similar isolates have been made in 1970. The original recovery was made from a pool of Culex pipiens collected near Hermiston, Oregon during investigation of an WE epizootic in horses. Subsequent isolates were obtained from both C. pipiens and Culex tarsalis from Oregon, Colorado, and Texas. Studies with one isolate selected as the prototype (69V2161) have shown it to be nonpathogenic for suckling mice, although the virus produces irregular deaths and marked stunting of survivors in suckling hamsters. Wet chicks inoculated subcutaneously with 69V2161 produce a viremia which persists for at least ten days. Laboratory C. tarsalis feeding on viremic chicks became infected with the virus and were able to transmit the infection to

susceptible wet chicks, albeit at a low level. Plaque production in Vero cell cultures has been achieved by alternate passage in DECC and Vero CC with increased clarity and size of plaques.

The 69V2161 isolate was not reduced in titer after standard treatment with chloroform and ether, and showed only a moderate reduction in titer ($10^{1.1}$) following DCA treatment. It was completely inactivated following exposure to pH3 buffers. Filtration studies suggest the particle size to be in the 45-100 mμ range. Electron microscopy performed at CDC in Atlanta, Georgia have shown the 69V2161 isolate to be reovirus-like in morphology and resembling other viruses in a group comprised of Bluetongue, Colorado tick fever, African horse sickness and other ungrouped arboviruses (personal communication from Dr. Fred Murphy, CDC).

Hale County, Texas

Studies on the ecology of WE and SLE viruses were continued in Hale County, Texas during 1969 and 1970. A total of 889 pools of 25 C. tarsalis each were tested in 1969 with 22% of the pools yielding WE virus. Minimum infection rates (MIR) for WE virus in C. tarsalis for June, July, and August were 1.9, 18.1, and 19.6/1,000 mosquitoes, respectively. The first WE isolation from nestling house sparrows in 1969 was during the week of July 13. Infection ratios for nestling house sparrows were 9.5% in July and 4.% in August. Infection ratios in nestling house sparrows appear to be much higher during years in which human cases of WE occur. Nestling sparrow infection ratios were 25.% and 26.2% during 1965 and 1966 in which 12 and 11 confirmed cases of WE occurred in Hale County. No confirmed human cases have occurred since 1966 and infection ratios in nestling sparrows for 1967-1969 have been less than 9%. During these years, WE infection rates in C. tarsalis have remained relatively stable. Preliminary tabulation of 1970 testing show 19% of 487 pools of C. tarsalis were positive for WE virus which is similar to previous years. Only 1.3% of 300 nestling house sparrows bled from May through August had demonstrable WE viremia, and again there were no confirmed WE cases.

During 1969, there was no SLE virus isolated from mosquitoes. In 1970, there were five SLE virus isolates from C. tarsalis with one each in July and August, and three in September.

During 1965, an unidentified virus was isolated from a jackrabbit (Lepus californicus) blood sample in Hale County, Texas. This isolate was subsequently identified as Main Drain (MD) virus, a Bunyamwera group virus first reported from Kern County, California. Identification was made using

immune serum kindly supplied by Dr. James Hardy, School of Public Health, U. C., Berkeley.

Multiple isolations of Main Drain or a closely related virus were made in 1969 and 1970 resulting from routine use of Vero cell cultures for testing field specimens. All isolations have come from pools of Aedes vexans, Aedes nigromaculis, and Psorophora signipennis with additional isolations from L. californicus blood samples. A limited serologic survey in July of 1969 showed 81% of 31 adult L. californicus and 50% of 18 juvenile jackrabbits with MD neutralizing antibody.

In addition to the Bunyamwera virus isolations, California virus (CEV) was isolated in 1969 and 1970 from pools of A. nigromaculis and P. signipennis, and has been isolated from L. californicus blood samples obtained in 1970. Neutralizing antibody to CEV was present in 90% of the 31 adult and 39% of the juvenile jackrabbits in the July 1969 survey.

House sparrows have been shown to be an important amplifying host for WE virus in Hale County and, for this reason, studies were initiated to determine the feasibility of a sparrow control program. Ornitrol, a chemosterilant produced by G.D. Searle and Company, was evaluated in colonized house sparrows during 1969 and 1970. Birds in one aviary were fed Ornitrol treated seed and birds in another aviary received untreated seed. Acceptance of the treated seed was good in the aviary with about 5 gm. of seed consumed per bird each day in both aviaries. Treated seed was fed in the south aviary from April 27 through June 15, and nestling activity was checked Monday, Wednesday, and Friday each week throughout the study. The following table summarizes results of the test:

	Egg Hatching Success in Clutches Started Prior to May 4[a]		Egg Hatching Success in Clutches Following May 4	
	No Eggs Laid	% Hatched	No. Eggs Laid	% Hatched
North Aviary (Untreated Seed)	82	56	72	64
South Aviary (Treated Seed)	30	63	30	0

a - May 4 was considered the earliest date at which the chemosterilant could have been effective.

The first nestling hatched in the south aviary (treated) on July 6 following discontinuation of feeding Ornitrol treated seed on June 16. Comparable hatching success continued in both aviaries thereafter with cessation of egg hatching August 19 in the north aviary and September 17 in the south aviary.

Two adult birds and five fledglings died in the south aviary between May 30 and June 11. No deaths occurred in the north aviary during this time. Autopsy specimens have been sent to G.D. Searle and Company to test for chemosterilant accumulations in the liver.

Exploratory studies on the use of Ornitrol to reduce breeding among field populations of house sparrows are planned for the coming season.

REPORT FROM THE DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION SAN ANTONIO, TEXAS

Serological tests for the prevalence of arbovirus antibodies in primates are being conducted. In the first series paired samples were selected from 85 baboons from our colony and tested for SLE, WEE, and EEE HI antibodies, after storage at -20° C for 1-5 years. Each pair included a sample taken upon arrival at San Antonio from Africa and one after exposure of this animal to possible infection in outdoor cages during 1-4 years. About 50% of the baboons had been exposed for three arbovirus transmission seasons. Forty-seven percent of the animals gave positive reactions. The trend was toward a decrease in titer for these group A and group B viruses, Table 1. However, 11% showed either no change or an increase in WEE antibody, Table 2. Twenty-six percent showed a decrease in antibody titer for SLE virus and none exhibited an increase.

Six selected WEE positive serum pairs were subjected to CF tests with WEE antigen and 11 proved to be negative. A sampling of the SLE positive animals gave initial positive CF tests with SLE antigen but all converted to negative.

Surveys of sera from various nonhuman primate species with twelve selected

Table 1

HI antibody titers to WEE and SLE Viruses

Sera from 85 baboons before and after exposure in outdoor cages*

WEE Antigen	Neg.	1:10	1:20	1:40	1:80	1:160
Before	75**	14	7	4	0	0
After	85	9	4	2	0	0
SLE Antigen						
Before	73	4	4	5	5	5
After	86	9	1	4	0	0

* Exposed 1 to 4 seasons, 50% 3 seasons

** Expressed as percent

Table 2

Paired sera from 85 baboons tested for HI antibodies

before and after exposure* in outdoor cages

Antigen	Negative	Increase	Decrease	No. change
WEE	71**	6	18	5
SLE	73	0	26	1
EEE	96	1	2	1

* Exposed 1-4 seasons, 50% 3 seasons

** Expressed as percent

African and North American arboviruses are in progress. Results of HI tests are incomplete at the present.

A program has been initiated, in collaboration with the National Communicable Disease Center in Atlanta, Georgia, on experiments with California group arboviruses in baboons. The purpose is to study the pathogenesis and immunological relationship of California arboviruses in a primate model system.

Banerjee reported in the Indian Journal of Medical Research 57:1003-1005, 1969, the loss of virulence to mice observed in the virus obtained from the carrier culture of Aedes albopictus infected with Chikungunya virus. Mouse virulence was lost after the 7th. passage in the cell line. We attempted serial passage of the California virus strain BFS-283 by parenteral injection of Culex quinquefasciatus. Mouse virulence was not diminished after the 8th. passage. Serial passages have been continued but tests are incomplete at this time.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY THE UNIVERSITY OF TEXAS (SOUTHWESTERN) MEDICAL SCHOOL AT DALLAS

Bats experimentally infected with Japanese B encephalitis (JBE) virus exhibit no overt signs of encephalitis even though virus replicates in brain tissue reaching levels equal to the JBE virus content of the brains of mice succumbing to the infection. Also, histologic evidence of encephalitis is minimal in the brain tissue of infected bats. JBE virus infection may persist in these animals for months or even years with recurrent viremias occurring spontaneously. Studies to define the mechanisms which permit multiplication of JBE virus in the tissues of bats in the absence of cellular injury have included investigations of various aspects of the immune response of these animals. Results of studies on the immune response of bats to a variety of antigenic stimuli have indicated that the antibodies formed by these animals differ quantitatively and qualitatively from those produced by other animals, suggesting an immunologic deficiency in bats.

In view of the rapidly accumulating evidence of an immunologic basis for

the tissue injury associated with certain persistent viral infections, experiments were initiated to determine if the response of the central nervous system (CNS) of bats infected with JBE virus could be modified by the administration of specific immune serum at a critical time during the course of infection. Hyperimmune serum against JBE virus (strain OCT-541) was prepared by infecting domestic pigs by subcutaneous inoculation. These animals received booster doses of virus 30 and again 60 days post inoculation and were bled 14 days after the 2nd booster dose. The hyperimmune pig serum had a neutralization index (NI) of 10,000 against JBE virus. Normal control serum was obtained from the pigs prior to infection.

Following the format of studies by Berge et al. on mice infected with Venezuelan equine encephalomyelitis virus (J. Immunol. 87:509-517, 1961) and by Webb et al. on mice infected with Langat virus (J. Hyg., Camb. 66: 343-354, 1968) bats were infected with JBE virus by intracerebral inoculation and at a time when previous studies had shown that maximum levels of virus were present in brain tissue the animals were divided into groups and treated with 0.2 ml of either normal or hyperimmune pig serum or saline administered intraperitoneally. Twenty-four hours later blood was obtained by cardiac puncture for virus assay and neutralizing antibody determinations; bats were then sacrificed and the brains were removed. Each brain was divided longitudinally; one hemisphere was placed in neutral formalin for histologic examination and the corresponding half frozen for subsequent virus assay.

In this preliminary study we found that the administration of potent specific immune serum to infected bats at a time when high levels of virus were present in brain tissue induces in virtually all animals brain lesions which are more intense and more widespread than observed in infected bats which received either normal serum or saline at the same time interval. These results indicate that the histological alterations observed may be allergic reactions to virus (antigen)-antibody complexes and the fact that they are not seen in bats during a "normal" course of infection may be due to the low quantity and poor quality of the antibodies formed by these animals in response to JBE virus infection.

(S.E. Sulkin and R. Allen)

REPORT FROM THE DEPARTMENT OF INFECTIOUS DISEASES
THE UNIVERSITY OF TEXAS SCHOOL OF PUBLIC HEALTH AT HOUSTON

Surveillance of arbovirus activity within the metropolitan area of Houston continued through September, 1970. The study sites were described in the March, 1970 Information Exchange and include a study area of about 4.5 square miles in the northwest area of Houston, the Houston Arboretum and other specific sites located throughout Houston. An additional study area, Camp Wallace, an abandoned military camp, is located approximately 15 miles from the Gulf of Mexico near Galveston, Texas. It has a scrub-grassland habitat with about a 10% covering of trees.

Mosquito collections were made almost entirely with CDC miniature light traps baited with dry ice or by resting capture. During June, July and August, certain mosquito collections were made from sites known to produce large numbers of Culex pipiens quinquefasciatus. These mosquitoes were not sorted or identified but were divided into pools of approximately 100 mosquitoes and processed for attempted virus isolation. This procedure, which was in addition to the normal collection and processing of mosquitoes, was employed in order to process a maximum number of mosquitoes in a short period of time to provide a more rapid means of detecting St. Louis virus.

Flanders virus was isolated from 33 out of 102 pools of unidentified mosquitoes processed in June, 7 out of 203 in July, and 3 out of 58 in August. A total of 28, 444 C. p. quinquefasciatus (including 10, 315 collected in May, June and July) was collected and processed in 283 pools from January through August, 1970. Of these pools, 11 contained Flanders virus. All 11 pools were collected in May, June and July. Flanders virus was also isolated from one pool of Psorophora ferox and 3 pools of C. restuans.

Virus strains belonging to the California Encephalitis Virus group were isolated as in 1969 from mosquitoes belonging to the Aedes (Ochlerotatus) Group F (scapularis group). Species belonging to this group and known to exist in the collection areas include Ae. atlanticus, Ae. tormentor, Ae. infirmatus and, to a limited extent, Ae. dupreei. The isolation of viruses belonging to the CEV group in Houston has been summarized as follows:

month of collection	number of mosq. collected	number of positive pools number of pools
April	2254	0/22
May	9071	27/94
June	7413	36/79
July	1467	1/15
August	226	0/3
The isolation of CEV from Camp Wallace is summarized as follows:		
June	258	2/3
July	2117	6/22
August	178	1/2

These isolates were from pools of Ae. infirmatus, Ae. atlanticus - Ae. tormentor, or Ae. atlanticus - Ae. tormentor - Ae. infirmatus. Of the 64 isolates obtained in Houston, 60 were from mosquitoes collected in the Houston Arboretum.

Preliminary studies in reciprocal cross complement fixation tests and gel diffusion tests indicate that at least 3 strains (one from 1969 and 2 from 1970) are closely related if not identical to Keystone virus. In addition, one strain isolated in 1970 appears to be different from Keystone and the 3 other strains studied to date.

Three isolations of a Cache Valley-like virus were made from Culiseta species of mosquitoes collected in January and February, 1970.

REPORT FROM THE ARBOVIROLOGY UNIT
CENTER FOR DISEASE CONTROL
ATLANTA, GEORGIA

I. Venezuelan Equine Encephalitis (VEE) Vector Studies in South and Central America, 1969-1970

A. Ecuador

In May 1969, 80,190 mosquitoes were collected by Dr. Roy Chamberlain from eight localities near Guayaquil, Ecuador. Culex nigripalpus, Mansonia titillans, and Aedes scapularis were the predominant mosquitoes collected; they accounted for more than 50% of the total catch, Table 1. VEE was isolated from a pool of Aedes sp. near scapularis, Table 2. Results of kinetic HI studies performed at MARU by Dr. Peter Franck indicate this isolate to be related to strain IB.

B. Guatemala

Mosquitoes were collected on the Pacific coastal plain in July 1969, and in the mountainous interior in August. Two-thirds of the total catch was Culex nigripalpus, Table 1. Twenty-one VEE isolations were made from six mosquito species, Table 2. Several isolates were selected for KHI studies and submitted to MARU. Their results showed that P. confinnis, C. nigripalpus, M. titillans, P. cilipes, and Culex (Mel.) sp. isolates were related to IB, and that a Culex (Mel.) sp. isolate from Morales was related to IE.

Mosquitoes were collected at Montufar, July 1970, in an effort to determine what type of VEE could be found one year after the epidemic. An isolation of VEE was made from Culex nigripalpus and it will be of interest to see if this strain is IB or IE.

C. El Salvador

Dr. Sam Breeland of CDC's Malaria Program collected 8,703 mosquitoes in July 1969, in El Salvador during the epidemic. Ninety percent of the mosquitoes collected were Culex nigripalpus, Table 1. No viruses were isolated.

Table 1
Mosquitoes Collected and Tested for Arboviruses during VEE Epidemic Studies in South and Central America, 1969-1970

Mosquito Species	ECUADOR May '69	GUATEMALA July-Aug. '69	GUATEMALA Aug. '70	EL SALVADOR July '69	NICARAGUA Nov. '69-Jan. '70	COSTA RICA July-Aug. '70
Aedomyia squamipennis	891	390	33	2		5
Aedes angustivittatus		184	21			28
bimaculatus-fulvus pallens	46					4
euplocamus ?						1
scapularis	11,768	1,389				
serratus						1
taeniorhynchus	6,070	832	319	10		557
terrens	15		2			10
vexans		175				
species near scapularis	7,959	12	599	54	8	2,216
Anopheles albimanus	1,418	608	114	65	700	1,021
kompi ?						1
pseudopunctipennis		363	2			767
punctimacula	5	257	18	6	1	766
vestitipennis		24				
species	11	26	4	1		18
Coquillettidia nigricans-like	145	723		1		1,034
Culex coronator-like			846			283
interrogator-like		52				24
janitor-like		40				
(Mel.) sp. #1	196		52	133		303
(Mel.) sp. #2	30					25
(Mel.) spp.	6,035	6,831	779	147	21	2,875
nigripalpus	20,161	51,077	25,961	7,779	212	15,073
pipiens quinquefasciatus						42
species	370	2,680	332	55	1,722	178
Deinocerites pseudes					2	11,543
spanius				1		79
species	2		5		3	20
Haemagogus species	19		2			9
Mansonia dyari	7,739	2,735	213	220	632	228
flaveola	4	36				
titillans	15,233	976	487	178	97	1,260
species	68					2
Orthopodomyia kummi						1
Psorophora albipes		199				
ciliata		38	2			
cilipes		203	10			11
confinnis		4,998	79	9	12	93
cyanescens		3				
ferox	1	6	5	1		74
varipes		50				
species	3	55				3
Trichoprosopon species		6				1
Uranotaenia geometrica						2
lowii	761	729	201	41		99
sapphirina	24	7				2
species	1,216	256	108		1	8
Wyeomyia species						3
TOTALS	80,190	75,510	30,194	8,703	3,411	38,670

Arbovirus Ecology Laboratory, CDC, 1970

Table 2
VEE Isolations from Mosquitoes Collected in South and Central America during 1969-1970 Epidemics

Location	Date	Species	No. Isolated
Ecuador			
Ceibos	May 1969	Aedes sp. near scapularis	1
Guatemala			
Montufar	July 1969	Psorophora confinnis	6
		Culex nigripalpus	5
		Mansonia titillans	2
		Psorophora cilipes	1
		Aedes taeniorhynchus	1
Montufar	July 1970	Culex nigripalpus	1
Agua Blanca	August 1969	Psorophora confinnis	2
Chiquimula	August 1969	Culex (Mel.) sp.	1
Zacapa	August 1969	Culex (Mel.) sp.	1
		Psorophora cilipes	1
Morales	August 1969	Culex (Mel.) sp.	1
Costa Rica			
Point Soley	August 1970	Deinocerites pseudes	12
		Aedes taeniorhynchus	4

Arbovirus Ecology Laboratory, CDC, 1970

D. Nicaragua

No virus was isolated from 3,411 mosquitoes collected in Nicaragua by MARU and Nicaraguan government personnel from November 1969 through January 1970. Unfortunately, a delay in shipment resulted in a thawing of these specimens.

E. Costa Rica

In anticipation of the spread of VEE into Costa Rica, cooperative studies with MARU were initiated in July 1970. Mosquitoes collected at that time were negative. In August, early in the epidemic, a second mosquito sampling was done. Little change in species or population was noted. One collection was made at Point Soley, a coastal habitat not previously sampled. In this instance, 11,543 Deinocerites pseudes were collected, Table 1. Twelve isolations of VEE were made from this species, and four additional isolations were made from A. taeniorhynchus. Although D. pseudes may account for VEE transmission to man and animals along the coast, it does not account for the transmission which occurred away from the coast.

Biting collections with man and horse as bait were made in Guanacaste Province, Table 3. Eight species were found to bite on horse and only three bit man. Aedes spp. were the most numerous biters. Possibly as many as three species were placed in this category due to taxonomic difficulties.

F. Summary

These and other published studies have shown that more than 20 species of mosquitoes are involved in the transmission of VEE, often making it impossible to single out the most important vector. Very likely, multiple vectors are responsible for transmission of VEE during epidemics; the number of vectors involved may depend on the ecological habitat, the mosquitoes' ability to become infected at virus levels occurring naturally in the field, and their proclivity for feeding upon man and horses.

II. Epidemiologic Studies of Dengue in Puerto Rico

A study of dengue, less intensive now than during the epidemic peak of the summer of 1969, is continuing. A few serologic conversions were detected in humans on the south side of the island in October-December 1969. More recently, the continued presence of dengue-2 has been shown by the isolation of this virus from a seven-year-old female bled in September 1970, in

Table 3

Man and Horse Biting Collections
Guanacaste Province, Costa Rica

Species	Man	Horse	Totals
Aedes taeniorhynchus	11 (1)*	40 (3)	51 (4)
spp.	407 (8)	710 (16)	1,117 (24)
Anopheles albimanus		7 (2)	7 (2)
punctimacula		6 (2)	6 (2)
Culex nigripalpus		195 (4)	195 (4)
(Mel.) sp. #3		15 (2)	15 (2)
Mansonia titillans		5 (2)	5 (2)
Psorophora ferox-like	11 (1)	12 (2)	23 (3)
Totals	429 (10)	990 (33)	1,419 (43)

* Number of mosquitoes collected - (Number of pools tested).

Arbovirus Ecology Laboratory, CDC, 1970

Aguada in western Puerto Rico. Accompanying serologic conversions in humans in this same area have been detected. Preliminary ecologic studies in the area suggest the possibility of the establishment of an endemic situation. Presence of the virus has now been detected through at least the 19th month.

III. Production of More Type-Specific Reagents to Group B Arboviruses

In an attempt to produce antisera useful for neutralization tests in cell cultures and antigens for complement-fixation tests, soluble antigens to a number of Group B arboviruses are being used to immunize monkeys. Preliminary results are quite promising.

(W. D. Sudia, V. F. Newhouse and C. H. Calisher)

REPORT FROM THE ENCEPHALITIS FIELD STATION AND VIRUS LABORATORY MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH BOSTON, MASSACHUSETTS

Massachusetts experienced an epizootic of Eastern equine encephalomyelitis amongst equines during the summer of 1970. The first equine case was reported July 14. Approximately 50 equines died from encephalitis. Geographically the case distribution was similar to the 1938 outbreak. All cases were located south of Boston in southeastern Massachusetts. No cases were detected on Cape Cod.

In cooperation with the Massachusetts Division of Animal Health, blood and brain specimens were collected. Seven horses have been confirmed by EEE virus isolations from brain specimens. EEE neutralizing antibody was detected in blood samples from two additional horses that were unvaccinated.

One human case was also recorded, a five month old child. Date of onset was August 23. The residence of this case was located one half mile from the first recognized equine case and the child had not traveled with his

foster parents.

Mosquito collecting by standard or battery-operated New Jersey light-traps supplemented with dry ice was conducted at 13 horse case sites and the site of an outbreak amongst penned pheasants. EEE virus has been isolated from Culiseta melanura, Coquillettidia perturbans, Culex pipiens and Culiseta morsitans. Positive pools of mosquitoes were collected at seven of the 14 collecting site. Testing of mosquitoes is continuing.

Both EEE virus and WEE virus have been isolated from wild birds captured at our regular study sites. Virus has been isolated from four species of birds (Northern yellowthroat, catbird, robin, and house sparrows). The house sparrows were picked-up sick outside the study areas.

No unusual weather pattern was detected. Annual rainfall has been above normal since 1967. However, during the months selected by Hayes and Hess 1964, * rainfall was far below the amounts that correlated with human epidemics.

A more detailed report of the outbreak will be prepared for the next issue.

* Hayes, Richard O. and Archie D. Hess. 1964 Climatological conditions associated with outbreaks of eastern encephalitis. Am. J. Trop. Med. & Hyg., 13: 851-858

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

A brief summary of the work completed in 1969 and 1970 to date is given in the attached tables. Sera tested by hemagglutination-inhibition (HI) were acetone treated before adsorption with goose cells. All sera were screened with HI antigens prepared with the following viruses: eastern and western encephalomyelitis (EE, WE), Powassan (POW), St. Louis encephalitis (SLE), Cache Valley (CV), and California encephalitis (CE). Neutralization tests (NT) in 2-day-old mice were run on all HI reacting sera; in addition, all human and deer sera were examined for CE and CV neutralizing antibody and goose sera were tested for EE, WE, POW, SLE, CE, and CV antibody. Patients' sera were also investigated by the complement-fixation technic (CF) with EE, WE, POW, SLE, CE and CV antigens. In CF and HI tests four-fold or greater antibody rises and increases of 2 $\log_{10}$ of the neutralization index were considered diagnostic.

(E. Whitney)

DIVISION OF LABORATORIES AND RESEARCH
N. Y. STATE DEPARTMENT OF HEALTH
NEW SCOTLAND AVE., ALBANY, N. Y.

Table 1

Surveys

Year	Samples	Virus Isolations		County
1969	1205 arthropods (138 pools)	CE: 1 pool of A. canadensis CV: 1 pool of A.sollicitans		Rockland Suffolk
	348 wildlife bloods or tissues	EE: 1 pheasant brain		Orange
1970 Jan. - Sept.	327 Arthropods (130 pools)	None		
	26 wildlife bloods or tissues	None		
		Antibody findings		
		HI	NT	
1969	139 birds' sera	EE: 3 pheasants	EE: 4 pheasants	Orange
	37 mammalian sera	None	EE, WE, and CE: 1 horse	Orange
	22 human sera	CE: 6 CV: 2	CE: 6 CV: 2	Albany and Columbia
1970 Jan. - Sept.	67 birds' sera (including 55 geese from St. Lawrence County)	None	None	
	103 deer sera from Seneca County	CE: 8 CV: 21	CE: 33 CV: 47	Seneca
	12 deer sera from Suffolk County	POW: 9 SLE: 6 CV: 2	POW: 11 CE : 4	Suffolk (Shelter Island)
	67 human sera	SLE: 5, CV: 1		Suffolk

EW:dr
10/27/70

DIVISION OF LABORATORIES AND RESEARCH
N. Y. STATE DEPARTMENT OF HEALTH
NEW SCOTLAND AVE., ALBANY, N. Y.

Table 2

Patients with signs of central nervous system involvement

Year	Patients		Age	County
	No. tested for arbovirus antibody	No. with diagnostic findings*		
1969	75	CE: 1	6 yrs.	Albany
		CE and CV: 1	33 yrs.	Columbia
1970 Jan. - Sept.	77	CE: 1	6 yrs.	Monroe
		CE: 1	6 yrs.	Schenectady

Key: * by at least two of the serologic procedures applied

EW:dr
10/27/70